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Research Article

Determination of Sildenafil Citrate in Powder Preparations Prepared from Revatio Tablets 20 mg for Infants with Persistent Pulmonary Hypertension of the Newborn

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Abstract: The aim of the present study was to develop and validate a HPLC method for the assay of sildenafil citrate (SIL) in powder preparations prepared from Revatio Tablets 20 mg for infants with persistent pulmonary hypertension of the newborn. The good recovery of SIL from preparations was achieved using a methanol:0.1N HCl =1:1 solution as the extraction solvent. A chromatographic system comprising a YMC AM12S05-1506WT column, mobile phase of CH_3CN:H_2O:$HClO_4$:$NaClO_4$=400:600:1:5 (V/V/V/W), flow rate of 1 mL/min, and UV detector set at 290 nm showed good chromatographic separation for SIL and the internal standard (propyl 4-hydroxybenzoate). A regression analysis revealed that the method was linear over the standard curve range from 0.01 to 1 mg/tube. Inter-day precision values between the ranges were 4.3% or better. Accuracy values between the ranges were -0.9% - 7.0%, except for the value of 0.01 mg/tube. The accuracy value at 0.01 mg/tube was 16.6%, which indicated that the limit of quantitation of this method was 0.01 mg/tube. We herein demonstrated that this method is useful for determining SIL in a powder preparation prepared from Revatio tablets 20 mg.
Keywords: Sildenafil citrate, Powder preparation, HPLC, Persistent pulmonary hypertension of the newborn, Revatio tablets 20 mg, Determination, Adsorption.

INTRODUCTION

Persistent pulmonary hypertension of the newborn (PPHN) is a life-threatening neonatal pathology due to poor hemodynamic and respiratory transition to extrauterine life. Inhaled nitric oxide (iNO) is currently used to treat PPHN. However, iNO is not available in many developing countries and approximately 50% of infants with PPHN do not respond to this therapy. Sildenafil is a phosphodiesterase type 5 inhibitor that selectively reduces pulmonary vascular resistance in animal models and adult humans. Recent studies reported that the administration of sildenafil significantly increased oxygenation and reduced mortality with no clinically important side effects in infants with PPHN [1].

A pharmaceutical preparation containing sildenafil citrate (SIL) for pulmonary arterial hypertension, Revatio Tablets 20 mg from Pfizer Japan Inc., is available for adults in Japan, whereas that for children is not. Therefore, when sildenafil is administered to infants with PPHN, Revatio Tablets 20 mg are ground in a mortar to make a powder. Lactose is added to the powder as a diluent, and is 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 drug loss [6, 7].

Although the same losses are considered to occur for SIL, there is currently no study in the literature that describes the loss of SIL in these processes or its content in the powder prepared by grinding tablets on dispensing. Therefore, in order to clarify the exact dose of sildenafil administered to infants with PPHN, we herein developed a determination method for SIL in the powder prepared from Revatio Tablets 20 mg.

MATERIALS AND METHODS

Materials

SIL made by Mylan Laboratories Limited (Andhra Pradesh, India) was donated by Kowa Shoji

Co., Ltd. (Kanagawa, Japan). Lactose, microcrystalline cellulose, anhydrous dibasic calcium phosphate, magnesium stearate, hypromellose, and titanium oxide in JP XVI were used. Croscarmellose sodium and triacetin in Japanese Pharmaceutical Excipients 2004 were used. Other chemicals were of special reagent or HPLC grade.

Apparatus and chromatographic conditions for preliminary studies

The HPLC system consisted of a Model LC-10AS pump, equipped with a Model SCL-10A system controller, Model SPD-10A UV spectrophotometric detector, Model CTO-10A column oven, Model C-R4A Chromatopac, and Model SIL-10A autoinjector, all from Shimadzu Corporation (Kyoto, Japan). The mobile phase was acetonitrile-water-perchloric acid (60%)-sodium perchlorate monohydrate=380:620:1:5, (V/V/V/W) for SIL. The chromatographic column was a YMC Pack AM12S05 ODS (150 mm x 6 mm I.D., particle diameter 5 µm) obtained from YMC Co., Ltd. (Kyoto, Japan). The flow rate and temperature of the column were 1 mL/min and 40°C. The wavelength for determining SIL was 290 nm. The injection volume for HPLC was 20 µL.

