Scholars Academic Journal of Pharmacy
Abbreviated Key Title: Sch Acad J Pharm
ISSN 2347-9531 (Print) | ISSN 2320-4206 (Online)
Journal homepage: http://saspublishers.com

OPEN ACCESS

Pharmaceutical Sciences

Development of a Validated HPLC Method for Candesartan Cilexetil to Evaluate the Process of Grinding Tablets on Dispensing in Japan

Hikaru Tabuchi[1], Rikako Nojima[1], Takuro Kurita[1, 2], Tadakazu Tokumura[1*]

[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

DOI: 10.36347/sajp.2022.v11i02.001 | **Received:** 29.12.2021 | **Accepted:** 06.02.2022 | **Published:** 11.02.2022

***Corresponding author:** Tadakazu Tokumura
Laboratory of Pharmaceutics, Kagawa School of Pharmaceutical Sciences, Tokushima Bunri University, Shido 1314-1, Sanuki, Kagawa 769-2193, Japan

Abstract **Original Research Article**

The aim of the present study was to develop and validate a HPLC method for assaying candesartan cilexetil (CAND) in powder preparations prepared from Blopress® Tablets 2 mg. A chromatographic system comprising a YMC AM12S05-1506WT column, the mobile phase of CH_3CN:H_2O:$HClO_4$:$NaClO_4$=700:300:1:5 (V/V/V/W), a flow rate of 1 mL/min, and a UV detector set at 254 nm was used. The retention time of CAND was approximately 8.8 min. A regression analysis revealed that the calibration curve of the method was linear in the range of 0.10 to 80 µg/mL. Intra-day precision and accuracy ranged between 0.1 and 1.6% and between -0.4 and 20.9%. The accuracy value at 0.10 µg/mL was 20.9%. Inter-day precision and accuracy ranged between 0.2 and 3.0% and between -0.3 and 20.6%. The accuracy value at 0.10 µg/mL was 20.6%. In both cases, accuracy values at 0.10 µg/mL were out of the range of -10% to 10%. Therefore, the limit of quantification was established to be 0.10 µg/mL. The content of Blopress® Tablets 2 mg was 99.20 ± 0.81% (mean ± SD, n=3). The ratios of CAND in powder removed from the mortar, remaining on the mortar and pestle surface, and scattered on the paper to total recovery were 85.48 ± 3.50, 14.27 ± 3.53, and 0.30 ± 0.02%, respectively.

Keywords: HPLC method, candesartan cilexetil (CAND), Grinding Tablets.

INTRODUCTION

In cases in which children need to be treated using pharmaceutical preparations that are only available for adults in Japan, "grinding tablets on dispensing" is performed. The grinding of Revatio® Tablets 20 mg from Pfizer Japan Inc. on dispensing is a typical example. Revatio® Tablets 20 mg are a pharmaceutical preparation containing sildenafil citrate (SIL) that is used to treat pulmonary arterial hypertension. When SIL is administered to infants with persistent pulmonary hypertension of the newborn (PPHN) [1], Revatio® Tablets 20 mg are ground in a mortar to make a powder. Lactose is added to the powder as a diluent and mixed well in the mortar. The mixed powder is packaged for each dose using an automatic packaging machine. These processes are typically performed as dispensing work in a prescription department and are referred to as "grinding tablets on dispensing" in Japan. The grinding of tablets is associated with weight and drug losses [2-5], while the automatic packaging process results in further drug loss [6, 7].

We previously reported the development of a method for assaying SIL in the powder prepared from Revatio® Tablets 20 mg, and found drug losses in the tablet grinding process [8, 9], which was consistent with the findings of other studies [2-5]. Therefore, we used the model tablet, Allegra® 60 mg tablets, to develop methods that reduce drug losses in the tablet grinding process using a mortar and pestle. The findings obtained showed that drug powder remaining on the surfaces of the mortar and pestle mainly contributed to drug loss during this process. The use of calcium monohydrogen phosphate was found to markedly reduce drug loss due to powder remaining on the surfaces of the mortar and pestle [10]. This method was applied to "grinding tablets on dispensing" for Revatio® Tablets 20 mg, and the effects of calcium monohydrogen phosphate were confirmed [11].

