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Establishment of Dissolution Specifications for Solid Oral Dosage Form

朝 鮮 大 學 校 大 學 院

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고시수재 의약품 중 용출규격 미설정 제제의 용출규격 설정에 관한 연구

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국문초록

고시수재 의약품 중 용출규격 미설정 제제의 용출규격 설정에 관한 연구 - 피라세탐 정, 브롬화수소산페노테롤 정, 소브레롤 캡슐, 염산딜라제프 정

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「대한약전(Korean Pharmacopoeia, KP)」및 「대한약전 외 의약품등 기준(Korean Pharmaceutical Codex, KPC)」은 현재 우리나라의 의약품 연구 및 분석에 있어서 기준이 되는 기준·규격서 라고 할 수 있다. 이는 의약품의 안정성 확보와 품질 관리를 위하여 과학의 발달과 급변하는 의약품 정책에 맞추어 주기적으로 제·개정되고 있다. 현재 국내 의약품의 품질 관리는 이러한 식품의약품안전청 고시에 수재된 기준·규격에 따라 의약품 제조의 제반 변수가 되는 제조 환경 및 제조 방법에 영향을 주는 품질관련 사항을 평가 및 관리하면서 이루어지고 있다.

최종 제품의 기준 규격에 포함되는 시험 항목 중 특히, 용출 시험은 일반적인 제제인 경구용 고형제제의 중요한 항목으로서 평가되고 있다. 경구용 고형제제의 용출 시험은 제형으로부터 약물의 방출 및 생리적 조건에서 약물의 용출을 예측하는 시험으로서 유효성분이 위장관내에서 흡수되어 작용하는 정도를 예측하는 지표가 될 수 있다. 또한 제제학적인 측면에서 제품 롯트별 품질의 균일성, 생산 공정의 밸리데이션, 제품의 안정성, 제제 조성 및 처방 개발, 유효기간 중 품질의 적합성 여부 판단 등에 대한 유용한 정보를 제공할 수 있다. 이러한 측면에서 경구용 의약품 제품이 판매되기 전에 실험실적

으로(*in vitro*) 용출시험이 수행되어야 하며, USP에서도 거의 대부분의 경구용 고형제제 의약품에서 붕해 시험 또는 용출 시험을 필수항목으로 설정하도록 하고 있다. 특히, 정제의 경우 유효성분을 방출하지 않고 작은 조각으로 깨질 수 있기 때문에 붕해 시험만으로 생체내(*in vivo*)에서 활성성분이 어느 정도로 작용할 수 있는지 평가할 수는 없다. 그러나 현재 식품의약품안전청 고시「대한약전외 의약품 기준(Korean Pharmaceutical Codex, KPC)에 수재된 의약품 중 많은 품목에서 용출 규격이 설정되어 있지 않다. 따라서 국내유통 의약품의 품질관리 향상과 의약품 규격의 국제화를 위해 용출 규격이 미설정된 품목에 대한 용출을 연구하여 규격을 새로 설정하는 것이 시급히 요구되고 있다. 이에 본 연구에서는 「대한약전외 의약품 기준(KPC)」에 수재된 의약품 중 용출 규격이 설정되지 않은 피라세탐 정, 브롬화수소산페노테롤 정, 소브레롤 캡슐, 염산딜라제프 정 등 4품목을 선정하여 「대한약전(KP)」일반정보에 수재된「경구용의약품의 용출 규격 설정 지침」에 따라 용출 규격을 설정하였다. 우선, 시험결과의 신뢰성을 위해 실험에 사용하는 용출시험 장치를 밸리데이션 한 후, 용출액 분석을 위한 고성능액체크로마토그래프(HPLC) 분석법을 확립하여 예비실험을 진행하였다. 설정된 분석 방법은 「대한약전(KP)」 일반정보에 수재된 「의약품등 분석법의 밸리데이션에 대한 지침」에 근거하여 특이성, 안정성, 직선성, 정확성, 정밀성, 정량한계와 같은 밸리데이션 파라미터를 이용하여 그 적정성을 평가하였다. 이러한 결과를 토대로 설정된 용출 시험 조건과 시험액 분석조건을 이용하여 각 제제에 맞는 용출시험 규격을 마련하였다. 용출 시험 결과는 타실험실과의 교차시험을 통해 그 신뢰성을 검증하였다. 또한 대조약과 동일한 성분을 갖는 시험약을 이용하여 확립된 시험방법으로 시험하여 예상 용출 규격에 적용해보았다. 본 연구결과는 「대한약전외 의약품 기준(KPC)」에 수재된 의약품 중 용출 시험이 미설정된 제제의 용출규격 설정을 위한 기초자료로 활용되어, 국내 유통 의약품의 품질경쟁력을 높이는데 기여할 것으로 사료된다.

