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

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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 aim of the present study was to develop and validate an HPLC method for assaying acetaminophen (AAP) for use as AAP cleaning validation on an automatic packaging machine. A chromatographic system comprised of 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 a UV detector set at 271 nm, was used. Theophylline (TEO) was used as an internal standard. The AAP and TEO retention times were approximately 6.2 and 6.8 min, respectively. Regressio analysis found that the method was linear over the standard curve range from 0.02 to 40 mg/tube. Inter-day precision and accuracy ranged between 0.34 and 26.86%, and -1.93 and 2.44%, respectively. The precision and accuracy values were under 10% and inside a range of -10% to 10% without 0.02 mg/tube. Therefore, the lower limit of quantification was considered to be 0.02 mg/tube. 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 98.4 ± 2.52% (mean ± SD, n=3) and 99.4 ± 0.27%, respectively.

Keywords: Acetaminophen, Automatic packaging machine, HPLC, Cleaning validation, Determination, Swabbing method.

INTRODUCTION

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].

An automatic packaging machine is used in many 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.

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 necessary.

Acetaminophen (AAP) is commonly used as the global standard for analgesics. For example, the

WHO lists AAP as an essential drug, and clinical guidelines in many countries include AAP as a first-line drug for pain relief because of its efficacy and safety profile. In particular, there is no significant risk of gastrointestinal disorders, renal dysfunction, bleeding, or cardiovascular events, and it is considered a safer option than non-steroidal anti-inflammatory drugs [3].

AAP is used alone or combined with other medications to treat acute primary headaches; it is combined with aspirin and caffeine for migraine and tension-type headaches and combined with tramadol for a cluster headaches.

AAP weakly inhibits cyclooxygenase-2 in the central nervous system, and is approximately as effective for pain and fever relief as aspirin, but has no anti-inflammatory action [4, 5].

Acetaminophen, an important drug as noted above, was selected as the second 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 a swabbing method, as well as a report on theophylline [6].

MATERIALS AND METHODS
Materials
Acetaminophen (AAP) was purchased from Neiyaku Kagaku Co. Ltd. (Nara, Japan). A pharmaceutical preparation (powders) of AAP used was purchased from Choseido Pharmaceutical Co. Ltd. (Tokushima, Japan). Theophylline anhydrous (TEO) was purchased from Sigma-Aldrich Co., LCC (St. Louis, USA). 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 AAP. 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 AAP was 271 nm. The injection volume for HPLC was 5 μL.

Calibration curve samples
AAP (400 mg) was dissolved in 10 mL of methanol: water=1:1 solution (diluted methanol). This AAP solution was diluted by diluted methanol, and

AAP solutions at 0.2, 4 and 40 mg/mL were prepared. 0.1, 0.5, 1.0, and 2.0 mL of AAP solution at 0.2 mg/mL were added to 50 mL centrifuge tubes. 0.5 and 1.0 mL of the AAP solution at 4 mg/mL were added to 50 mL centrifuge tubes. 0.2, 0.5, 0.75 and 1.0 mL of the AAP solution at 40 mg/mL were added to 50 mL centrifuge tubes. As a result, centrifuge tubes containing 0.02, 0.1, 0.2, 0.4, 2, 4, 8, 20, 30, and 40 mg of AAP were prepared. 1 mL of internal standard (IS) solution and 39 mL of diluted methanol were added to the centrifuge tubes. A 2 mg/mL solution of TEO in water was used as an IS solution. Each centrifuge tube was well stirred. Each solution (5 μL) was injected into the HPLC column. One set of these solutions was prepared on each experiment day. Concentrations from 0.02 to 4 mg/tube were used for a lower range calibration curve, and from 4 to 40 mg/tube for a higher range calibration curve. Values of the peak area ratio, AAP/TEO, were calculated and the values were used for a calibration curve and to calculate the amount of AAP.

Swabbing procedure
15 mg of the pharmaceutical preparation of AAP was scattered on a stainless steel tray and a plastic tray. The areas of the base of the trays were both 236 cm^2. AAP in the preparation 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 39 mL of diluted methanol, and 1 mL of IS solution were added to the centrifuge tubes. Each centrifuge tube was well stirred. After an ultrasonic treatment for 5 min, each centrifuge tube was well stirred. 5 mL of the solution in the centrifuge tube was withdrawn by 5 mL syringe, and then filtered using syringe filter GLCT-HPTFE1345 from Shimadzu GLC Ltd. (Tokyo, Japan). 4 mL of filtrate for each syringe was discarded, and the next 1 mL of filtrate was used for the HPLC assay.

RESULTS AND DISCUSSION
The retention times of AAP and TEO were approximately 6.2 and 6.8 min. A linear regression analysis gave slope, intercept, and correlation coefficients of Y=0.15356X + 0.00661, and r=0.99998, respectively. Linearity was confirmed at concentrations from 0.02 to 40 mg/tube. When a calibration curve for determining samples was prepared in the concentration range, no acceptable values for accuracy may be observed around the original. Therefore, two calibration curves, for lower concentrations from 0.02 to 4 mg/tube

and for higher concentrations from 4 to 40 mg/tube, were calculated.

Inter-day precision and accuracy for lower concentrations were assessed by analyzing each drug concentration 6 times on different days, as shown in Table 1. Precision ranged between 0.34% and 26.86%. The accuracy value ranged between -0.41% and 2.44%. The values without 26.86% were acceptable. The precision and accuracy values were under 10% and inside the range of -10% to 10%, respectively, without

0.02 mg/tube. Therefore, the lower limit of quantification was considered to be 0.02 mg/tube, which was the lowest concentration providing validation data.

Inter-day precision and accuracy for higher concentrations were assessed by analyzing each drug concentration 6 times on different days, as shown in Table 2. Precision ranged between 0.67% and 1.64%. The accuracy value ranged between -1.93% and 0.711%. All values were acceptable.

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

Actual concentration (mg/tube)	Concentration found (mg/tube) (mean ± SD, n=6)	Precision (%)	Accuracy (%)
0.02	0.0205 ± 0.0055	26.86	2.44
0.1	0.0996 ± 0.0045	4.52	-0.41
0.2	0.2037 ± 0.0064	3.12	1.87
0.4	0.3994 ± 0.0040	1.00	-0.15
2	1.9979 ± 0.0228	1.14	-0.10
4	4.0073 ± 0.0136	0.34	0.18

Precision and accuracy values were calculated using the following equations:

$$\text{Precision (\%)} = (\text{SD/mean}) \times 100.$$
$$\text{Accuracy (\%)} = ((\text{concentration found} - \text{actual concentration})/ \text{actual concentration}) \times 100.$$

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

Actual concentration (mg/tube)	Concentration found (mg/tube) (mean ± SD, n=6)	Precision (%)	Accuracy (%)
4	3.9227 ± 0.0308	0.78	-1.93
8	8.0417 ± 0.1321	1.64	0.52
20	20.1058 ± 0.1670	0.83	0.53
30	30.2145 ± 0.2297	0.76	0.71
40	39.9760 ± 0.2665	0.67	-0.06

Precision and accuracy values were calculated using the following equations:

$$\text{Precision (\%)} = (\text{SD/mean}) \times 100.$$
$$\text{Accuracy (\%)} = ((\text{concentration found} - \text{actual concentration})/ \text{actual concentration}) \times 100.$$

Recoveries of AAP from an AAP preparation on a stainless steel tray and a plastic tray were 98.4 ± 2.52% (mean ± SD, n=3) and 99.4 ± 0.27%, 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 AAP 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 AAP swab samples. This method may make an important

contribution to the cleaning validation of automatic packaging machines in Japan.

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