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Evaluation method for drug sorption to PVC- and non-PVC-based tubes in administration sets using a pump

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Abstract:	Administration sets are delivery tools for direct application of drugs into the body and are composed of a spike, a drip chamber, tubes, luer adapters (connectors), a needle cover for protection, and other accessories. Drug sorption to tubes of the administration sets is a critical issue in terms of safety and efficacy. Although drug sorption is an important factor in the quality of an administration set, there are no standard evaluation methods for the regulation of drug sorption to the tubes. Here, we describe an evaluation protocol for drug sorption to tubes of administration sets. Tubes made of polyvinyl chloride (PVC)- and non-PVC-based polymeric materials were cut to 1 m in length. Diazepam and tacrolimus were used as model drugs. In the kinetic sorption study, we selected drug concentration and flow rate based on the clinical usage of these drugs. After dilution of each drug in a glass bottle, the diluted drug solution was delivered through tubes of administration sets using a pump. Samples were collected in amber vials at appropriate time points and drugs were analyzed using high-performance liquid chromatography. Drug concentrations and sorption levels to tubes of the administration sets were calculated. Acceptable criteria to ensure the quality of administration sets are recommended.
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Dear Editor-in-Chief,

Please find enclosed our manuscript entitled "**Evaluation method of drug sorption to PVC- and non-PVC-based tubes in administration sets using a pump**" that we would like to be considered for publication in **Journal of Visualized Experiments**. This paper highlights **a protocol for drug sorption to tubes in administration sets**. The techniques presented in this paper and demonstrated in video format will be highly useful for researchers working in the field of **making standards for evaluation of drug sorption to tubes of administration sets and studying chemical and physical interactions between drugs and polymeric materials used in tubes of administration sets**.

Dr. Su-Eon Jin, Dr. Seungho Jeon, Prof. Hyo-Jin Byon and Prof. Sung-Joo Hwang designed the procedures described in the manuscript. **Dr. Su-Eon Jin and Siwon You** performed the experiments and analyzed the data. Finally, **Dr. Su-Eon Jin and Prof. Sung-Joo Hwang** (I) wrote the manuscript.

During the preparation and submission of this manuscript, we have been kindly assisted by **Dr.Benjamin Werth, Science Editor - Chemistry**.

Thank you for your consideration of this manuscript. My address, phone number, fax number, and e-mail address are listed on the manuscript title page and last page of this cover letter. Please let me know if you will need anything more to process this paper. We look forward to hearing from you.

Sincerely yours,

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TITLE:
Evaluation of drug sorption to PVC- and non-PVC-based tubes in administration sets using a pump

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Drug, Sorption, Tubes, PVC, Non-PVC, Administration set

SHORT ABSTRACT:
We report a method for drug sorption evaluation using a pump and model drugs (diazepam and tacrolimus). After drug analysis using high-performance liquid chromatography, drug concentrations and sorption levels in the tubes of administration sets are calculated.

LONG ABSTRACT:
Administration sets are delivery tools for the direct application of drugs into the body and are composed of a spike, a drip chamber, tubes, Luer adapters (connectors), a needle cover for protection, and other accessories. Drug sorption to tubes of administration sets is a critical issue in terms of safety and efficacy. Although drug sorption is an important factor in the quality of an administration set, there are no standard evaluation methods for the regulation of drug sorption to the tubes. Here, we describe an evaluation protocol for drug sorption to tubes of administration sets. Tubes made of polyvinyl chloride (PVC)- and non-PVC-based polymeric materials were cut to 1 m in length. Diazepam and tacrolimus were used as model drugs. In the kinetic sorption study, we selected the drug concentration and flow rate based on the clinical usage of these drugs. After the dilution of each drug in a glass bottle, the diluted drug

solution was delivered through tubes of administration sets using a pump. Samples were collected in amber vials at appropriate time points and the drugs were analyzed using high-performance liquid chromatography. Drug concentrations and sorption levels to tubes of the administration sets were calculated. Acceptable criteria to ensure the quality of administration sets are recommended.

INTRODUCTION:

Administration sets are composed of a spike, a drip chamber, tubes, Luer adapters (connectors), and a needle cover for protection. Other accessories, such as an airway check valve, a regulating clamp, an in-line filter, a Y-tube with cap (an injection port), and a needle, can also be attached to administration sets. Drug sorption to tubes is a critical issue in the delivery of injectable drugs[1]. Sorption describes the adsorption of a drug to the polymer surface and the absorption of a drug into the polymeric matrix[2]. Drug sorption to tubes in administration sets causes unpredictable drug loss and makes it difficult to control the delivered drug concentration. Drug sorption to polymeric tubes is therefore a major impediment to the precise transfer of injectable drugs into the body. However, there are no standard methods or regulatory guidelines for the evaluation of drug sorption to tubes in administration sets.

The levels of drug sorption to tubes in administration sets have been reported using various evaluation methods[1-5]. Test methods and model drugs are key factors in sorption evaluation. The pump method[1, 3] and drip method[4, 5] have been widely used for sorption tests. In general, the pump method should be used in the case of drugs with low concentrations and low flow rates as the infusion conditions. Using various evaluation methods, many studies of drug sorption have been reported for polyvinyl chloride (PVC)- and non-PVC-based tubes in administration sets[1-3, 5]. Many sorptive drugs can be selected to investigate whether the tubes of the administration sets have drug sorption potential or not. Diazepam (Figure 1a)[1, 2], tacrolimus (Figure 1b)[5], nitroglycerin[2], and cyclosporin A[3] are representative drugs with high sorption in PVC- and non-PVC-based tubes.

For the evaluation of drug sorption to the tubes, test conditions such as flow rate and drug concentration are based on the clinical use of the selected drugs[1, 6, 7]. In the case of diazepam, a high concentration of 100 µg/mL was used at a flow rate of 1 mL/min to mimic the initial dose for the treatment of status epilepticus[1]. For tacrolimus, a concentration of 10 µg/mL was delivered at a flow rate of 10 mL/h. Dextrose solution (5%) was used for the dilution of drug injections, and tube length was fixed at 1 m. Glass bottles and vials should be used to prevent additional sorption during the experiment and storage.

In this study, we conducted a kinetic sorption study with the model drugs, diazepam and tacrolimus, using a pump method. Specific details of this protocol, from tube preparation to sorption evaluation, were described previously. Methods for the evaluation of drug sorption have already been used to confirm drug properties for injections and to recommend the clinical use of injections with administration sets on a case-by-case basis[1-7]. This protocol may be used as a standard technique for the sorption evaluation

of administration sets. International standards for the evaluation of drug sorption to tubes may be necessary to ensure the safety and efficacy of drug delivery.

PROTOCOL:

1. Preparation of tubes in administration sets

Note: Precisely perform the cutting step to eliminate the effect of differences in tube length on drug sorption.

1.1) Label the end of the tubes with the tube type (*e.g.,* PVC, polyurethane (PU), and polyolefin (PO)) using a marker.

1.2) Remove all detachable accessories, such as connectors and needle covers.

1.3) Using a sharp razor to ensure clean edges, cut the tubes to 1 m in length from the connection of the drip chamber.

2. Dilution of drug injections

Note: Use a glass bottle (1 L) as the container for the injected drug solution. Perform the dilution step precisely. Verify the composition of the marketed drug product and use the same batch number for a whole experimental set.

2.1) Label bottles with the drug names (*e.g.,* diazepam or tacrolimus).

2.2) Dilute the drug injections with 5% dextrose solution, from 1 mg/mL to 100 µg/mL for diazepam injections (10 mL of diazepam injection in 100 mL of 5% dextrose solution) and from 5 mg/mL to 10 µg/mL for tacrolimus injections (200 µL of tacrolimus injection in 100 mL of 5% dextrose solution).

Note: Set up the tested concentrations of drugs and solvents based on the clinical usage.

2.3) Gently mix the diluted solutions in the bottle by swirling to obtain homogenous drug solutions.

2.4) Collect diluted solutions (1-10 mL) in amber vials using a glass graduated cylinder.

2.5) Verify the concentration, as described in step 4.

Note: Use these concentrations of drugs as concentrations at the starting points.

3. Kinetic sorption study using an infusion pump

Note: Confirm the tube-dependent flow rate using a pump prior to the sorption test due to the hardness of tubes. Collect samples at precise time points and use glass bottles and vials to prevent additional drug sorption during storage. Perform the test as shown

in Figure 2. Protect the drug solution against light if the drug has photosensitivity. Perform the experiments in triplicate.

3.1) Without creating air bubbles, preload a diluted solution of the drug into the tube using a syringe.

3.1.1) Connect one end of the tube to a syringe.

3.1.2) Put the other end of the tube into the bottled drug solution.

3.1.3) Pull back the syringe plunger until the tube is completely filled with the drug solution.