ABSTRACT

Development of Dissolution testing method for Piracetam Tablets, Fenoterol Hydrobromide Tablets, Sobrerol capsules and Dilazep Hydrochloride Tablets in Korean Pharmaceutical Codex

A dissolution testing of oral dosage forms, such as the capsule and tablet, can serve as an effective tool for quality control of a drug product and predictor of *in vivo* performance. However, there are a number of drugs with no established dissolution specifications in Korean Pharmaceutical Codex (KPC). So, the purpose of this study is to develop the dissolution testing method of oral dosage forms among commercially available drug preparations which have no dissolution specifications. Four monographs in the KPC were selected for this study: Piracetam Tablets, Fenoterol hydrobromide Tablets, Sobrerol Capsules and Dilazep hydrochloride Tablets. The appropriate dissolution medium were determined by the dissolution profile observed with various types of dissolution media, pH 1.2, pH 4.0, pH 6.8 buffer solution and distilled water, according to the "Guidelines on Specifications of Dissolution tests for Oral dosage forms" of Korean Pharmacopoeia (KP). And the apparatus and rotational speed for dissolution testing were also investigated. The dissolution test for Piracetam Tablets and Fenoterol hydrobromide Tablets was carried out under sink condition with distilled water as dissolution medium at 37° C and KP apparatus II (paddle) at 50rpm was applied. For Sobrerol Capsules, pH 6.8 buffer was found to be suitable to ensure the best in vitro dissolution profile.

The rotation speed of paddle was determined to be 100 rpm. In case of Dilazep hydrochloride Tablets, the best dissolution conditions were achieved using a paddle at a rotation speed of 100 rpm and medium containing pH 1.2 buffer. The media volume used in all drugs was 900 mL. The in vitro dissolution samples were analyzed using a high performance liquid chromatography (HPLC) method and the validation was performed to verify the newly established method for categories including accuracy, precision, specificity, linearity, quantitation limit and range. The validation results were within the acceptable range, demonstrating to be suitable to measure the dissolution rate of each drug. Based on the dissolution conditions and results, the dissolution specifications of them were proposed and those could be utilized in the revised version of KPC. Moreover, the analysis method was demonstrated to be adequate for quality control of that.

1. Introduction

Dissolution testing is a test that determines not only the release of the drug substances from the drug product but also the dissolution or solubilization of the drug under the physiological condition. This has emerged in the pharmaceutical field as a very important tool to estimate the performance of the drug product in the biological system[1]. It provides the measurements of a bioavailability of a drug as well as can demonstrate bioequivalence from bath-to-batch. Although the dissolution testing can not be confirmed the in vitro-in vivo correlation, it is used to guide development of new drug products and assess variability of drug products. Therefore, the quality of oral dosage forms should be verified preferably with an in vitro dissolution test before the product is released to the market[2,3]. In case of some fast-dissolving drugs, disintegration is only test required. However, disintegration is not a good indicator of drug performance in vivo, since tablets can break apart in small pieces without releasing the active components. Moreover, the significance of dissolution testing could be also supported by the bioequivalence guidances of Korea Food & Drug Administration(KFDA) that the in-vivo bioequivalence studies of biopharmaceutics classification system (BCS) class Ⅰ(having high solubility and high permeability) drugs could be replaced by the dissolution testing[4].

