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Validated HPLC Theophylline Assay Method for Cleaning Validation on an Automatic Packaging Machine

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Original Research Article

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Abstract: Automatic packaging machines are used for preparing one-dose packages with powders, granules, tablets and capsules in pharmacies in Japan. The packaging machines are not dedicated to an individual patient, which leads to contamination of the packaging for the next patient. Cleaning validation for pharmaceutical manufacturing plants is therefore considered essential for packaging machines. The purpose of the present study was to develop and validate an HPLC method for assaying theophylline (TEO) for use as TEO cleaning validation on an automatic packaging machine. A chromatographic system comprising a YMC AM12S05-1506WT column, mobile phase of CH_3CN:H_2O:$HClO_4$:$NaClO_4$=100:900:1:5 (V/V/V/W), flow rate of 1 mL/min, and UV detector set at 271 nm was used. The TEO retention time was approximately 6.8 min. Regression analysis found that the method was linear over the standard curve range from 0.024 to 120 μg/mL. Inter-day precision and accuracy ranged between 0.20 and 6.59%, and -7.18 and 0.93%, respectively. The precision and accuracy values were under 10% and inside a range of -10% to 10%. Therefore, the lower limit of quantification was inferred to be 0.024 μg/mL. A swabbing procedure using non-woven fabric swabs containing ethanol for disinfection was validated. Mean recoveries from a stainless steel tray and a plastic tray were 102.4 ± 2.2% (mean ± SD, n=3) and 102.5 ± 1.5%, respectively.
Keywords: Theophylline, Automatic packaging machine, HPLC, Cleaning validation, Determination, Swabbing method.

INTRODUCTION

An automatic packaging machine is used in pharmacy dispensaries in Japan to prepare one dose packages for each patient. The machine can prepare one dose packages containing tablets, capsules, powders or granules. However, the machine is not dedicated to an individual patient, which is the general operating method in Japan, and this may lead to contamination of the package for the next patient.

For pharmaceutical manufacturing plants, documented equipment maintenance and cleaning is required to establish the cleanliness of equipment before its subsequent release for use in the manufacture of intermediates and active pharmaceutical ingredients [1]. Non-dedicated equipment should be cleaned at product changeover to prevent cross-contamination. Cleaning procedures should contain sufficient detail to enable operators to clean each type of equipment in a reproducible and effective manner, and these procedures should include a complete description of the methods and materials, including dilution of cleaning agents used to clean equipment. In addition, the cleaning validation master plan requires that detergent used to clean the manufacturing equipment in the cleaning validation phase is shown to be removed to an acceptable level in terms of commercial manufacturing [2].

Cleaning validation must be done for the machines to avoid cross-contamination. However, there is no report on drug levels remaining on the surfaces of the machine after use for one patient. Particularly, after preparing powders and granules, the drug levels remaining on the surfaces of the machine are important because operation with powders and granules carries the highest risk of cross-contamination. Therefore, we examined cleaning validation for an automatic packaging machine. First, the development of determination methods for drugs by HPLC from swab samples using a swabbing method was considered necessary.

Theophylline (dimethylxanthine) has been used to treat airway diseases for more than 80 years. It was originally used as a bronchodilator, but the relatively

high doses required are associated with frequent side effects, so use declined as inhaled β_2-agonists became more widely used. More recently, theophylline was shown to have anti-inflammatory effects in asthma and chronic obstructive pulmonary disease (COPD) at a lower concentration. The molecular mechanism of bronchodilatation is inhibition of phosphodiesterase (PDE) 3, but the anti-inflammatory effect may be due to inhibition of PDE4 and histone deacetylase-2 activation, resulting in the switching off of activated inflammatory genes. Through this mechanism, theophylline also reverses corticosteroid resistance, and this may be of particular value in severe asthma and COPD, in which histone deacetylase-2 activity is reduced. Theophylline is given systemically (orally as slow-release preparations for chronic treatment and intravenously for acute exacerbations of asthma). Efficacy is related to blood concentrations, determined mainly by hepatic metabolism, which may be increased or decreased in several diseases and by concomitant drug therapy. Theophylline is now usually used as an add-on therapy in patients with asthma not well controlled on inhaled corticosteroids with or without long-acting β_2-agonists, and in patients with COPD with severe disease not controlled by bronchodilator therapy. Side effects are related to plasma concentrations and include nausea, vomiting, and headaches due to PDE inhibition, and at higher concentrations to cardiac arrhythmias and seizures due to adenosine A_1-receptor antagonism. In the future, low-dose theophylline may be useful in reversing corticosteroid resistance in COPD and severe asthma [3].

Theophylline, an important drug as noted above, was selected as the first drug to develop the determination method for cleaning validation of the machine. In this report, we describe linearity, precision, accuracy and the limit of quantification, and report the percentage recovery from surfaces of a stainless steel tray and a plastic tray using the swabbing method.

MATERIALS AND METHODS

Materials

Theophylline anhydrous (TEO) was purchased from Sigma-Aldrich Co., LCC (St. Louis, USA). THEODUR® Dry syrup 20% (TDS) made by Tanabe Mitsubishi Pharma Corporation (Osaka, Japan) was used. Other chemicals were of special reagent or HPLC grade.