Candesartan cilexetil (CAND) was approved for the treatment of hypertension in children in Japan in 2019. Oral doses of 0.05-0.3 mg/kg CAND were approved for infants aged between 1 and 6 years old. However, there is currently no suitable dosage form of CAND for administration to infants. Therefore, the grinding of tablets, such as Blopress® Tablets, on dispensing is performed, similar to SIL. We herein developed a method to assay CAND in a powder prepared from Blopress® Tablets 2 mg, and measured the amount of CAND in powder removed from the mortar, remaining on the mortar and pestle surfaces, and scattered on the paper after Blopress® Tablets 2 mg were ground in a mortar to make a powder. We also described a validated HPLC assay method for CAND and the amounts of CAND in the powder removed from the mortar, remaining on the mortar and pestle surfaces, and scattered on the paper.

MATERIALS AND METHODS

Materials

CAND was purchased from Yungjin Pharm. Co., Ltd. (Seoul, Korea). Blopress® Tablets 2 mg from Teva Takeda Pharma Ltd. (Aichi, Japan) were used. Other chemicals were of special reagent or HPLC grade.

Apparatus and chromatographic conditions

The HPLC system consisted of a Model LC-20AD pump, equipped with LC solution on PC, a Model SPD-20A UV spectrophotometric detector, Model CTO-20A column oven, and Model SIL-20A autoinjector, all from Shimadzu Corporation (Kyoto, Japan). The chromatographic column was YMC Pack AM12S05 ODS (150 mm × 6 mm I.D., particle diameter of 5 µm) obtained from YMC Co., Ltd. (Kyoto, Japan). The mobile phase was acetonitrile-water-perchloric acid (60%)-sodium perchlorate monohydrate=700:300:1:5, (V/V/V/W) for CAND. The flow rate and temperature of the column were 1 mL/min and 40℃. The wavelength used to measure CAND was 254 nm. The injection volume for HPLC was 20 µL.

Standard solution

CAND (100.03 mg) was dissolved in 100 mL of ethanol, and a solution at 1 mg/mL was used in each experiment and stored at 4℃.

Calibration curve samples

CAND solution at 1 mg/mL was diluted using ethanol to make CAND solutions at 0.10, 0.20, 0.50, 1.0, 2.0, 5.0, 10, 20, 40, 60, and 80 µg/mL. Each solution was injected into the HPLC column, and a linear regression analysis was performed.

Content of CAND in one Blopress® Tablet 2 mg

Approximately 10 mL of water was added to one Blopress® Tablet 2 mg and it was completely disintegrated in the solution. After an ultrasonic treatment for 10 min, 30 mL of ethanol was added to the suspension. The ultrasonic treatment of the suspension was performed again for 10 min. Ethanol was added and the volume was adjusted to 50 mL. The solution was stirred well and then filtered. Hydrophilic PTFE membrane filters (diameter 13 mm, hole diameter 0.45 µm) from Shimadzu GLC Ltd. (Tokyo, Japan) were used for filtration. A filtrate was assayed by HPLC. Four milliliters of the standard solution was added to 96 mL of ethanol. This solution was also assayed by HPLC. The content of CAND in Blopress® Tablets 2 mg was calculated from a comparison of the peak areas of the solutions.

Method to assess the grinding process

The grinding of tablets was performed according to a previously reported method [12]. Briefly, a mortar and pestle was set on the center of A3 paper. One Blopress® Tablet 2 mg was placed in the mortar and ground to a powder. The powder was removed from the mortar and added to a 50-mL flask. Approximately 30 mL of ethanol was added to the flask, and an ultrasonic treatment was performed for 10 min. Ethanol was added, and the volume was adjusted to 50 mL. The solution was stirred well and then filtered using the Hydrophilic PTFE membrane filter. The filtrate was assayed by HPLC. Scattered powder on the paper was placed into a 50-mL flask, and ethanol was added to prepare a sample solution for HPLC assay using the same method. Powder remaining on the mortar and pestle surfaces was washed with ethanol. The ethanol used was collected and added to a 50-mL flask, and ethanol was added to prepare a sample solution for HPLC assay using the same method.