The Korean Pharmacopoeia (KP) and Korean Pharmaceutical Codex (KPC) have been revised to reflect and harmonize between international standards and our specification. Currently, KP requires dissolution testing for developed drug products in oral dosage forms, but there are a number of drugs with no established dissolution specifications in KPC. In these respects, we selected 4 items for establishing dissolution

specification: Piracetam Tablets, Fenoterol hydrobromide Tablets, Sobrerol Capsules and Dilazep hydrochloride Tablets listed in KPC which have no dissolution specification[5,6].

Piracetam (2-oxo-1-pyrrolidine acetamide) is a cyclic derivative of GABA (Figure 1-(a)). It has been studied in an extensive number of clinical experiments, and has shown positive results in the treatment of post-stroke aphasia, epilepsy, cognitive decline following heart and brain surgery, dementia[7,8] and myoclonus[9]. Piracetam's mechanism of action is not fully understood. Several reviews indicate that the drug influences neuronal and vascular functions improves the function of the neurotransmitter acetylcholine via muscarinic cholinergic (ACh) receptors which are implicated in memory processes[10,11].

Fenoterol hydrobromide (5-(1-Hydroxy-2-[[2-4-hydroxyphenyl)-1-methyl]ethyl]-1,3-benzenediol hydrobromide), has been introduced into clinical use as a sympathomimetic bronchodilator drug (Figure 1-(b))[12]. The drug is a calcium-channel blocker and classified as a class IV anti-arrhythmic agent[13]. It has been proven efficacy in the control of supra ventricular tachyarrhythmias and in the management of classical and variant angina pectoris[14].

Sobrerol((1S)-5-(1-hydroxy-1-methylethyl)-2-methylcyclohex-2-en-1-ol) (Figure 1-(c)) has been used as mucosecretolytic drug and treatment of respiratory disease with bronchial mucus hypersecretion and obstruction. The reports about its absorption, distribution and excretion in animals shown favourable clinical activity by a decreased in the viscosity of sputum[15-17].

Dilazep hydrochloride (Bis[3,4,5-trimethoxybenzoic acid] [2,3,6,7-tetrahydro-1H-1,4-diazepine-1,4(5H)-diyl]di(trimethylene) ester)(Figure 1-(d)) is used clinically for the treatment of kidney disease and ischemic heart disease as a vasodilator. Moreover, Deguchi

et al.(1997) reported that it suppress tissue factor mRNA expression and its activity in human umbilical vein endothelial cells stimulated by tumour necrosis factor-α (TNF-α), thrombin, or phorbol 12-myristate 13-acetate[18].

There are few official or analytical methods for determination of piracetam in pharmaceutical formulations although it is listed as the piracetam tablets in KPC. For the quantitative estimation of sobrerol, gas chromatographic method has been usually presented due to its structure including the monograph of KPC[19] and several studies were published to analyze dilazep hydrochloride in biological samples[20,21]. However, the dissolution rate studies of those in oral dosage forms have not been reported in the literature.

In case of fenoterol hydrobromide tablets, El-Shabrawy et al.(2003)[22] and W. Beyene et al.(2004)[23] used spectrophotometric analysis of fenoterol hydrobromide in pure form and dosage forms. It has been determined by various analytical methods such as HPLC[24,25], voltammetry[26], immuno-assay[27], GC[28] and flow-injection analysis[29]. The HPLC method for dissolution studies of verapamil in oral dosage forms has been also reported, but drug content and range for analysis were different with drug preparation in our market[30].

The purpose of the present work is to study the dissolution profiles of four monographs (piracetam tablets, fenoterol hydrobromide tablets, sobrerol capsules and dilazep hydrochloride tablets) and to propose the dissolution specification that could be applied in the KPC. And a simple and accurate HPLC method for determination of each drug substance is developed and validated to ensure that it is suitable for analysis of dissolution samples. Overall procedure in this study was conducted based on the KP guidelines "Guidelines on Specifications of Dissolution tests for Oral dosage forms" and "guidelines of validation

of analytical procedures of pharmaceuticals".