Adsorption of SIL to tubes with operating conditions of stirring and changing tubes

A total of 7.60 mg of SIL was dissolved in CH_3OH:H_2O =1:1 solution (diluted methanol) to make a 1.0 mg/mL SIL solution. A total of 0.5 mL of the SIL solution was diluted with water to make a 10 µg/mL SIL solution in water. Five milliliters of the 10 µg/mL SIL solution in water was added to a 10-mL glass tube using a 5-mL glass measuring pipette. The solution in the tube was stirred for 15 s using a vortex mixer. The solution in the tube was transferred to a new tube (the second) using a 5-mL glass measuring pipette, and stirred in this tube in the same manner. This operation was repeated a further 3 times. As the result, the solution in the fifth tube underwent the stirring and transferring operations 5 times.

As a sample, 0.2 mL of the solution was withdrawn from the fifth tube using a continuously adjustable air displacement pipette to 200 µL, and the sample solution was assayed by HPLC. As a reference to calculate recoveries, the 10 µg/mL SIL solution was assayed by HPLC. Recoveries (%) were calculated using equation [1].

Recovery (%) = Peak area of the sample/mean peak area of the reference x 100 [1]

The same experiment was performed using 15-mL polypropylene tubes. The adsorption of SIL in diluted methanol was examined under the same experimental conditions for glass and polypropylene tubes.

Adsorption of SIL in diluted methanol to a syringe filter

The syringe filter, E131 from Pall Corporation (Tokyo, Japan) was used. A total of 0.5 mL of the 1 mg/mL SIL solution was diluted in diluted methanol to make a 10 µg/mL SIL solution. Three milliliters of the solution was passed through the filter, which was then discarded. Approximately 1 mL of the solution that passed through the filter was then collected into a sample cup for HPLC. This sample was assayed by HPLC. As a reference to calculate recoveries, the 10 µg/mL SIL solution was assayed by HPLC. Recoveries (%) were calculated using equation [1].

Recovery of SIL from lactose when SIL solution was added to its powder

One hundred microliters of the 1 mg/mL SIL solution in diluted methanol was added to 0.1 g of lactose. Diluted methanol (10 mL) was then added to lactose in order to extract SIL. After being stirred well using a vortex mixer, the solution was filtered by the syringe filter E131. The filtrate was assayed by HPLC. As a reference to calculate recoveries, the solution prepared by 100 µL of the 1 mg/mL SIL solution and 10 mL of diluted methanol was assayed by HPLC. Recoveries (%) were calculated using equation [1].

Preparation of a SIL and lactose mixture and determination of the SIL content in the mixture

One hundred milligrams of SIL and 900 mg of lactose were mixed well in a mortar. Ten milligrams of the mixture was added to a 15-mL centrifuge tube, and 10 mL of diluted methanol was then added. After being stirred well using a vortex mixer, the solution was filtered by the syringe filter E131. The filtrate was assayed by HPLC. As a reference to calculate recoveries, the solution prepared by mixing 1 mL of the 1 mg/mL SIL solution and 9 mL of diluted methanol was assayed by HPLC. Recoveries (%) were calculated using equation [1].

Effects of additives on recoveries of SIL from the SIL and lactose mixture

Ten milligrams of the SIL and lactose mixture was added to a 15-mL centrifuge tube, and 10 mg of an additive was then added. Tubes without additives were prepared as a reference to calculate recoveries. Anhydrous dibasic calcium phosphate, magnesium stearate, titanium oxide, croscarmellose sodium, and hypromellose were used as additives. After each additive had been added to the tube, 10 mL of diluted methanol was added to each tube. After an ultrasonic treatment for 15 s, the solution in the tubes was stirred well on a vortex mixer. The solution was filtered by the syringe filter E131. The filtrate was assayed by HPLC. Recoveries (%) were calculated using equation [1].

Enhanced recovery of SIL from the powder with croscarmellose sodium

One hundred milligrams of the SIL and lactose mixture and 100 mg of croscarmellose sodium were mixed well in a mortar. Twenty milligrams of the mixture was added to a 15-mL centrifuge tube. As a reference to calculate recoveries, 10 mg of the SIL and lactose mixture was added to a 15-mL centrifuge tube. Ten milliliters of each of the following solvents: methanol:0.1N HCl=1:1, methanol:saline=1:1, ethanol:water=1:1, and diluted methanol, was added to the tubes. The determination of SIL in these solutions was performed using the same method described above. Recoveries (%) were calculated using equation [1].