3.2) Install the tube into an infusion pump.

3.2.1) Open the door of the infusion pump and push the release lever.

3.2.2) Insert the preloaded tube into the infusion pump and keep it straight.

3.2.3) Remove the syringe at the end of the tube after installation.

3.2.4) Put the end of tube into a chemically resistant borosilicate glass graduated cylinder to collect the drug solution after it passes through the tube.

3.3) Set the flow rate based on the type of tube in the administration set (PVC, PU, or PO) and the drug (*e.g.,* 1 mL/min for diazepam and 10 mL/h for tacrolimus).

3.4) Collect samples into amber vials at various time points, at room temperature.

3.4.1) Collect 1-mL diazepam samples at 0.05, 0.30, 0.55, and 1.05 h.

3.4.2) Collect 10-mL tacrolimus samples at 1.05, 2.05, 3.05, and 4.05 h.

4. Analysis of drug using high-performance liquid chromatography (HPLC)

Note: Recommended HPLC methods for drug analysis are described in references [1, 8, 9]. Use tandem mass spectrometry (MS/MS) and immunoassay after sample preparation as alternative methods[10, 11]. Perform the experiments in triplicate.

4.1) Weigh the drugs and dissolve them in organic solvents at a concentration of 1 mg/mL as stock solutions.

4.1.1) Use methanol as a solvent for diazepam stock solution due to the low solubility of diazepam in 5% dextrose.

4.1.2) Use acetonitrile as a solvent for tacrolimus stock solution due to the low solubility of tacrolimus in 5% dextrose.

4.2) Prepare standard solutions by diluting the stock solutions.

4.2.1) Dilute diazepam stock solutions with methanol to 0.3125, 0.625, 1.25, 2.5, 5.0, 10.0, and 20.0 µg/mL.

4.2.2) Dilute tacrolimus stock solutions with 5% dextrose to 2.5, 5.0, 10.0, 15.0, and 20.0 µg/mL.

4.3) Analyze standards using the HPLC method with UV detection[1, 8, 9].

Note: Use an appropriate detection method (UV, fluorescence, etc.) for drug analysis.

4.3.1) Inject 10 µL of standards into an HPLC system with UV detection equipped with a C_{18} column (1.5 mm × 250 mm, 5 µm). See Table 1 for analysis conditions.

4.3.2) Confirm the specificity and linearity[1, 8, 9].

4.3.2.1) For specificity, monitor the drug peak (*i.e.,* whether it was separated from other peaks in the chromatogram) to identify the drug.

4.3.2.2) Confirm linearity at the calibration range (*i.e.,* whether the peak area results are directly proportional to the concentrations).

4.3.3) Obtain calibration curves.

4.3.3.1) Create graphs based on concentration versus peak area value from chromatograms of the standards[1, 8, 9].

4.3.3.2) Obtain linear regression equations with R^2 for the calibration curves (*e.g.,* y = ax + b, x: concentration of drug, y: peak area)[1, 8, 9].

4.4) Dilute the samples from the sorption study with methanol for diazepam or 5% dextrose for tacrolimus as appropriate so that they fall within the calibration range and directly inject 10 µL of diluted sample into the HPLC system.

5. Calculation of drug concentration and sorption level

5.1) Calculate the drug concentrations of the samples using calibration curves (unknown x and known y).

5.2) Calculate the sorption levels of the drugs using the following equation:

$$S = \left(1 - \frac{Cp}{Co}\right) \times 100$$

where S: Sorption level (%)

Co: Drug concentration after the dilution of the injections (µg/mL)

Cp: Drug concentration passed through the tubes (µg/mL)

REPRESENTATIVE RESULTS:

The sorption to tubes in the administration set was kinetically monitored using the model drugs, diazepam (Figure 1a) and tacrolimus (Figure 1b), and the pump method (Figure 2). Diluted drug (Figure 2a) was passed through PVC- and non-PVC-based tubes (Figure 2b) at a fixed flow rate using an infusion pump (Figure 2c). The glass bottle was opened slightly to allow the insertion of the administration set tube. After the drug was delivered through the tubes (Figure 2d), samples were collected into amber vials (Figure 2e). All samples, including samples at the starting points, were analyzed using an HPLC method with UV detection (Figure 3a). Analysis conditions are listed in Table 1. For the preparation of drug standards, diazepam and tacrolimus were dissolved in methanol and acetonitrile because of their insolubility in 5% dextrose. The drug concentrations at the starting points of the sorption study were calculated from the analysis of the samples after the drug dilution. Sorption levels in PVC- and non-PVC-based tubes of administration sets were determined by calculating the percentage of remaining drug content after passage through the tubes from the calibration curves (Figure 3b) and subtracting these values from 100% (Figure 3c). The recommended acceptable range of drug sorption percentages was less than 10%, based on the content of injections from pharmacopoeias[12]. In addition, the specific drugs (*e.g.,* anticancer drugs) should be confirmed with clinical guidelines. We determined whether drug sorption levels of the samples were appropriate (Figure 3d). Figure 4 and Figure 5 show representative chromatograms at low and high concentrations of drugs for standards and sample solutions, respectively. Retention time of each drug was 8.2 min for diazepam and 6.8 min for tacrolimus. There were no interfering peaks from the matrix. Specifically, in samples of the diazepam sorption study, the interfering peaks did not overlap with drug peak in the chromatogram, although a different solvent (5% dextrose) was used than in the standards. Table 2 shows calculations of representative sorption levels of diazepam and tacrolimus. The sorption level of each drug was the highest in PVC-based tubes, lower in PU-based tubes, and lowest in PO-based tubes at the initial phase of the kinetic sorption test.

FIGURE LEGENDS:

Figure 1. Chemical structures of model drugs: (a) diazepam and (b) tacrolimus. Diazepam is a benzodiazepine derivative and tacrolimus is a 23-membered macrolide lactone. This figure has been modified[1].

Figure 2. Test set of a kinetic sorption study using a pump. (a) Drug diluted with 5% dextrose in a bottle, (b) tube of administration set (1 m in length), (c) infusion pump, (d) drug passed through the tube, and (e) amber vials for storage. To minimize additional drug sorption, drug solutions and samples were prepared and stored in a glass bottle and amber vials for injections, respectively. This figure has been modified[1].

Figure 3. Key steps for evaluating drug sorption level to tubes of administration sets. (a) Analysis of drug using an HPLC method with UV detection, calculation of (b) drug concentration from the calibration curve and (c) sorption level (%), and (d) recommendation of acceptable criteria for drug sorption. Several steps in the sorption evaluation, from drug selection to the sorption test, such as the analysis of drug concentration and the calculation of sorption levels, are described.

Figure 4. Representative chromatograms of diazepam. (a) Blank (methanol), standards at (b) 0.3125 µg/mL and (c) 20 µg/mL, and (d) sample (5% dextrose). The peak of diazepam was detected at 8.2 min, and fluctuations in retention time occurred within 1 min in the chromatograms. The peaks from the solvent were presented before the diazepam peak in the blank and standard chromatograms.

Figure 5. Representative chromatograms of tacrolimus. (a) Blank (acetonitrile with 5% dextrose), standards at (b) 2.5 µg/mL and (c) 20 µg/mL, and (d) sample. The tacrolimus peak at 6.8 min was successfully separated in a chromatogram. There were no peaks that overlapped with the tacrolimus peak. The peak was detected at 2.5 µg/mL, with a signal-to-noise ratio greater than 10.

Table 1. HPLC conditions.

Table 2. Representative sorption results for diazepam and tacrolimus in PVC- and non-PVC-based tubes (n = 3)[1].

DISCUSSION:

Drug sorption to administration sets is a cause of unexpected drug loss in intravenous drug delivery. During sorption, drugs are generally partitioned to polymeric materials of tubes at the early phase of infusion; after sorption equilibrium is reached, the delivered amount of drug is stabilized[1]. The sorption levels of drugs should be evaluated and minimized. Several evaluation methods for drug sorption have been studied, such as a pump method and a drip method. Compared to the drip method, the pump method can be easily manipulated without bias. Although administration sets with flow regulators (conventional form) are used in the drip method, desired flow rates less than 5 mL/h are hard to achieve. Therefore, we recommend the pump method for the sorption evaluation of tubes in administration sets.