Apparatus and chromatographic conditions

The HPLC system consisted of a Model LC-20AS pump, equipped with an LC-solution on a PC, a Model SPD-20A UV spectrophotometric detector, a Model CTO-20A column oven, and a Model SIL-20A autoinjector, all from Shimadzu Corporation (Kyoto, Japan). The mobile phase was acetonitrile-water-perchloric acid (60%)-sodium perchlorate monohydrate=100:900:1:5, (V/V/V/W) for TEO. The chromatographic column was a YMC Pack AM12S05 ODS (150 mm x 6 mm I.D., particle diameter of 5 μm) obtained from YMC Co., Ltd. (Kyoto, Japan). The flow rate and temperature of the column were 1 mL/min and 40°C, respectively. The wavelength used to measure FEX was 271 nm. The injection volume for HPLC was 10 μL.

Calibration curve samples

TEO (12 mg) was dissolved in 50 mL of water. This solution at 240 μg/mL was diluted using water to make TEO solutions at 0.024, 0.048, 0.24, 0.48, 2.4, 12, 24, 60, and 120 μg/mL. Each solution (10 μL) was injected into the HPLC column. One set of these solutions was prepared on each experiment day. Concentrations from 0.024 to 2.4 μg/mL were used for a lower range calibration curve, and from 2.4 to 120 μg/mL for a higher range calibration curve.

Swabbing procedure

15 mg of TDS was scattered on a stainless steel tray and a plastic tray. The areas of the base of the trays were both 236 cm^2. TEO in TDS on the trays was recovered by wiping the surfaces of the trays using swab pad® ethanol for disinfection (SWP, Libatape Pharmaceutical Co., Ltd., Kumamoto, Japan), which is a non-woven fabric wet swab containing ethanol for disinfection. The surfaces of the trays were wiped with one side of the SWP. After this operation, the surface was wiped again using a new SWP with the same method. The two SWPs used were put into a 50 mL centrifuge tube.

Determination method for swabbing samples

Two SWPs were contained in each centrifuge tube. Approximately 15 mL of water was added to the centrifuge tubes. The centrifuge tubes were shaken at 200 rpm for 18 h to extract TEO from the SWP. The solution in the tubes was transferred to a 50 mL measuring flask. 15 mL of water was added to the tubes again, and the tubes were shaken at 200 rpm for 10 min. The solution in the tubes was transferred to the 50 mL measuring flask. The same operation was then performed again. An appropriate quantity of water was added to the flasks to adjust the volume to 50 mL. The solution in the flask was filtered using a syringe filter. The filtrate was assayed by HPLC.

RESULTS AND DISCUSSION

The retention time of TEO was approximately 6.8 min. A linear regression analysis gave slope, intercept, and correlation coefficients of Y=31853X + 1732.3, and r=1.000, respectively. The linearity was confirmed at concentrations from 0.048 to 120 μg/mL. When a calibration curve for determining samples is prepared in the concentration range, no acceptable values for accuracy may be observed around the original. Therefore, two calibration curves, for lower concentrations from 0.024 to 2.4 μg/mL and for higher concentrations from 2.4 to 120 μg/mL, were calculated.

Inter-day precision and accuracy for lower concentrations were assessed by analyzing each drug concentration 11 times on different days, as shown in Table 1. Precision ranged between 0.20% and 6.59%. The accuracy value ranged between -0.08% and 0.93%. All values were acceptable. The precision and accuracy values were under 10% and inside the range of -10% to 10%, respectively. Therefore, the lower limit of quantification was inferred to be 0.024 μg/mL, which was the lowest concentration providing validation data.

Inter-day precision and accuracy for higher concentrations were assessed by analyzing each drug concentration 11 times on different days, as shown in Table 2. Precision ranged between 0.24% and 2.67%. The accuracy value ranged between -7.18% and 0.61%. All values were acceptable.

Table-1: Inter-day precision and accuracy of TEO measurements for lower concentrations

Actual concentration (μg/mL)	Concentration found (μg/mL) (mean ± SD, n=11)	Precision (%)	Accuracy (%)
0.024	0.0242 ± 0.0016	6.59	0.88
0.048	0.0484 ± 0.0011	2.19	0.93
0.24	0.2402 ± 0.0008	0.32	0.07
0.48	0.4796 ± 0.0016	0.32	-0.08
2.4	2.4020 ± 0.0048	0.20	0.08

Precision and accuracy values were calculated using the following equations:

Precision (%) = (SD/mean) x 100.
Accuracy (%) = ((concentration found – actual concentration)/ actual concentration) x 100.

Table-2: Inter-day precision and accuracy of TEO measurements for higher concentrations

Actual concentration (μg/mL)	Concentration found (μg/mL) (mean ± SD, n=11)	Precision (%)	Accuracy (%)
2.4	2.2277 ± 0.0595	2.67	-7.18
12	11.9592 ± 0.0448	0.37	-0.34
24	24.1474 ± 0.0695	0.29	0.61
60	60.2697 ± 0.1667	0.28	0.45
120	119.9614 ± 0.2866	0.24	-0.03

Precision and accuracy values were calculated using the following equations:

Precision (%) = (SD/mean) x 100.
Accuracy (%) = ((concentration found – actual concentration)/ actual concentration) x 100.

Recoveries of TEO from TDS on a stainless steel tray and a plastic tray were 102.4 ± 2.2% (mean ± SD, n=3) and 102.5 ± 1.5%, respectively. These values were acceptable. It was found from the recovery data that the swabbing procedure using SWP for stainless steel and plastic surfaces, as well as the extraction method, were appropriate and effective. The procedure may be useful to confirm the amount of residual drugs on the surfaces of automatic packaging machines.

CONCLUSION

A method to measure TEO in swab samples used in a cleaning validation procedure was developed. The results suggested that this method is accurate and has a sufficiently low limit of quantification for TEO swab samples. This method may make an important contribution to the cleaning validation of automatic packaging machines in Japan.

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