Content of SIL in one Revatio Tablet 20 mg

One Revatio Tablet 20 mg was added to 40 mL of 0.1N HCl, and disintegrated in the solution. After an ultrasonic treatment for 5 min, 40 mL of methanol was added to the suspension. The ultrasonic treatment of the suspension was performed again for 5 min. Diluted methanol was added, and the volume was adjusted to 100 mL. One milliliter of the suspension was added to a 50-mL centrifuge tube. One hundred microliters of the IS solution (100 mg/mL in methanol) and 20 mL of methanol:0.1N HCl=1:1 solution were also added to the centrifuge tube. The solution in the tube was stirred well on a vortex mixer, and then filtered by the syringe filter E131. The filtrate was assayed by HPLC. A total of 29.79 mg of SIL was dissolved in 100 mL of the methanol:0.1N HCl=1:1 solution. One milliliter of the solution was withdrawn and added to a 50-mL centrifuge tube. The same treatment described above was performed. The solution was assayed by HPLC as the standard. The content of SIL was calculated from a comparison of the peak area ratio of SIL and IS between sample solutions and the standard solution. The amount of SIL in each tablet was also converted to the amount of sildenafil, and then compared with 20 mg.

Optimized extraction and chromatographic conditions

The powder containing 0.5 mg sildenafil (0.7 mg as SIL) in a package, which was packaged automatically, was withdrawn into a 50-mL centrifuge tube. After weighing the powder, 20 mL of the methanol:0.1 N HCl =1:1 solution and 100 µL of the IS solution (10 mg/mL in diluted methanol) were added to the 50-mL centrifuge tube. The solution in the tube was stirred well on a vortex mixer, and then filtered by the syringe filter E131. The filtrate was assayed by HPLC. The package after the powder had been withdrawn was washed with 2 mL of the methanol:0.1 N HCl =1:1 solution. The solution obtained was then added to a 15-mL centrifuge tube. One hundred microliters of the IS solution (10 mg/mL in diluted methanol) was added to the 15-mL centrifuge tube. The solution in the tube was stirred well on a vortex mixer, and then filtered by the syringe filter E131. The filtrate was assayed by HPLC.

The HPLC system consisted of a Model LC-20AS 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 mobile phase was acetonitrile-water-perchloric acid (60%)-sodium perchlorate monohydrate=400:600:1:5, (V/V/V/W) for SIL. The chromatographic column was a YMC Pack AM12S05 ODS (150 mm x 6 mm I.D., particle diameter 5 µm) obtained from YMC Co., Ltd. (Kyoto, Japan). The flow rate and temperature of the column were 1 mL/min and 40°C. The wavelength used for the determination of SIL was 290 nm. The injection volume for HPLC was 20 µL.

SIL (100 mg) was dissolved in 50 mL of diluted methanol. This solution was stored at 4°C for 1 month. Diluted SIL solutions at 1 and 0.1 mg/mL were prepared from this solution using the methanol:0.1 N HCl =1:1 solution. Appropriate volumes of the 2 kinds of solutions were added to 50-mL centrifuge tubes in order to make tubes with 0.01 - 1 mg SIL. Twenty milliliters of the methanol:0.1 N HCl =1:1 solution and 100 µL of the IS solution (10 mg/mL in diluted methanol) were added to the 50-mL centrifuge tubes. The solution in the tube was stirred well on a vortex mixer and assayed by HPLC to make a calibration curve.

Confirmation of specificity for the determination method of SIL by HPLC

One hundred milligrams of each additive was added to a 15-mL centrifuge tube. Ten milliliters of the methanol:0.1N HCl =1:1 solution was then added to these tubes. The solution in the tubes was stirred well on a vortex mixer, and then subjected to the ultrasonic treatment for 15 s. The solution was filtered using the syringe filter E131. The filtrate was assayed by HPLC. The additives contained in Revatio Tablets 20 mg, including lactose, microcrystalline cellulose, anhydrous dibasic calcium phosphate, magnesium stearate, hypromellose, titanium oxide, croscarmellose sodium, and triacetin, were examined.

RESULTS AND DISCUSSION

Results of preliminary studies

The column, YMC Pack AM12S05 ODS, and the mobile phase, the $CH_3CN/H_2O/HClO_4/NaClO_4$ system, were employed because of the large amount of data for developing determination methods of drugs [8-11], and detailed HPLC conditions for preliminary studies were then examined. CH_3CN concentrations in the mobile phase were arbitrarily varied and the retention time of FLT was confirmed. The retention time was observed at 7.6 min when the concentration of CH_3CN was 38%. Based on this result, the mobile phase for the study was determined to be $CH_3CN:H_2O:HClO_4:NaClO_4$=380:620:1:5.