When using the pump method, major factors that affect drug sorption to tubes of administration sets are drug properties (*e.g.,* hydrophobicity and charge), conditions of the sorption test (*e.g.,* drug concentration, flow rate, solvent compatibility, tube length, and temperature), analytical methods for drugs (*e.g.,* HPLC and MS), and the tube polymers in the administration sets (*e.g.,* PVC, PU, and PO)[2-12]. First, the selection of the model drugs is critical for obtaining precise and accurate experimental results. Even though diazepam management is tracked by the Psychotropic Drugs Control Act, we selected diazepam (Figure 1a) and tacrolimus (Figure 1b) as model drugs because of their high sorption levels to polymeric tubes of administration sets[1] or containers[13, 14]. In this case, drugs at high concentrations showed less sorption than those at low concentrations in the early phase of infusion[1, 2]. These drugs have high log P values (diazepam: 2.82[15], tacrolimus: 3.96[16]) and low solubility, as categorized by the Biopharmaceutical Classification System (BCS class 2). Because of their hydrophobicity, these drugs can interact with tubes of administration sets, leading to sorption. Other drugs showing high sorption levels (*e.g.,* nitroglycerin[2] and cyclosporin A[3]) can be used as alternative model drugs for sorption evaluation. Furthermore, macromolecular drugs,

such as biologics (antibody therapeutics, insulin, etc.), can be applied for the quality evaluation of administration sets regarding drug sorption[12].

We set up a simple kinetic sorption study, using a pump to easily obtain precise results and to minimize artifacts. (Figure 2). In the pump method, drug solution (Figure 2a) was passed through a tube cut from the administration set (Figure 2b) after installation into an infusion pump (Figure 2c). Except for the tubes from administration sets, all devices (bottle, graduated cylinder, and sampling vials) were composed of chemically resistant borosilicate glass to prevent additional drug sorption to polymers. In this study, tubes without other accessories at a fixed length of 1 m were used to simplify the factors of drug sorption to tubes of administration sets. If a clinical condition requires it, a multiplication factor for the length of tube can be used. In the sorption test, the diluted drug solutions were used as the starting concentrations[1,7]. After delivery, the drug solution (Figure 2d) was collected into vials at various time points (Figure 2e). Drug solutions for sampling were passed completely through the tube at the preselected conditions of flow rate and sampling time points. Sorption generally occurs in the early phase of infusion, and the pattern is followed by a convection-interfacial resistance-diffusion model[7]. Diazepam sorption results are comparable to the double-lumen extension tube model[17] when the initial drug concentration is considered before delivery. Therefore, sampling time points can be modified so that sorption evaluation takes less time. All factors of the test conditions were confirmed based on the clinical usage of drugs.

In this protocol, we chose the HPLC method for drug analysis based on previous reports[1, 8, 9]. Simple and reproducible HPLC methods have been developed. The HPLC conditions are listed in Table 1. Various other techniques, such as MS and immunoassay, have also been developed as alternative analysis methods of drug concentrations[10, 11]. MS/MS and immunoassay are highly sensitive for the detection of drugs and their metabolites. Specifically, an immunoassay can easily be performed without requiring large and expensive equipment for drug analysis.

Regarding the quality evaluation of administration sets, drug sorption to PVC- and non-PVC-based materials used in the tubes of administration sets has been studied. The evaluation of sorption to tubes in administration sets started with drug selection and ended with the consideration of acceptable criteria of sorption levels, as illustrated (Figure 3). PVC-based tubes showed high sorption levels for many drugs such as diazepam, tacrolimus (Table 2), nitroglycerin[2], and cyclosporin A[3]. Among approaches to minimize drug sorption to the tubes of administration sets to less than 10%, alternative materials or polymeric combinations have been developed, such as PO-based materials and layer-by-layer designs[2, 13]. The PE/PB/PP blended PO-based tube of an administration set used in this study showed low sorption levels, as a non-PVC-based tube. On the other hand, PE-based tubes are not used for administration sets, but they are commercially used in the market as a syringe extension tube due to their hardness.

This protocol can be applied to the quality control of administration sets with respect to drug sorption. More drugs classified by sorption level (highest, lower, and lowest) should be used in sorption evaluations for the quality assurance of administration sets. This protocol can also be used in scientific research for the development of new alternative polymeric materials or new designs for tubes in administration sets that do not result in drug sorption[1, 13].

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DISCLOSURES:
The authors have nothing to disclose.

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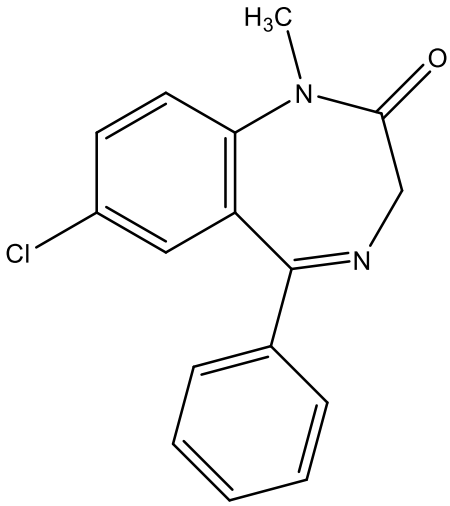

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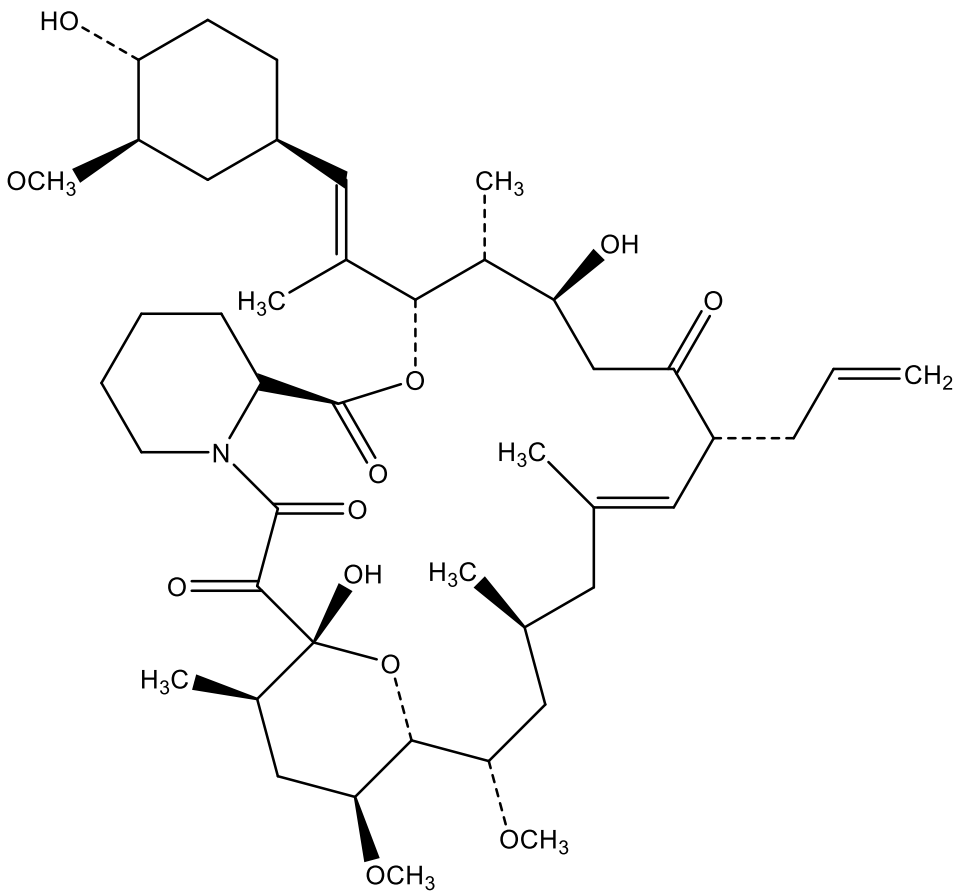

