

A Validated HPLC Ketotifen Fumarate Assay Method for Cleaning Validation on an Automatic Packaging Machine

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Original Research Article

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Article History

Received: 05.11.2018

Accepted: 09.11.2018

Published: 30.11.2018

DOI:

10.21276/sajp.2018.7.11.1



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 ketotifen fumarate (KTF) for use as KTF cleaning validation on an automatic packaging machine. A chromatographic system comprising a YMC AM12S05-1506WT column, mobile phase of CH₃CN:H₂O:HClO₄:NaClO₄=400:600:1:5 (V/V/V/W), flow rate of 1 mL/min, and UV detector set at 300 nm was used. Propyl parahydroxybenzoate (PPB) was used as an internal standard. The KTF and PPB retention times were approximately 6.4 and 10.8 min, respectively. Regression analysis found that the method was linear over the standard curve range from 0.1 to 100 µg/tube. Inter-day precision and accuracy ranged between 1.70 and 22.80%, and -4.37 and 6.50%, respectively. The precision and accuracy values were under 10% and inside a range of -10% to 10% without 0.1 µg/tube. Therefore, the lower limit of quantification was inferred to be 0.1 µg/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 96.5 ± 6.41% (mean ± SD, n=3) and 97.1 ± 4.93%, respectively.

Keywords: Ketotifen fumarate, 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 drug determination methods by HPLC from swab samples using a swabbing method was considered necessary.

Ketotifen fumarate (KTF) is a non-bronchodilator anti-asthmatic drug that inhibits the effects of

determined endogenous substances known as inflammatory mediators, and thereby possesses antiallergic activity. KTF possesses a powerful and sustained non-competitive histamine (H₁) blocking property [3]. KTF is widely and commonly used for treating allergic diseases. Interestingly, it has also been applied to non-allergic diseases, such as improving sperm quality [4], treating irritable bowel syndrome [5] and reducing joint capsule fibrosis [6]. In addition, it has been suggested that KTF may be a novel medication for diabetes by stabilization of mast cells in an animal model and humans [7-9].

KTF, an important drug as noted above, was selected as the third drug to develop the determination method for cleaning validation of the machine. In this report, we describe the 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, following on the reports for theophylline [10] and acetaminophen [11].

MATERIALS AND METHODS

Materials

Ketotifen fumarate (KTF) was purchased from Sigma-Aldrich Co., LCC (St. Louis, USA). Zaditen[®] Dry Syrup 0.1% as a pharmaceutical preparation of KTF was purchased from Mitsubishi Tanabe Pharma Corporation (Osaka, Japan). Propyl parahydroxybenzoate (PPB) was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). 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=400:600:1:5, (V/V/V/W) for KTF. 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 KTF was 300 nm. The injection volume for HPLC was 0.1 mL.

Calibration curve samples

KTF (10 mg) was dissolved in 10 mL of methanol. This KTF solution was diluted by methanol: water =1:1 solution (diluted methanol), and KTF solutions at 0.1 and 0.002 mg/mL were prepared. Then, 0.05, 0.1, 0.2, and 0.5 mL of the KTF solution at 0.002 mg/mL were added to 50-mL centrifuge tubes. Next, 0.05, 0.1, 0.2, 0.5, 0.75, and 1.0 mL of the KTF solution at 0.1 mg/mL were added to 50-mL centrifuge tubes. As

a result, centrifuge tubes containing 0.0001, 0.0002, 0.0004, 0.001, 0.005, 0.010, 0.020, 0.050, 0.075, and 0.10 mg of KTF were prepared. After that, 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 PPB in diluted methanol was used as an IS solution. Each centrifuge tube was well stirred. Each solution (0.1 mL) was injected into the HPLC column. One set of these solutions was prepared on each experiment day. Concentrations from 0.0001 to 0.005 mg/tube were used for a lower range calibration curve, and from 0.005 to 0.10 mg/tube for a higher range calibration curve. Values of Peak area ratio, KTF/PPB were calculated, and the values were used for a calibration curve and to calculate the amount of KTF.

Swabbing procedure

Fifteen mg of the KTF pharmaceutical preparation was scattered on a stainless steel tray and a plastic tray. The base areas of the trays were both 236 cm². KTF 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 by 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 ultrasonic treatment for 5 min, each centrifuge tube was well stirred. Then, 5 mL of the solution in the centrifuge tube was withdrawn using a 5-mL syringe, and filtered using a syringe filter GLCT-HPTFE1345 from Shimadzu GLC Ltd. (Tokyo, Japan). Finally, 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 KTF and PPB were approximately 6.4 and 10.8 min, respectively. A linear regression analysis gave slope, intercept, and correlation coefficients of $Y=0.02768X + 0.00549$, and $r=0.9998$, respectively. The linearity was confirmed at concentrations from 0.1 to 100 µg/tube. When a calibration curve to determine 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 0.1 to 5 µg/tube and for higher concentrations from 5 to 100 µg/tube, were calculated.

Inter-day precision and accuracy for lower concentrations were assessed by analyzing each drug concentration 10 times on different days, as shown in

Table 1. Precision ranged between 1.79% and 22.80%. The accuracy value ranged between -4.37% and 6.50%. The values without 22.80% were acceptable. The precision and accuracy values were under 10% and inside the range of -10% to 10%, respectively, without 0.1 µg/tube. Therefore, the lower limit of quantification was inferred to be 0.1 µg/tube, which was the lowest concentration providing validation data.

Inter-day precision and accuracy for higher concentrations were assessed by analyzing each drug concentration 10 times on different days, as shown in Table 2. Precision ranged between 1.70% and 4.18%. The accuracy value ranged between 0.33% and 4.48%. All values were acceptable.

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

Actual concentration (µg/tube)	Concentration found (µg/tube) (mean ± SD, n=10)	Precision (%)	Accuracy (%)
0.1	0.096 ± 0.022	22.80	-4.37
0.2	0.198 ± 0.019	9.61	-0.99
0.4	0.410 ± 0.025	6.13	2.48
1.0	1.065 ± 0.040	3.80	6.50
5.0	5.184 ± 0.093	1.79	3.68

Precision and accuracy values were calculated using the following equations:

$$\text{Precision (\%)} = (\text{SD}/\text{mean}) \times 100.$$

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

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

Actual concentration (µg/tube)	Concentration found (µg/tube) (mean ± SD, n=10)	Precision (%)	Accuracy (%)
5	5.017 ± 0.210	4.18	0.33
10	10.364 ± 0.307	2.97	3.64
20	20.809 ± 0.451	2.17	4.05
50	52.242 ± 1.471	2.82	4.48
75	77.624 ± 1.482	1.91	3.50
100	103.735 ± 1.758	1.70	3.74

Precision and accuracy values were calculated using the following equations:

$$\text{Precision (\%)} = (\text{SD}/\text{mean}) \times 100.$$

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

Recoveries of KTF from KTF preparation on a stainless steel tray and a plastic tray were 96.5 ± 6.41% (mean ± SD, n=3) and 97.1 ± 4.93%, 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, was 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 KTF 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 KTF swab samples. This method may make an important contribution to the cleaning validation of automatic packaging machines in Japan.

ACKNOWLEDGMENT

The authors are very grateful to Messrs. Tatsuki Tanioka, and Yuji Tsutsumi for their assistance in experimental work.

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