The recoveries of SIL from glass tubes in water and diluted methanol were 68.7 ± 0.6% (mean ± SD, n=3), and 97.9 ± 0.9%, respectively. The adsorption of SIL in water to glass was observed, and adsorption to glass was prevented by using diluted methanol. The same experiment was performed for polypropylene tubes. The recoveries of SIL in water and diluted methanol were 96.3 ± 0.7% and 98.7 ± 0.3%, respectively. The adsorption of SIL to the polypropylene tubes was not observed not only in diluted methanol, but also in water. Based on these results, diluted methanol and polypropylene tubes were employed for subsequent experiments.

When the diluted methanol solution of SIL was passed through the syringe filter, E131, the recovery of SIL was 100.3 ± 0.9% (mean ± SD, n=3). No adsorption of SIL to E131 was confirmed. When the SIL solution was added to lactose, the recovery of SIL was 100.9% (mean, n=2). Recovery from the powder mixture of SIL with lactose was 97.8 ± 0.9% (mean ± SD, n=3).

The recoveries of SIL from the mixed powder of SIL and lactose with additives were 98.0 – 100.5%, except for that of the mixture with croscarmellose sodium, from which recovery was 79.4%. In an attempt to improve recovery, extraction solvents were examined. In the solvents examined, the largest value was 97.7% using the methanol:0.1N HCl =1:1 solution. Therefore, this solution was considered suitable as the extraction solvent for SIL.

A compound for the internal standard was selected from parabens that had a longer retention time than that of SIL in the HPLC conditions. When the retention time of SIL was approximately 7.6 min that of propyl4-hydroxbenzoate was approximately 10 min. Based on this result, propyl 4-hydroxbenzoate was selected as the internal standard (IS). Solutions of 100 mg/mL propyl 4-hydroxbenzoate in methanol and 10 mg/mL propyl 4-hydroxbenzoate in diluted methanol were used as the internal standard solutions.

The content of Revatio Tablets 20 mg was 99.9 ± 2.6% (mean ± SD, n=3). This value is acceptable, indicating the suitability of the extraction system of SIL.

Analysis of optimized conditions and applications

Typical chromatograms of SIL obtained under the conditions described above are shown in Figure 1. The retention times of SIL and IS were approximately 8 min and 13 min, respectively. A linear regression analysis gave a slope, intercept, and correlation coefficient of Y=6.2099X – 0.01518, r=0.99998. Intra-day precision and accuracy were determined by analyzing five replicates at each drug amount, which are shown in Table 1. Precision was ranged from 0.0% to 0.6%. The accuracy value ranged from -2.1% to 16.5%. Inter-day precision and accuracy were determined by analyzing each standard amount over 8 different days. The result for the calibration curve is shown in Table 2. Precision from 0.01 mg/tube to 1 mg/tube was from 4.3% to 0.2%. Accuracy ranged from -0.9% to 16.6%. The values of the intra-day and inter-day precision and accuracy, except for the lowest amount, ranged within 10% and from -10% to 10%, respectively, which were acceptable. The lower limit of quantification was established to be 0.01 mg/tube from the validation data, as shown in Tables 1 and 2, because accuracy at 0.01 mg/tube was greater than 10%.

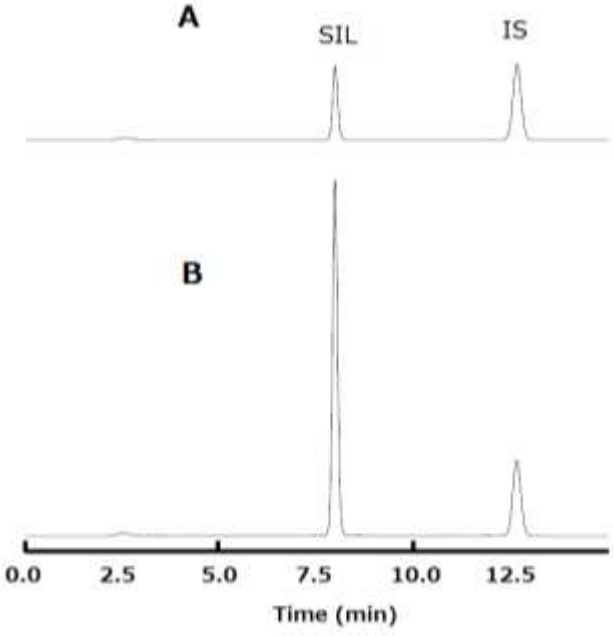

Fig 1: HPLC chromatograms for SIL and IS in standard solution and extracted solutions from the powder, A: Standard solution at 0.1 mg/tube, B: Extracted solution from the powder. The amount of SIL calculated was 0.49 mg.