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Figure 1

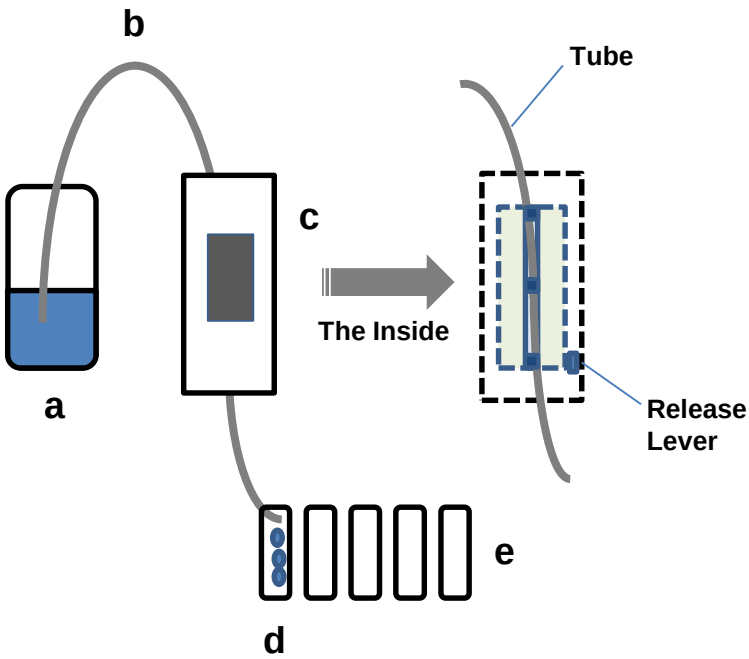

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Figure 2

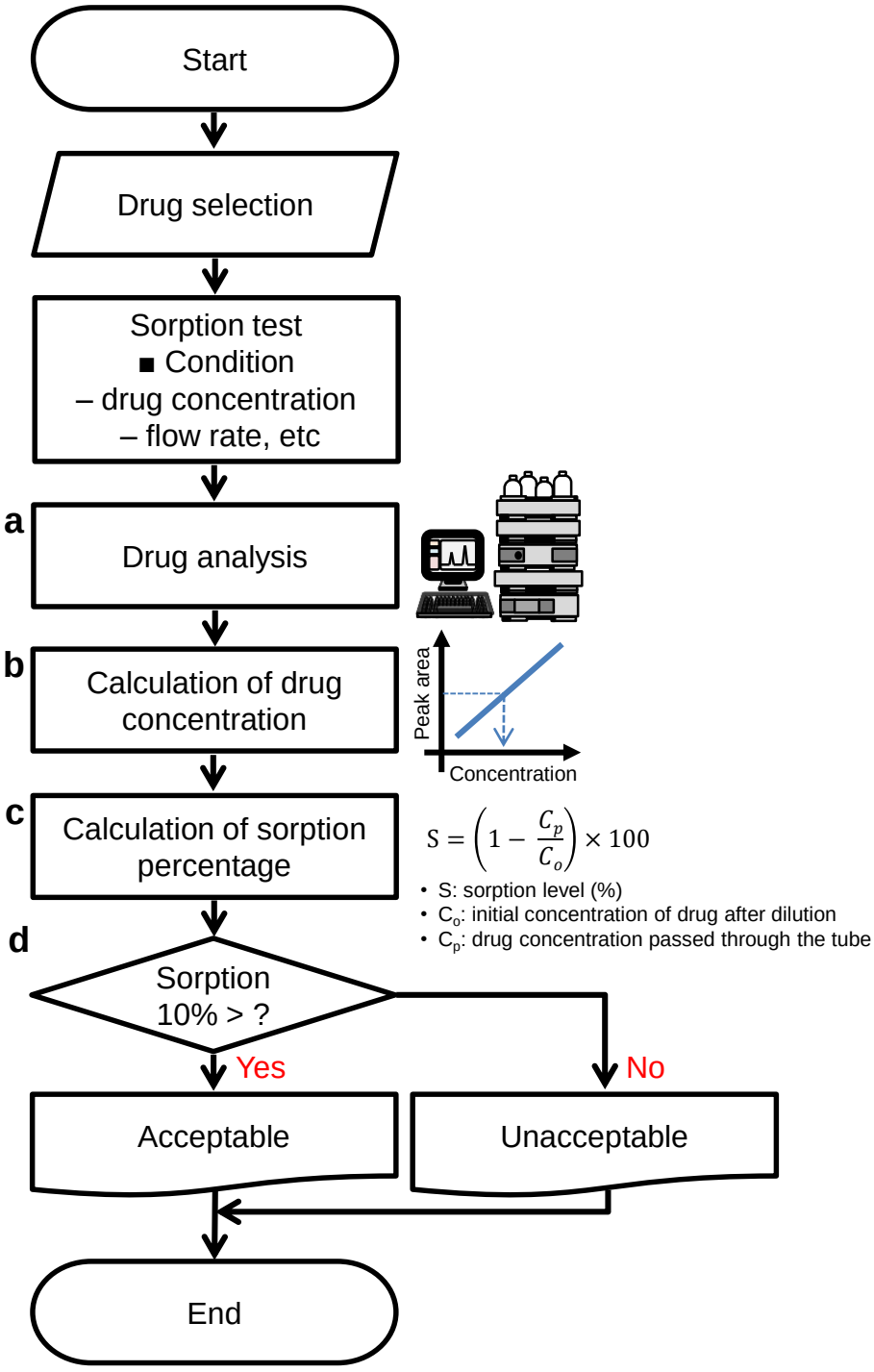

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Figure 3

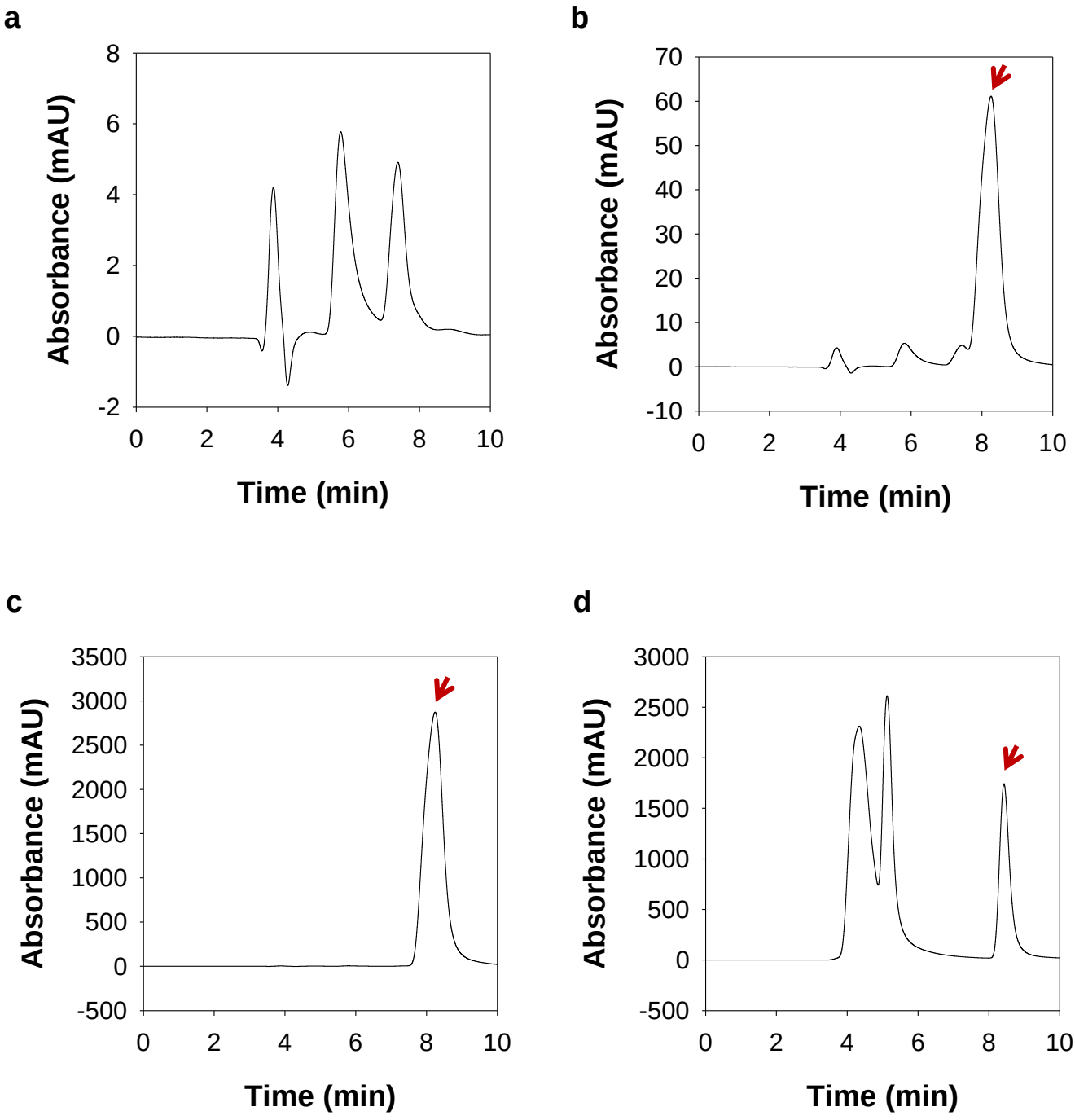

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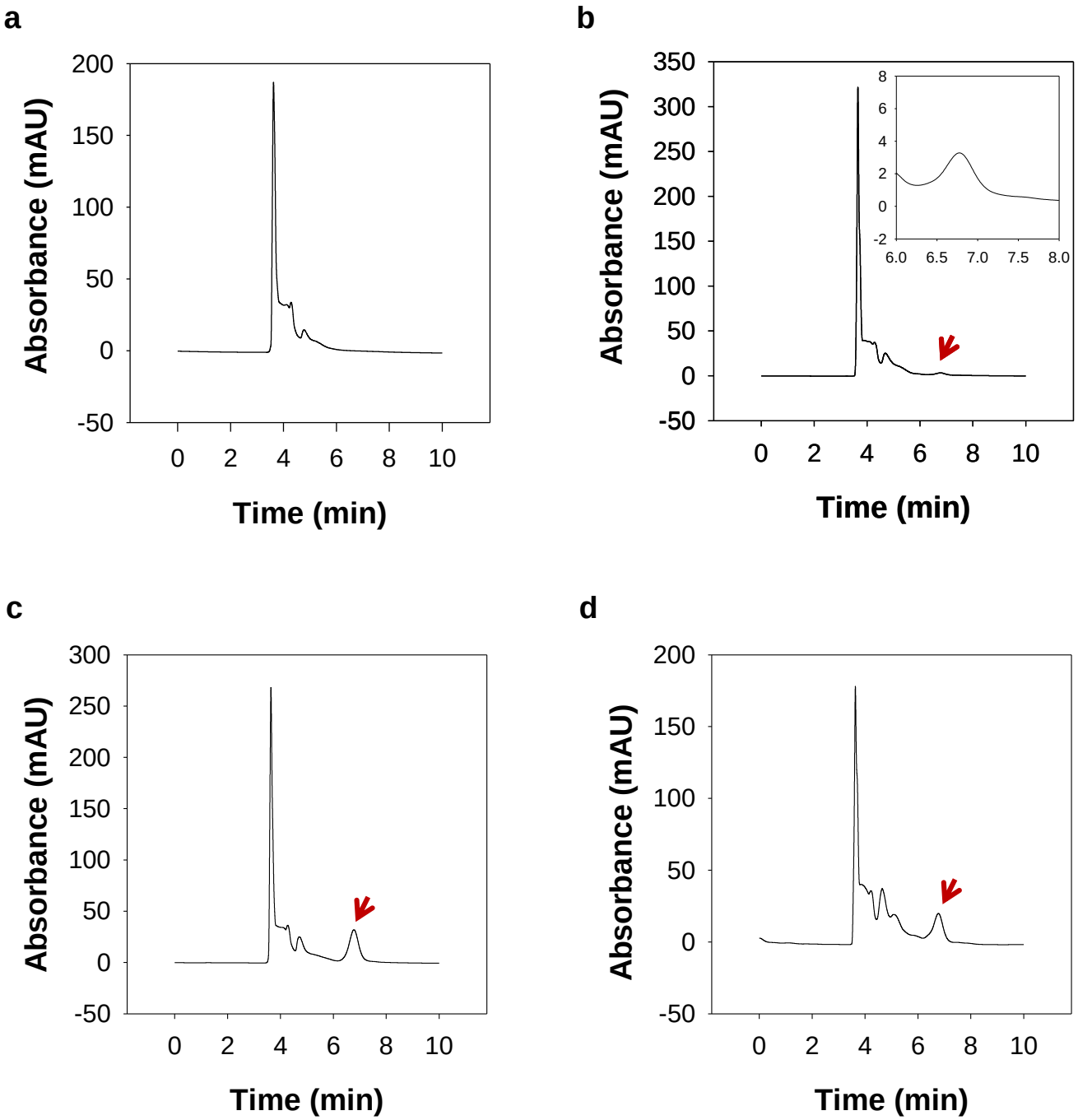
Figure 4



Jin et al.
Figure 5

Table1

HPLC condition	Drug	
	Diazepam	Tacrolimus
Mobile phase	A mixture of acetonitrile, methanol, and sodium phosphate buffer* (29:47:24, v/v/v, adjusted to pH 3.1 with phosphoric acid) *Sodium dihydrogen phosphate 1.2 g/L in distilled water	Acetonitrile (100%)
Flow rate	0.1 mL/min	0.1 mL/min
UV wavelength	232 nm	213 nm
Run time	10 min	10 min
Retention time	8.2 min	6.8 min

Table2

Click here to download Table Table 2 Representative sorption results_PR2.xls

Drug	Sorption (%)		
	PVC	PU	PO
Diazepam (0.05 h)	27.6 ± 3.0	21.9 ± 12.6	11.3 ± 4.6
Tacrolimus (1.05 h)	15.1 ± 3.3	10.3 ± 6.3	0.6 ± 0.9

Excel Spreadsheet- Table of Materials/Equipment

Click here to download Excel Spreadsheet- Table of Materials/Equipment Evaluations_Materials_Equipment

Name of Reagent/ Equipment	Company	Catalog Number	Comments/ Description
PVC IV sets	Becton Dickinson (BD) Co. Ltd. (Franklin Lakes, NJ, USA)		Internal diameter: 2.54 mm
PU IV sets	Tianjin Hanaco Medical (THM) Co.Ltd. (Tianjin, China)		Non-PVC Internal diameter: 2.54 mm
Non-PVC Polyolefin IV sets	Polyscientech, Co. Ltd (Anseong, Korea)		Non-PVC [PE elastomer/PP elastomer/PB elastomer(25/50/25, weight ratio) blend] Internal diameter: 2.54 mm
Syringe	Korea Vaccine Co. Ltd. (Seoul, Korea)	KOVAX-SYRINGE 1 mL 26G 1/2"	
Daewon diazepam injection	Daewon Pharma. Co. Ltd. (Hwaseong, Gyunggi, Korea)		1 mg/mL, total 2 mL Batch No.: P003 Composition: diazepam, propylene glycol, ethanol, benzyl alcohol, sodium benzoate, bezoic acid, water for injection
Tacrobel injection	Chong Keun Dang, Co. Ltd. (Seoul, Korea)		5 mg/mL, total 1 mL Batch No.: AG002 Composition: tacrolimus hydrate, polyoxyl 60 hydrogenated castor oil (HCO-60), dehydrated alcohol
5% Dextrose	JW Pharmaceutical (Seoul, Korea)	500 mL	
