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

Tadakazu Tokumura[1*], Ai Yasumoto[1], Takuro Kurita[1,2]
[1]Laboratory of Pharmaceutics, Kagawa School of Pharmaceutical Sciences, Tokushima Bunri University, Shido 1314-1, Sanuki, Kagawa 769-2193, Japan
[2]Division of Clinical Pharmaceutics, Department of Pharmaceutical Sciences, Nihon Pharmaceutical University, Komuro 10281, Ina-machi, Kitaadachi-gun, Saitama, 362-0806, Japan

Original Research Article

***Corresponding author**
Tadakazu Tokumura

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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 nicotinamide (NA) for use as NA cleaning validation on an automatic packaging machine. A chromatographic system comprised of a YMC AM12S05-1506WT column, mobile phase CH_3OH:H_2O = 100:900 (V/V), flow rate 1 mL/min, and a UV detector set at 261 nm, was used. Tyrosine (TY) was used as an internal standard. The NA and TY retention times were approximately 6.8 and 4.1 min, respectively. Regression analysis found that the method was linear over the standard curve range from 0.01 to 20 mg/tube. Inter-day precision and accuracy ranged between 0.22 and 6.45%, and -4.62 and 5.84%, respectively. The precision and accuracy values were under 10% and inside a range of -10% to 10%. Therefore, the lower limit of quantification was considered to be 0.01 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 101.9 ± 9.24% (mean ± SD, n=3) and 103.4 ± 2.67%, respectively.
Keywords: Nicotinamide, 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.

Nicotinamide (NA) is the amide form of vitamin B_3 (niacin). Niacin is required for the synthesis of coenzymes, nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate

(NADP). Niacin binds with G protein-coupled receptor (GPR) 109A on cutaneous Langerhans cells and causes vasodilation that leads to flushing in head and neck area. Niacin deficiency due to excessive alcohol consumption, certain drugs or inadequate dietary uptake causes pellagra, a kind of photosensitivity dermatitis. Recently, several studies have revealed the mechanism of photosensitivity in niacin deficiency, and this may open up new therapeutic approaches. The expression level of prostaglandin E synthase (PTGES) is up-regulated in the skin of both pellagra patients and niacin-deficient pellagra mouse models. In addition, pellagra is mediated through prostaglandin E_2-EP4 (PGE_2-EP4) signaling via reactive oxygen species (ROS) production in keratinocytes [3].

NA, an important drug as noted above, was selected as the fourth 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 a swabbing method, following on the reports on theophylline [4], acetaminophen [5], and ketotifen fumarate [6].

MATERIALS AND METHODS

Materials

Nicotinamide (NA) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). As a pharmaceutical preparation of NA, nicotinamide powder 10% Zonne® was purchased from Zonnebodo Pharmaceutical Co. Ltd. (Tokyo, Japan). L-Tyrosine (TY) 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 methanol: water = 100:900, (V/V) for NA. 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 NA was 261 nm. The injection volume for HPLC was 10 µL.

Calibration curve samples

NA (500 mg) was dissolved in 50 mL of methanol: water = 1:1 solution (diluted methanol). This NA solution at 10 mg/mL was diluted by diluted methanol, and NA solutions at 0.2 mg/mL were prepared. Next, 0.05, 0.1, 0.2, and 0.5 mL of NA solution at 0.2 mg/mL were added to 50-mL centrifuge tubes. After that, 0.05, 0.1, 0.2, 0.5, 0.75, 1.0 and 2.0 mL of the NA solution at 10 mg/mL were added to 50-mL centrifuge tubes. As a result, centrifuge tubes containing 0.01, 0.02, 0.04, 0.1, 0.5, 1, 2, 5, 7.5, 10 and 20 mg of NA were prepared. Then, 1 mL of internal standard (IS) solution and 39 mL of diluted methanol were added to the centrifuge tubes. A 4-mg/mL solution of TY in 0.1 M HCl: methanol = 1:1 was used as an IS solution. Each solution (10 µL) was injected into the HPLC column. One set of these solutions was prepared on each experiment day. Concentrations from 0.01 to 1 mg/tube were used for a lower range calibration curve, and from 1 to 20 mg/tube for a higher range calibration curve. Values of the peak area ratio, NA/TY, were calculated and these values were used for a calibration curve and to calculate the amount of NA.

Swabbing procedure

Fifteen mg of the NA pharmaceutical preparation was scattered on a stainless steel tray and a plastic tray. The areas of the base of the trays were both 236 cm^2. NA 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 by a 5-mL syringe, and filtered using an E131 syringe filter from Pall Corporation (Tokyo, Japan). Finally, 4 mL of filtrate from each syringe was discarded, and the next 1 mL of filtrate was used for the HPLC assay.

RESULTS AND DISCUSSION

The retention times of NA and TY were approximately 6.8 and 4.1 min. A linear regression analysis gave slope, intercept, and correlation coefficients of Y=2.81157X + 0.07624, and r=0.999959, respectively. Linearity was confirmed at concentrations from 0.01 to 20 mg/tube. When a calibration curve for determining samples was prepared in the concentration range, no acceptable values for accuracy were observed around the original. Therefore, two calibration curves, for lower concentrations from 0.01 to 1 mg/tube and for higher concentrations from 1 to 20 mg/tube, were calculated.

Inter-day precision and accuracy for lower concentrations were assessed by analyzing each drug

concentration seven times on different days, as shown in Table 1. Precision ranged between 0.22% and 6.45%. The accuracy values ranged between -0.83% and 5.84% and these 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 considered to be 0.01 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 seven times on different days, as shown in Table 2. Precision ranged between 0.58% and 4.71%. The accuracy values ranged between -4.62% and 0.65% and all values were acceptable.

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

Actual concentration (mg/tube)	Concentration found (mg/tube) (mean ± SD, n=7)	Precision (%)	Accuracy (%)
0.01	0.0106 ± 0.0007	6.45	5.84
0.02	0.0207 ± 0.0007	3.53	3.36
0.04	0.0408 ± 0.0008	1.87	1.98
0.1	0.0997 ± 0.0012	1.24	-0.31
0.5	0.4959 ± 0.0030	0.60	-0.83
1	1.0010 ± 0.0022	0.22	0.10

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 NA measurements for higher concentrations

Actual concentration (mg/tube)	Concentration found (mg/tube) (mean ± SD, n=7)	Precision (%)	Accuracy (%)
1	0.9538 ± 0.0450	4.71	-4.62
2	1.9761 ± 0.0344	1.74	-1.20
5	5.0264 ± 0.0291	0.58	0.53
7.5	7.5356 ± 0.0476	0.63	0.47
10	10.0654 ± 0.0781	0.78	0.65
20	19.8134 ± 0.2558	1.29	-0.93

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 NA from an NA preparation on a stainless steel tray and a plastic tray were 101.9 ± 9.24% (mean ± SD, n=3) and 103.4 ± 2.67%, 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 NA 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 Na swab samples. This method may make an important contribution to the cleaning validation of automatic packaging machines in Japan.

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