Table 1: Intra-day precision and accuracy of SIL measurements

Actual amount (mg/tube)	Amount found (mg/tube) (mean ± SD, n=5)	Precision (%)	Accuracy (%)
0.01	0.0117 ± 0.0001	0.6	16.5
0.02	0.0213 ± 0.0000	0.2	6.4
0.05	0.0502 ± 0.0001	0.1	0.4
0.1	0.0979 ± 0.0001	0.1	-2.1
0.2	0.1968 ± 0.0002	0.1	-1.6
0.5	0.5037 ± 0.0002	0.0	0.7
0.7	0.6986 ± 0.0004	0.1	-0.2
1	1.0000 ± 0.0008	0.1	0.0

Precision and accuracy values were calculated using the following equations:
Precision (%) = (SD/mean) x 100.
Accuracy (%) = ((amount found – actual amount)/actual amount) x 100.

Table 2: Inter-day precision and accuracy of SIL measurements

Actual amount (mg/tube)	Amount found (mg/tube) (mean±SD, n=5)	Precision (%)	Accuracy (%)
0.01	0.0117 ± 0.0005	4.3	16.6
0.02	0.0214 ± 0.0004	2.0	7.0
0.05	0.0503 ± 0.0013	2.6	0.5
0.1	0.0991 ± 0.0019	2.0	-0.9
0.2	0.1988 ± 0.0013	0.6	-0.6
0.5	0.4979 ± 0.0029	0.6	-0.4
0.7	0.6985 ± 0.0029	0.4	-0.2
1	1.0023 ± 0.0022	0.2	0.2

Precision and accuracy values were calculated using the following equations:
Precision (%) = (SD/mean) x 100.
Accuracy (%) = ((amount found – actual amount)/actual amount) x 100.

The extracted solutions from additives containing Revatio Tablets 20 mg were analyzed by HPLC. There were no peaks in the retention times of SIL and IS on the chromatogram (data not shown).

Table 3 shows the results of the assay for the powders prepared from Revatio Tablets 20 mg by "grinding tablets on dispensing". The amounts of SIL in the powder withdrawn from the package and in the cleaning solution for the package after the powder had been withdrawn were determined exactly. The mean values for the powder and cleaning solution were 0.48 ± 0.03 mg (mean ± SD, n=9) and 0.127 ± 0.004 mg, respectively. The mean SIL amount in one package was 0.607 mg. This amount corresponded to 0.43 mg of sildenafil, which was lower than the designed value of 0.5 mg. This reason for this currently remains unclear, and was not solely attributed to the weight of the powder packaged. Previous studies have implicated the process of preparing powder from the tablet [2-5]. This developing determination method will be a strong tool for clarify the reasons for drug loss.

Table 3: Amount of SIL in packages packing the powder prepared from Revatio tablets for infants with PPHN

Package Number	Weight of the powder withdrawn (mg)	Amount of SIL in the powder withdrawn (mg)	Amount of SIL in the cleaning solution for each package (mg)
1	194.63	0.49	0.13
2	196.16	0.50	0.12
3	201.01	0.50	0.13
4	206.39	0.53	0.13
5	194.98	0.50	0.12
6	179.27	0.45	0.13
7	179.30	0.45	0.13
8	178.88	0.43	0.13
9	182.80	0.45	0.12
Mean	190.38	0.48	0.127
SD	10.48	0.03	0.004
RSD (%)	5.5	6.3	3.1

CONCLUSION

A determination method of SIL in powder preparations prepared from Revatio Tablets 20 mg for infants with persistent pulmonary hypertension of the newborn was developed herein. The results obtained indicated that this method was accurate and had a sufficient lower limit of quantification for the powder preparations of SIL. This method will make an important contribution to the prevention of drug loss in the process of "grinding tablets on dispensing" in Japan.

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