2. Experimentals

2-1. Materials

Commercially available drug preparations of piracetam, fenoterol hydrobromide, sobrerol and dilazep hydrochloride in Korea were obtained from each pharmaceutical company. The 3 lots of each drug product for reference were purchased to validate the analysis methods, which the 1 lot for additional test was studied(Table 1). Piracetam, fenoterol hydrobromide and dl-sobrerol for standard were obtained from Sigma-Aldrih (Saint Louis, MO, USA). Dilazep hydrochloride was a gift from Bukwang Pharm (Seoul, South Korea). All other chemicals were reagent-grade and were used without further purification. Purified water(NanoPure Diamnond, Barnstead Thermolyne, USA) was used for dissolution and throughout analysis. Buffer solution for dissolution test, pH 1.2, pH 4,0 and pH 6.8, was prepared by guideline in the KP IX.

2-2. Instrumentation

Dissolution testing was performed using a DISTEK EVOUTION 6300(Distek, USA) dissolution tester equipped with USP apparatus 1 (basket) and USP apparatus 2 (paddles). The suitability test of dissolution apparatus was conducted according to the "Guidance for setting dissolution specification for oral dosage forms" of KP, previously. The test for the rotary basket or for the paddle method was carried out using two standard calibrator (USP dissolution calibrator, 300 mg salicylic acid standard tablet and 10 mg prednisone standard tablet).

A liquid chromatography with a Nanospace SI-2 HPLC system (Shiseido, Tokyo, Japan), a series 3017 PDA detector, series 3001 pump, a series

3023 automatic injector and a series 3004 column oven was used to quantify samples.

The ultrasonic bath used for deaeration was the model Powersonic 420 (Hwashin Tech, Seoul, Korea).

2-3. Methods

2-3-1. Assay tests

Piracetam Tablets - The contents of piracetam in Reference A-1, 2, 3 and Test B tablets were determined. Twenty tablets, each containing 800 mg Piracetam, were accurately weighed and finely powdered. A quantity of powder equivalent to about 50mg piracetam was weighed and transferred to 50 mL volumetric flask. And about 30 mL of water was added to the flask, shaken for 10 min, added to make 50 mL and filter through 0.45 ㎛ PVDF syringe filter(Whatman, Brentford, United Kingdom). Aliquots of 2mL of the solution was transferred to the 50 mL volumetric flask and diluted with water obtaining the final concentration of 40 ㎍/mL. The standard solution was also prepared by same procedure as sample solution. The peak areas of sample solution and standard solution were analyzed as directed under the liquid-chromatography at 214 nm according to the monograph in the KPC.

Fenoterol Hydrbromide Tablets - The contents of fenoterol hydrobromide in Reference C-1, 2, 3 and Test D tablets were determined. Twenty tablets, each containing 2.5 mg fenoterol hydrobromide, were accurately weighed and finely powdered. A quantity of powder equivalent to about 5 mg fenoterol hydrobromide was taken and transferred to 100 mL volumetric flask containing 50 mL of 0.01 mol/L hydrochloric acid. They were shaken for 30 min and the volume was completed with 0.01 mol/L hydrochloric acid. About 20 mg fenoterol hydrobromide was weighed and the standard solution was prepared with 0.01 mol/L hydrochloric acid obtaining the final concentration of 50 ㎍/mL. The solutions were

filtered with 0.45 ㎛ PVDF syringe filter(Whatman, Brentford, United Kingdom) in before analysis. Finally, the absorbances of sample solution and standard solution were determined at 276 nm under the ultraviolet-visible(UV) spectrophotometry according to the monograph in the KPC.

Soberérol Capsules - The contents of sobrerol in Reference E-1, 2, 3 and Test F tablets were determined. Twenty capsules, each containing 0.2 g sobrerol, were accurately weighed in a volumetric flask and add ethanol to make 100 mL. Aliquots of 10 mL of the solution was transferred to the 100 mL volumetric flask and diluted with ethanol. This solution was used as the test solution and the standard solution was prepared by dissolving 20 mg of sobrerol standard to be 0.2 mg/mL solution. Aliquots of 1 mL each of test solution and standard solution were filtered with 0.45㎛ PVDF syringe filter(Whatman, Brentford, United Kingdom) and analyzed as directed under the gas-chromatography according to the monograph in the KPC.