RESULTS AND DISCUSSION

The retention time of CAND was approximately 8.8 min. In the linear regression analysis, the slope, intercept, and correlation coefficient were Y=35402X - 1077.3, and r=0.99999, respectively. Intra-day precision and accuracy were assessed by analyzing three replicates at each concentration, which are shown in Table 1. Precision ranged between 0.1 and 1.6%, and accuracy between -0.4 and 20.9%. The accuracy value at 0.10 µg/mL was 20.9%, which was out of the range from -10 to 10%. Therefore, the lower limit of quantification was inferred to be 0.10 µg/mL. Inter-day precision and accuracy were assessed by analyzing each concentration over five days. This result is shown in Table 2. Precision ranged between 0.2 and 3.0%, and accuracy between -0.3 and 20.6%. Accuracy at 0.10 µg/mL was 20.6%, which was out of the range from -10 to 10%. Based on accuracy values at 0.10 µg/mL in intra- and inter-day data, the limit of quantification was established to be 0.10 µg/mL.

The content of Blopress® Tablets 2 mg was 99.20 ± 0.81% (mean ± SD, n=3). This result is acceptable, indicating the suitability of the extraction method for CAND.

The concentrations of sample solutions from powder removed from the mortar, remaining on the mortar and pestle surfaces, and scattered on the paper were 33.05, 4.45, and 0.12 µg/mL, respectively. The amount of CAND in these powders calculated from concentration data were 1.653, 0.223, and 0.006 mg, respectively. Calculations using these data revealed that the ratios of CAND in sample solutions prepared from the powder removed from the mortar, remaining on the mortar and pestle surface, and scattered on the paper to total recovery were 85.48 ± 3.50, 14.27 ± 3.53, and 0.30 ± 0.02% (mean ± SD, n=4), respectively. Therefore, the developed method is useful for assessing the loss of CAND in the grinding process of Blopress® Tablets 2 mg.

Table 1: Intra-day precision and accuracy of CAND

Actual concentration (µg/mL)	Calculated concentration (µg/mL) (mean ± SD, n=3)	Precision (%)	Accuracy (%)
0.10	0.1209 ± 0.0008	0.6	20.9
0.20	0.2169 ± 0.0012	0.5	8.5
0.50	0.5107 ± 0.0083	1.6	2.1
1.0	1.0037 ± 0.0007	0.1	0.4
2.0	1.9918 ± 0.0025	0.1	-0.4
5.0	4.9953 ± 0.0217	0.4	-0.1
10	9.9766 ± 0.0441	0.4	-0.2
20	19.9951 ± 0.0336	0.2	0.0
40	39.9550 ± 0.1843	0.5	-0.1
60	60.0310 ± 0.1875	0.3	0.1
80	80.0051 ± 0.2721	0.3	0.0

Precision and accuracy were calculated using the following equations.

Precision (%) = (SD/mean) × 100.

Accuracy (%) = ([Calculated concentration – Actual concentration]/Actual concentration) × 100

Table 2: Inter-day precision and accuracy of CAND

Actual concentration (µg/mL)	Calculated concentration (µg/mL) (mean ± SD, n=5)	Precision (%)	Accuracy (%)
0.10	0.1207 ± 0.0036	3.0	20.6
0.20	0.2194 ± 0.0032	1.5	9.7
0.50	0.5145 ± 0.0043	0.8	2.9
1.0	1.0083 ± 0.0047	0.5	0.8
2.0	2.0038 ± 0.0117	0.6	0.2
5.0	4.9962 ± 0.0241	0.5	-0.1
10	9.9655 ± 0.0445	0.4	-0.3
20	19.932 ± 0.0420	0.2	-0.3
40	39.9651 ± 0.1153	0.3	-0.1
60	60.1465 ± 0.2411	0.4	0.2
80	79.9305 ± 0.4663	0.6	-0.1

Precision and accuracy were calculated using the following equations.