5% Dextrose	Daehan Pharmaceutical (Seoul, Korea)	200 mL	Bottle (glass)
Amber vials		20 mL	Glass
Terumo infusion pump	Terumo (Medical Corp., USA)	TE-135	
HPLC system with UV detector	Agilent (Santa Clara, CA, USA)	Agilent 1260	
CAPCELL PAK C_{18} column	Shiseido (Japan)	90404	1.5 mm x 250 mm, 5 µm
Diazepam			From Daewon Pharma. Co., Ltd.
Tacrolimus	Teva Czech industries (Czech Republic)		From Chong Keun Dang, Co. Ltd.
Acetonitrile	Burdick and Jackson Co., Ltd. (MI, USA)	3/1/9017	

Methanol	Burdick and Jackson Co. Ltd. (MI, USA)	AH230-4	
Water	Burdick and Jackson Co. Ltd. (MI, USA)	3/1/4218	
Sodium dihydrogen phosphate	Sigma (St. Louis, MO, USA)		
Phosphoric acid	Sigma (St. Louis, MO, USA)		

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Author(s): Su-Eon Jin, Siwon Yoo, Seungho Jeon, Hyo-Jin Byon, Sung-Joo Hwang

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• 1.3: How are the tubes cut precisely? Sharp razor?
→ Recommendation added.
PROTOCOL
1. Preparation of tubes in administration sets
Note: Perform the cutting step precisely to eliminate the effect of differences in tube length on drug sorption. Use a sharp razor.

• 2.1: Please re-write for grammar and clarity.
2.1) Label the bottle with the dame (e.g., diazepam and tacrolimus).
→2.1) Add a label indicating drug name (e.g., diazepam or tacrolimus) to the bottle.

• 2.2. Note: Please re-write for grammar and clarity.
2.2) Dilute drug injections to the following concentrations in bottled 5% dextrose solution: diazepam injections, 1 mg/mL to 100 µg/mL (10 mL of diazepam injection in 100 mL) and tacrolimus injections, 5 mg/mL to 10 µg/mL (200 µL of tacrolimus injection in 100 mL)
→2.2) Dilute drug injections with 5% dextrose solution: from 1 mg/mL to 100 µg/mL for diazepam injections (10 mL of diazepam injection in 100 mL of 5% dextrose solution) and from 5 mg/mL to 10 µg/mL for tacrolimus injections (200 µL of tacrolimus injection in 100 mL of 5% dextrose solution).

• Protocol section 3.2: Please clarify how to insert the middle of the preloaded tube into the infusion pump. It is not clear what the "middle" of the tube indicates.
→To clarify the protocol 3.2, we added the inside of an infusion pump in Figure 2 and corrected the unclear part.