Dilazep Hydrochloride Tablets - The contents of dilazep hydrochloride in Reference G-1, 2, 3 and Test H tablets were determined. Twenty tablets, each containing 50 mg dilazep hydrochloride, were accurately weighed and finely powdered. A quantity of powder equivalent to about 25 mg dilazep hydrochloride was weighed and dissolved in water to make exactly 100 mL. The standard solution was also prepared by same procedure as sample solution. After, filtering with 0.45 ㎛ PTFE syringe filter(Advantec Toyo, Tokyo, Japan), the absorbances of sample solution and standard solution were determined at 265 nm under the ultraviolet-visible(UV) spectrophotometry according to the monograph in the KPC.

2-3-2. Dissolution tests

Preliminary dissolution test

Firstly, the preliminary test for establishment of dissolution condition was studied based on the "Guidance for setting dissolution specification for oral dosage forms" of KP. The dissolution testings of piracetam in Reference A-1 and fenoterol hydrobromide in Reference C-1 were performed using a calibrated dissolution apparatus with paddles at 50rpm in pH 1.2, 4.0, 6.8 and distilled water medium. In case of sobrerol in Reference E-1, dissolution tests with paddles at 50 rpm and 100 rpm in 4.0, 6.8 and distilled water medium were studied for medium and paddle speed selection. At pH 1.2, a change of sobrerol was observed, resulting in increased impurity peak in HPLC chromatogram during the period of experiments. So, pH 1.2 medium was not included in the tests. In the preliminary test of dilazep hydrochloride, pH 6.8 dissolution medium was excluded based on the stability results and rotation speed of paddles was investigated at 50rpm and 100rpm. The bath temperature was maintained at 37± 0.5℃. The media volume used in all drugs was 900 mL. The glass dissolution vessels were covered to minimize evaporation. At predetermined time intervals(5, 10, 15, 30, 45, 60, 90, 120, 180 min),a 5 mL of sample from each vessel was withdrawn, filtered and immediately replaced with identical volume of same media. The sample solutions were diluted down to appropriate concentration with dissolution medium. According to guideline, the tests were terminated at a point, 180 min or 360 min, when the final rate of dissolution reached not less than 85%. At each sampling time, the concentration of drug in the dissolution medium was determined by HPLC method and the profile of drug in each dissolution medium was studied.

Filter suitability for Dilazep Hydrochloride

To investigate the filter adsorption of dilazep hydrochloride, five types of hydrophilic filters, PVDF(polyvinylidenedifluoride), PTFE(polytetrafluoroethylene), mixed cellulose acetate, cellulose acetate and nylon, were compared with centrifugation method in the distilled water, pH 1.2 and pH 4.0 medium. Regardless of filter materials, syringe filters of 25 mm of the diameter and 0.45 ㎛ of the pore size were used in filtration experiments. The concentrations of test solutions in each medium were 3.4 ㎍/mL equivalent to the released amount at 5 min. These solutions were stirred at 37 ° C for 24 h prior to being filtered. The final concentrations were determined using same method used for the dissolution study after discarding the first 1 mL of filtrate. As control, the sample solution was centrifuged at 3000 rpm for 10 min and supernatant obtained was analyzed. These studies were repeated in triplicate.

Final dissolution test and Cross test

The final dissolution testing was conducted using each 12 tablets of piracetam of Reference A-1, 2, 3 lots based on the dissolution condition determined by preliminary test (Table 2). The conditions in the final dissolution tests of fenoterol hydrobromide tablets (Reference C-1, 2, 3 lots), sobrerol capsules (Reference E-1, 2, 3 lots) and dilazep hydrochloride tablets (Reference G-1, 2, 3 lots) were presented in Table 3, Table 4 and Table 5. To verify the results, the cross tests among other laboratory were performed in the same conditions with final dissolution testing. Additionally, the dissolution profiles of test B in piracetam tablets, test D in fenoterol hydrobromide tablets, test F in sobrerol capsules and test H in dilazep hydrochloride tablets were also investigated in same dissolution conditions. In case addition tests, each 6 tablets for 1

lot were tested.