Precision (%) = (SD/mean) × 100.

Accuracy (%) = ([Calculated concentration – Actual concentration]/Actual concentration) × 100.

CONCLUSIONS

A method to measure CAND in powder preparations prepared from Blopress® Tablets 2 mg for infants with hypertension was developed herein. The results obtained indicated that this method is accurate and has a sufficient limit of quantification for powder preparations of CAND. This method will make an important contribution to the assessment of drug loss in the process of "grinding tablets on dispensing".

REFERENCES

1. Yaseen, H., Darwich, M., & Hamdy, H. (2012). Is sildenafil an effective therapy in the management of persistent pulmonary hypertension?. *Journal of clinical neonatology*, *1*(4), 171-175.
2. Murakami, M., Tanoue, N., Nakamura, T., Tsuruta, S., Iwaoku, R., & Nakano, M. (1991). Investigation of loss in weight by grinding of tablets. *Jpn J Hosp Pharm*, *17*, 381-387.
3. Kuwana, Y., Hiramatsu, H., Furuichi, K., & Sumida, K. (1978). Loss in weight by grinding tablets on dispensing. *Jpn J Hosp Pharm*, *4*(1), 16-19.
4. Yano, H., Ishimaru, Y., Hidaka, N., Tatsukawa, T., Matsuoka, A., Tanaka, A., ... & Araki, H. (2014). Comparison of the mass loss rate and the divided variability with warfarin potassium crushing tablet and powder medicine. *J. Japanese Society Hospital*

Pharmacists, *50*(4), 461-465.

5. Miyamoto, S., Suzuki, M., and Kohda, Y. (1999). Drug-loss in dispensing and ingestion processes: digoxin products as model drug. *Jpn. J. Hosp. Pharm*, *25*(3), 292-298.
6. Sasaki, M., Sano, M., Tanaka, Y., & Yamamoto, I. (2006). Drug loss due to grinding, sifting and automatic packaging of carvedilol tablets. *Jpn. J. Pharm. Health Care Sci*, *32*(5), 420-423.
7. Wakui, N., Ookubo, T., Iwasaki, Y., Ito, R., Kobayashi, T., Hayakawa, K., ... & Nakazawa, H. (2011). Estimation of infant medicine content and drug loss after grinding small amount of tablets. *Jpn J Pharm Health Care Sci*, *37*, 425-430.
8. Tokumura, T., Kawakami, M., Kitada, R., Yamamoto, H., Yamamoto, H., & Kurita, T. (2015). Determination of sildenafil citrate in powder preparations prepared from revatio tablets 20 mg for infants with persistent pulmonary hypertension of the newborn. *Sch. Acad. J. Pharm*, *4*(8), 370-375.
9. Yamamoto, H., Kawakami, M., Kitada, R., Yokoi, R., Toyofuku, Y., Kurita, T., Senba, Y., Yamamoto, H., & Tokumura, T. (2019). Effects of tablet grinding methods on sildenafil content in each package prepared by an automatic packaging machine for patients in the neonatal intensive care unit. *Research Bulletin Tokushima Bunri University*, 98, 1-8.
10. Kawakami, M., Kitada, R., Kurita, T., & Tokumura, T. (2017). A method for decreasing the amount of the drug remaining on the surfaces of the mortar and pestle after grinding small amount of tablets. *Yakugaku Zasshi*, *137*(8), 1017 – 1025.
11. Kitada, R., Kawakami, M., Yamamoto, H., Yamamoto, H., Kurita, T., & Tokumura, T. (2020). Developing an optimized method for grinding sildenafil tablets for children to use in the neonatal intensive care unit. *J Parm Sci Technol Jpn*, 80, 307-314.
12. Tokumura, T., Kawakami, M., Kitada, R., & Kurita, T. (2016). Validated assay method for fexofenadine hydrochloride in powder preparations of Allegra® 60 mg tablets to develop a new method for grinding tablets on dispensing in Japan. *Sch. Acad. J. Pharm*, *5*(8), 359-362.