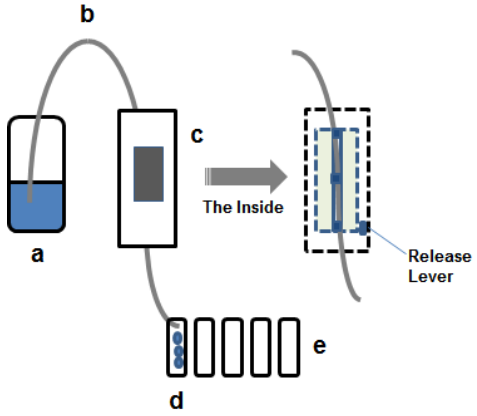

Figure 2. Test set of kinetic sorption study using a pump. (a) Drug diluted with 5% dextrose in a bottle, (b) tube of administration set (1 m in length), (c) infusion pump, (d) drug passed through the tube, and (e) amber vials for storage. To minimize additional drug sorption, drug solutions and samples were prepared and stored in a glass bottle and amber vials for injections, respectively. This figure has been modified[1].

• 3.2.4: What does the glass graduated cylinder contain?

→The glass graduated cylinder is made of chemically resistant borosilicate glass and used to collect the drug solutions without additional sorption to polymeric materials.

3.2.4) Put the end of tubes into a glass graduated cylinder after removing the syringe.

→3.2.4) Put the end of tube into a chemically resistant borosilicate glass graduated cylinder, which is used to collect drug solution after passing through the tube.

• Figure 4 legend: Please clarify "In the chromatogram, solvent peak was presented before diazepam peak".

Figure 4. Representative chromatograms of diazepam. (a) Blank (methanol), standard at (b) 0.3125 µg/mL and (c) 20 µg/mL, and (d) sample in 5% dextrose. The peak of diazepam was detected at 8.2 min and fluctuations in retention time occurred within 1 min in the chromatograms. In the chromatogram, solvent peak was presented before diazepam peak.

→**Figure 4. Representative chromatograms of diazepam.** (a) Blank (methanol), standards at (b) 0.3125 µg/mL and (c) 20 µg/mL, and (d) sample (5% dextrose). The peak of diazepam was detected at 8.2 min, and fluctuations in retention time occurred within 1 min in the chromatograms. The peaks from solvent were presented before the diazepam peak in the chromatograms of blank and standard.

• Table 2: Please provide at least replicate data. Data from previous publication is acceptable.

→We added the data in Table 2. Results are expressed the mean ± SD (n=3).

Table 2. Representative sorption results for diazepam and tacrolimus in PVC- and non-PVC-based tubes (n=3)[1].

Drug	Sorption (%)		
	PVC	PU	PO
Diazepam (0.05 h)	27.6 ± 3.0	21.9 ± 12.6	11.3 ± 4.6
Tacrolimus (1.05 h)	15.1 ± 3.3	10.3 ± 6.3	0.6 ± 0.9

• Please expand your discussion to cover the following in detail and in paragraph form: 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol. Several points have been deleted and must be added.

→We clarify the limitations of the technique, significance with respect to existing methods, future applications and critical steps within the protocol.

DISCUSSION

Drug sorption to administration sets is a cause of unexpected drug loss in intravenous drug delivery. During sorption, drugs are generally partitioned to polymeric materials of tubes at the early phase of infusion; after sorption equilibrium is reached, the delivered amount of drugs is stabilized[1]. The sorption levels of drugs should be evaluated and minimized. Several evaluation methods for drug

sorption have been studied, such as a pump method and a drip method. Compared to the drip method, the pump method can be easily manipulated without bias. Although administration sets with flow regulators (conventional form) are used in the drip method, desired flow rates less than 5 mL/h are hard to achieve. Therefore, we recommend the pump method for sorption evaluation of tubes in administration sets.*(3) significance with respect to existing methods)*

When using the pump method, major factors that affect drug sorption to tubes of administration sets are classified by drug properties (e.g., hydrophobicity, charge), conditions of sorption test (e.g., drug concentration, flow rate, solvent compatibility, tube length, temperature), analytical methods for drugs (e.g., HPLC, MS), and polymers of tubes in the administration sets (e.g., PVC, PU, PO)[2-12].*(5) critical steps within the protocol)* First, selection of the model drugs is critical for obtaining precise and accurate experimental results. Even though diazepam management is tracked by the Psychotropic Drugs Control Act, we selected diazepam (Figure 1a) and tacrolimus (Figure 1b) as model drugs because of their high sorption levels to polymeric tubes of administration sets[1] or containers[13, 14]. In this case, drugs at high concentrations showed less sorption than those at low concentrations in the early phase of infusion[1, 2]. These drugs have high log P values (diazepam - 2.82[15], tacrolimus - 3.96[16]) and low solubility as categorized by the Biopharmaceutical Classification System (BCS class 2). Because of their hydrophobicity, these drugs can interact with tubes of administration sets, leading to sorption. Other drugs showing high sorption levels (e.g., nitroglycerin[2] and cyclosporin A[3]) can be used as alternative model drugs for sorption evaluation. Furthermore, macromolecular drugs such as biologics (antibody therapeutics, insulin, etc.) can be applied for quality evaluation of administration sets regarding drug sorption[12]. *(1) modifications and troubleshooting)*

Next, we set up a simple kinetic sorption study using a pump to easily obtain the precise results, and minimize artifacts. (Figure 2). In the pump method, drug solution (Figure 2a) was passed through a tube cut from the administration set (Figure 2b) after installation into an infusion pump (Figure 2c). Except for the tubes from administration sets, all devices (bottle, graduated cylinder, and sampling vials) were composed of chemically resistant borosilicate glass to prevent additional drug sorption to polymers.*(5) critical steps within the protocol)* In this study, tubes without other accessories at a fixed length of 1 m were used to simplify the factors of drug sorption to tubes of administration sets. *(2) limitations of the technique)* If a clinical condition requires it, we can use a multiplication factor for a length of tube.*(1) modifications and troubleshooting)* In the sorption test, the diluted drug solutions were used as starting concentrations[1,7]. After delivery, the drug solution (Figure 2d) was collected into vials at various time points (Figure 2e). Drug solutions for sampling were passed completely through the entire tube at the preselected conditions of flow rate and sampling time points. Sorption generally occurs in the early phase of infusion and the pattern is followed by convection-interfacial resistance-diffusion model[7]. Comparing with double-lumen extension tube model[17], diazepam sorption results are comparable when the initial drug concentration is considered before delivery. Therefore, sampling time points can be modified so that sorption evaluation takes less time. All factors of test conditions were confirmed based on the clinical usage of drugs. *(1) modifications and troubleshooting)*

In this protocol, we chose the HPLC method for drug analysis based on previous reports[1, 8, 9]. Simple and reproducible HPLC methods have been developed. The HPLC conditions are listed in Table 1. Various other techniques such as MS and immunoassay have also been developed as alternative analysis methods of drug concentrations[10, 11]. MS/MS and immunoassay are highly sensitive for detection of drugs and their metabolites. Specifically, immunoassay can be easily performed without requiring large and expensive equipment for drug analysis.*(1) modifications and troubleshooting)*

Regarding quality evaluation of administration sets, drug sorption to PVC- and non-PVC-based materials used in the tubes of administration sets has been studied. Evaluation of sorption to tubes in administration sets started with drug selection and ended with consideration of acceptable criteria of sorption levels as illustrated (Figure 3). PVC-based tubes showed high sorption levels for many drugs such as diazepam, tacrolimus (Table 2), nitroglycerin[2], and cyclosporin A[3]. Among approaches to minimize drug sorption in the tubes of administration sets to less than 10%, alternative materials or designs of polymeric combinations have been developed, such as PO-based materials and layer-by-layer designs[2, 13]. PE/PB/PP blended PO-based tube of administration set used in this study, showed

low sorption level as non-PVC-based tube. On the other hand, PE-based tubes are not used for the administration sets, but commercially used in the market as a syringe extension tube due to their hardness.

This protocol can be applied for the quality control of administration sets with respect to drug sorption. More drugs classified by sorption level (highest, lower, and lowest) should be used in sorption evaluation for quality assurance of administration sets. This protocol can also be used in scientific research for development of new alternative polymeric materials or new designs for tubes in administration sets that do not result in drug sorption[1, 13]. ***(4) future applications)***

• Please copy edit your manuscript. There are a number of grammatical errors throughout. We recommend that you have a native English speaker copy-edit the manuscript.
→We have an English editing service with a native English speaker. Grammatical errors have been corrected.

• Please discuss the limitations of the method in the Discussion section.
→We discussed 2) limitations of the method as mentioned above.

DISCUSSION
~. In this study, tubes without other accessories at a fixed length of 1 m were used to simplify the factors of drug sorption to tubes of administration sets. If a clinical condition requires it, we can use a multiplication factor for a length of tube.~

• Figure 1: Is this Figure from Wikipedia? If you are re-using figures (e.g. Figures 1 and 2) from a previous publication, please obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section.
→We redraw chemical structures of diazepam and tacrolimus.

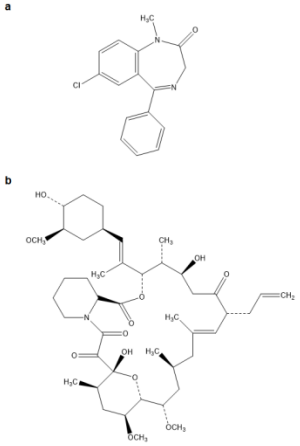

Figure 1. Chemical structures of model drugs: (a) diazepam and (b) tacrolimus. Diazepam is a benzodiazepine derivative, and tacrolimus is a 23-membered macrolide lactone. This figure has been modified[1].

• Please note that the reviewers raised some significant concerns regarding your method and your manuscript. Please address and/or expand on the following comments in the manuscript:

Reviewer #1:
• The main part step 3.2 is not clear enough. Subsequently the related figure (#2) is not well

illustrated. The "middle part" is nonsense and also after "removing the syringe" the open end is left inside the drug solution or what?
→To clarify the protocol 3.2, we added the inside of an infusion pump in Figure 2 and corrected the unclear part as mentioned above.

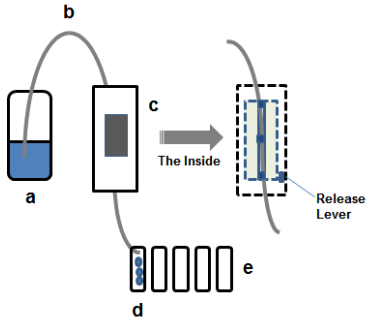

Figure 2. Test set of kinetic sorption study using a pump. (a) Drug diluted with 5% dextrose in a bottle, (b) tube of administration set (1 m in length), (c) infusion pump, (d) drug passed through the tube, and (e) amber vials for storage. To minimize additional drug sorption, drug solutions and samples were prepared and stored in a glass bottle and amber vials for injections, respectively. This figure has been modified[1].

• Step 4.3.1: Not all drugs can be quantified using UV detector. It should be considered here.
→We added the note for the detection method.

4.3) Analyze standards using the HPLC method with UV detection[1, 8, 9].
Note: Use an appropriate detection method (UV, fluorescence, etc) for drug analysis.

Reviewer #2:
• The sorption model proposed by Su-Eon Jin et al isn't an innovative concept. Provided data are inadequate in view of recent literature on the subject, e.g. the article of Foinard et al (PlosOne, 2016). This model doesn't correspond to a standardized method or guidelines because it couldn't be applied to any drug solution. It has been specifically performed on 2 drugs wellknown to absorb in PVC material : diazepam and tacrolimus, in very specific clinical conditions. The latter may be very different according the drugs tested. Here, specific parameters are tested:
- administration procedure : with a pump procedure,using a syringue pump
- length of tubes (which could influence the sorption)
- flow rate : because they are plenty of different flow rates used in clinical practice (especially considerating adults or children patients and also considerating the indication)
- specific tested concentrations: they may represent the therapeutic use of drugs. If several dosings do exist according the indication, they all have to be tested (especially for diazepam in this study)
- the acceptable criteria of drug sorption (±10%) : nowadays, some drugs' stabilities have to match an acceptable range less than 5% beacause of the physicochemical nature and the toxicty of the drugs. Such is the acs for instance of anticancer agents, as it is recommended in the last SFPO (Société Française de Pharmacie Oncologique) guidelines (Bardin et al, Annales Pharmaceutiques Françaises 2011)
- etc…
****This comment was not satisfactorily addressed in the manuscript. Please discuss the points made in the rebuttal letter in the manuscript. Please note that the authors' rebuttal was not clear and did not address the reviewer's comments.***
→ Sorption model used in Jin et al. is already proposed by Roberts (reference: 7. Roberts, M.S. Modeling solute sorption into plastic tubing during organ perfusion and intravenous infusions. *J. Pharm. Sci.* **85**, 655-665, doi:10.1021/js9500621 (1996).). This model is designed as nonlinear regression with a convection-interfacial resistance-diffusion based on the preloaded tube model with

solution. This model has been confimed. We also checked the test conditions (Jin et al.). We used this model to explain our data set and confrimed whether the pattern was matched or not.

The model proposed by Foinard et al. is not new. Foinard et al. didn't consider the initial point of drug concentration before delivery in sorption study. If they consider the concentration of drug at real "0" time of starting point, their concentration level should be started with 100%. Then, it is the same as Roberts. We think the model from Roberts can correspond to a standardized method or regulatory guidelines because sorption level should be compared with the drug concentration at starting point.

Although diazepam and tacrolimus are highly sorptive drugs categorized by BCS class II, their sorption levels are different in various types of tubes. Specifically sorption levels of diazepam and tacrolimus are lowered in PO-based tubes of administration sets than other tubes of administration sets. We recommended the protocol to minimize the affecting factors for drug sorption by fixing several parameters. This protocol is designed to perform easily. The acceptable criteria of drug sorption using our protocol with recommended drugs were lower than 10%. In the case of specific drugs, it should be included in pharmacopoeia or prescribing information.

→ We added the reviewer's comment in protocol and discussion of the manuscript.

REPRESENTATIVE RESULTS

~The recommended acceptable range of drug sorption percentages was less than 10%, based on the content of injections from pharmacopoeias[12]. In addition, the specific drugs (e.g., anticancer drugs) should be confirmed with clinical guidelines.~

DISCUSSION

~ When using the pump method, major factors that affect drug sorption to tubes of administration sets are classified by drug properties (e.g., hydrophobicity, charge), conditions of sorption test (e.g., drug concentration, flow rate, solvent compatibility, tube length, temperature), analytical methods for drugs (e.g., HPLC, MS), and polymers of tubes in the administration sets (e.g., PVC, PU, PO)[2-12]. First, selection of the model drugs is critical for obtaining precise and accurate experimental results. Even though diazepam management is tracked by the Psychotropic Drugs Control Act, we selected diazepam (Figure 1a) and tacrolimus (Figure 1b) as model drugs because of their high sorption levels to polymeric tubes of administration sets[1] or containers[13, 14]. In this case, drugs at high concentrations showed less sorption than those at low concentrations in the early phase of infusion[1, 2]. These drugs have high log P values (diazepam - 2.82[15], tacrolimus - 3.96[16]) and low solubility as categorized by the Biopharmaceutical Classification System (BCS class 2). Because of their hydrophobicity, these drugs can interact with tubes of administration sets, leading to sorption. Other drugs showing high sorption levels (e.g., nitroglycerin[2] and cyclosporin A[3]) can be used as alternative model drugs for sorption evaluation. Furthermore, macromolecular drugs such as biologics (antibody therapeutics, insulin, etc.) can be applied for quality evaluation of administration sets regarding drug sorption[12].

Next, we set up a simple kinetic sorption study using a pump to easily obtain the precise results, and minimize artifacts. (Figure 2). In the pump method, drug solution (Figure 2a) was passed through a tube cut from the administration set (Figure 2b) after installation into an infusion pump (Figure 2c). Except for the tubes from administration sets, all devices (bottle, graduated cylinder, and sampling vials) were composed of chemically resistant borosilicate glass to prevent additional drug sorption to polymers. In this study, tubes without other accessories at a fixed length of 1 m were used to simplify the factors of drug sorption to tubes of administration sets. If a clinical condition requires it, we can use a multiplication factor for a length of tube. In the sorption test, the diluted drug solutions were used as starting concentrations[1,7]. After delivery, the drug solution (Figure 2d) was collected into vials at various time points (Figure 2e). Drug solutions for sampling were passed completely through the entire tube at the preselected conditions of flow rate and sampling time points. Sorption generally occurs in the early phase of infusion and the pattern is followed by convection-interfacial resistance-diffusion model[7]. Comparing with double-lumen extension tube model[17], diazepam sorption results are comparable when the initial drug concentration is considered before delivery. Therefore, sampling time points can be modified so that sorption evaluation takes less time. All factors of test conditions

were confirmed based on the clinical usage of drugs.

In this protocol, we chose the HPLC method for drug analysis based on previous reports[1, 8, 9]. Simple and reproducible HPLC methods have been developed. The HPLC conditions are listed in Table 1. Various other techniques such as MS and immunoassay have also been developed as alternative analysis methods of drug concentrations[10, 11]. MS/MS and immunoassay are highly sensitive for detection of drugs and their metabolites. Specifically, immunoassay can be easily performed without requiring large and expensive equipment for drug analysis.

Regarding quality evaluation of administration sets, drug sorption to PVC- and non-PVC-based materials used in the tubes of administration sets has been studied. Evaluation of sorption to tubes in administration sets started with drug selection and ended with consideration of acceptable criteria of sorption levels as illustrated (Figure 3). PVC-based tubes showed high sorption levels for many drugs such as diazepam, tacrolimus (Table 2), nitroglycerin[2], and cyclosporin A[3]. Among approaches to minimize drug sorption in the tubes of administration sets to less than 10%, alternative materials or designs of polymeric combinations have been developed, such as PO-based materials and layer-by-layer designs[2, 13]. PE/PB/PP blended PO-based tube of administration set used in this study, showed low sorption level as non-PVC-based tube. On the other hand, PE-based tubes are not used for the administration sets, but commercially used in the market as a syringe extension tube due to their hardness. ~

• Administration sets in polyethylene have not be tested here. Why? If such material is marketed, it should also be evaluated for drug sorption. Please mention the details presented in the rebuttal letter in the Discussion section. It is not clear what the authors are trying to convey here.

→ Polyethylene (PE)-based tubes are not currently available in the market of administration sets because only PE-based tubes are too hard to use as tubes of administration sets. They are used in syringe extension sets. We used blended PO tubes of administration sets.

DISCUSSION

~ Among approaches to minimize drug sorption in the tubes of administration sets to less than 10%, alternative materials or designs of polymeric combinations have been developed, such as PO-based materials and layer-by-layer designs[2, 13]. PE/PB/PP blended PO-based tube of administration set used in this study, showed low sorption level as non-PVC-based tube. On the other hand, PE-based tubes are not used for the administration sets, but commercially used in the market as a syringe extension tube due to their hardness.~

• The administration sets are cut to 1m length. Why? It doesn't reflect clinical conditions because the devices often are more than 1.5m length: Author response (from the rebuttal letter): To remove the accessories of administration sets except tubes, we cut the tubes. If we use shorter length of tubes like 1 m in length, it takes shorter time to evaluate the drug sorption to tubes of administration sets. For considering a length factor of tube, it can reflect clinical conditions. Overall, it makes a protocol simple and easy to perform based on the clinical conditions. This comment was not satisfactorily addressed. Is the tube cut to 1 m for simplicity and easy of performance? In that case, it does not reflect the clinical settings.

→We already mentioned the length of tubes for sorption test. The tube cut to 1 m is not a clinical setting, but it can be easily multiplied for the total length of tubes to reflect the clinical settings. We mentioned it in discussion.

DISCUSSION

~Next, we set up a simple kinetic sorption study using a pump to easily obtain the precise results, and minimize artifacts. (Figure 2). ~ In this study, tubes without other accessories at a fixed length of 1 m were used to simplify the factors of drug sorption to tubes of administration sets. If a clinical condition requires it, we can use a multiplication factor for a length of tube. After delivery, the drug solution

(Figure 2d) was collected into vials at various time points (Figure 2e).~

***• There is no kinetic study (results) as mentioned by the authors. The results show only a percentage value of sorption at the end of the study. Values at each kinetic time have to be presented (table or graph).* Please elaborate on your published kinetic study in the results section. Please provide the reference.**
→We already provided the kinetic data for sorption study (Jin et al., 2016). Highest sorption level from the previous study (Jin et al., 2016) showed in the early phase of kinetic study. Thus, we provided the initial sorption levels of the drugs as representative results. We added the reference in Table 2 and mentioned the kinetics in discussion.

REPRESENTATIVE RESULTS
Table 2. Representative sorption results for diazepam and tacrolimus in PVC- and non-PVC-based tubes (n=3)[1].

Drug	Sorption (%)		
	PVC	PU	PO
Diazepam (0.05 h)	27.6 ± 3.0	21.9 ± 12.6	11.3 ± 4.6
Tacrolimus (1.05 h)	15.1 ± 3.3	10.3 ± 6.3	0.6 ± 0.9

DISCUSSION
Drug sorption to administration sets is a cause of unexpected drug loss in intravenous drug delivery. During sorption, drugs are generally partitioned to polymeric materials of tubes at the early phase of infusion; after sorption equilibrium is reached, the delivered amount of drugs is stabilized[1]. ~ Drug solutions for sampling were passed completely through the entire tube at the preselected conditions of flow rate and sampling time points. Sorption generally occurs in the early phase of infusion and the pattern is followed by convection-interfacial resistance-diffusion model[7]. Comparing with double-lumen extension tube model[17], diazepam sorption results are comparable when the initial drug concentration is considered before delivery. Therefore, sampling time points can be modified so that sorption evaluation takes less time. All factors of test conditions were confirmed based on the clinical usage of drugs.~

Reviewer #4:
***• At any point during the process, are you testing the starting concentration of the drug solution in the glass bottle? If not, this should be included as a baseline (control).* Author response: We tested the starting concentration of drug solution in Jin et al. 2016. We collected the samples after drug dilution in a bottle and analyzed. Please mention if and when the starting concentration of drug solution is measured in the JoVE manuscript results section. Please provide the reference.**
→Thank you for the comments. We used the starting concentration of the drug solution in a glass bottle as a control. We also clarified the results from protocol 5.2. References are added in the discussion.

REPRESENTATIVE RESULTS
~ All samples including samples at starting points were analyzed using an HPLC method with UV detection (Figure 3a). Analysis conditions are listed in Table 1. ~ The drug concentrations at starting points of the sorption study were calculated from the analysis of samples after drug dilution. Sorption levels in PVC- and non-PVC-based tubes of administration sets were determined by calculating the percentage of remaining drug content after passage through the tubes from the calibration curves (Figure 3b) and subtracting these values from 100% (Figure 3c).~

DISCUSSION

~ In the sorption test, the diluted drug solutions were used as starting concentrations[1,7]. After delivery, the drug solution (Figure 2d) was collected into vials at various time points (Figure 2e). Drug solutions for sampling were passed completely through the entire tube at the preselected conditions of flow rate and sampling time points. Sorption generally occurs in the early phase of infusion and the pattern is followed by convection-interfacial resistance-diffusion model[7]. Comparing with double-lumen extension tube model[17], diazepam sorption results are comparable when the initial drug concentration is considered before delivery. Therefore, sampling time points can be modified so that sorption evaluation takes less time. All factors of test conditions were confirmed based on the clinical usage of drugs.~

• Was the glassware used in this experiment silanized or otherwise coated? If not, are there any scenarios in which it may need to be? Please address this comment in the Discussion section.

→The glassware was made of chemically resistant borosilicate glass. We mentioned it in the manuscript at protocol 3, specifically 3.2.4 and discussion section.

PROTOCOL

~3. Kinetic sorption study using an infusion pump

Note: Confirm tube-dependent flow rate using a pump prior to the sorption test due to the hardness of tubes. Collect samples at precise time points and use glass bottles and vials to prevent additional drug sorption during storage. Perform the test as shown in Figure 2. Protect the drug solution against light if the drug has photosensitivity. Perform the experiments in triplicate.

3.1) Without creating air bubbles, preload a diluted solution of the drug into the tube using a syringe.
3.1.1) Connect one end of the tube to a syringe.
3.1.2) Put the other end of the tube into the bottled drug solution.
3.1.3) Pull back the syringe plunger until the tube is completely filled with the drug solution.
3.2) Install the tube into an infusion pump.
3.2.1) Open the door of the infusion pump and push the release lever.
3.2.2) Insert the middle of the preloaded tube into the infusion pump and keep it straight.
3.2.3) Remove the syringe at the end of the tube after installation of tube.
3.2.4) Put the end of tube into a chemically resistant borosilicate glass graduated cylinder, which is used to collect drug solution after passing through the tube.~

DISCUSSION

~Next, we set up a simple kinetic sorption study using a pump to easily obtain the precise results, and minimize artifacts. (Figure 2). In the pump method, drug solution (Figure 2a) was passed through a tube cut from the administration set (Figure 2b) after installation into an infusion pump (Figure 2c). Except for the tubes from administration sets, all devices (bottle, graduated cylinder, and sampling vials) were composed of chemically resistant borosilicate glass to prevent additional drug sorption to polymers.~

• Step 4.2.1: The authors use diazepam stock solutions and standards for calibration that are made up in methanol, but are testing sample concentrations of diazepam made up in 5% dextrose. Please comment on the different solvents and what differences might be expected in HPLC chromatograms and/or quantitation of diazepam concentration. Author response: We already checked the diazepam concentration in samples using calibration curves from diazepam standards. Diazepam standards were insoluble in 5% dextrose, so they were dissolved in methanol and diluted with methanol. In samples, using a different solvent from standards, other interfering peaks may be presented in the chromatograms. In this case, although there are peaks at 4 - 6 min before diazepam peak, they were not overlapped to

diazepam peak in the chromatogram. Please discuss your response in the Results or Discussion section.
→We added the response in results.

REPRESENTATIVE RESULTS
~ Analysis conditions are listed in Table 1. For the preparation of drug standards, diazepam and tacrolimus were dissolved in methanol and acetonitrile because of their insolubility in 5% dextrose. ~ There were no interfering peaks from the matrix. Specifically in samples of diazepam sorption study, the interfering peaks were not overlapped to drug peak in the chromatogram although the different solvent (5% dextrose) was used from standards.~