2-3-3. HPLC methods

Piracetam Tablets - An HPLC method of piracetam listed in assay tests of KPC monograph was employed. Chromatographic seperation was carried out at 40℃ with capcellpak C18 MG(250 × 4.6 mm, 5 μm) column from Shiseido(Japan). The different mobile phase depending on the dissolution media was prepared (shown as in Table 6). The rate of mobile phase flow was 0.8 mL/min and injection volume was 10 ㎕. The UV detection wavelength was 214 nm.

Fenoterol Hydrbromide Tablets - To determine the dissolved amount of Fenoterol hydrbromide tablets, HPLC analytical method was established instead of uv/vis spectrophotometer measurement listed in assay tests of KPC monograph. For the mobile phase, pH 3.2 buffer solution containing 0.25% of heptanesulfonic acid and adjusted with phosphoric acid was prepared and mixed with acetonitrile (1000 : 270(v/v)) at a flow rate of 1 mL/min. Capcellpak C_{18} (5 ㎛, 4.6× 250 mm) from Shiseido(Japan) was used as stationary phase and uv wavelength was monitored at 276 nm. The column temperature was controlled at 40℃ and the injection volume was 100 ㎕.

Sobrerol Capsules - The gas-chromatography method used in the assay tests of KPC monograph was replaced with HPLC for the analysis of sobrerol in dissolution samples. For chromatographic separation, capcellpak C18 MG(250 × 4.6 mm, 5 μm) column from Shiseido(Japan) was used. The temperature of the autosampler was maintained at 40 ° C. A mobile phase used mixture of 0.02 mol/L potassium phosphate monobasic solution (adjusted to pH 3.5) and methanol (1:1, v/v) for analysis at a wavelength of 210 nm. The rate of mobile phase flow was 1.0 mL/min and injection

volume was 10 ㎕.

Dilazep Hydrochloride Tablets - The HPLC analysis method of dilazep hydrochloride was also established instead of uv/vis spectrophotometer measurement listed in assay tests of KPC monograph. Chromatographic analysis was carried out using a capcellpak C18 MG(250 × 4.6 mm, 5 μm) and the column temperature was controlled at 40℃. The mobile phase consisted of a mixture of 0.02 mol/L sodium phosphate monobasic solution (adjusted to pH 3.0):acetonitrile (30:70, v/v). The flow rate was 1.0 mL/min and the injection volume was 10 ㎕. The detection of drug was carried out by ultraviolet absorption at 264 nm.

2-4. Method Validation

The developed HPLC method of in vitro dissolution test was validated according to KP guidelines: Specificity, stability, linearity, accuracy, precision and quantitation limits were evaluated.

Piracetam tablets

1)Specificity : It was demonstrated that active compound has no interference from excipients which take part in the commercial formulations of tablets by comparing the photodiode array (PDA) of standard solution and dissolution solution. The concentration of those solution was the specification of dissolution 100%.

2)Stability : Piracetam stability was evaluated in each dissolution media. The standard and sample solutions (44 ㎍/mL) were stored in HPLC tube and kept at an ambient temperature. Aliquots of the samples were tested at time 0, and after 1, 2 and 3 h. The responses for the aged solutions were investigated with the chromatograms and peak area obtained by proposed HPLC method.

3)Linearity : Piracetam stock solution was prepared by dissolving 53

mg of piracetam in a 100 mL distilled water in a 100 mL volumetric flask. Standard calibration solutions were prepared by dilution of the piracetam stock solution with distilled water to obtain five different concentrations within the range 5.3, 15.9, 26.6, 37.2, 53 ㎍/mL. Each solution was prepared in triplicate. The calibration curve for HPLC analysis was determined by plotting the ratio of the peak area of the drug against the drug concentration by the linear least square regression analysis.

4)Accuracy : The accuracy of the method was evaluated through the application of analytical procedure to drug products and pure drug in the known amounts. Piracetam stock solution was prepared by dissolving 44 mg of piracetam in a 100 mL distilled water in a 100 mL volumetric flask. Sample solution was prepared by adding 1 tablet of piracetam of Reference A-1 in a 900 mL distilled water. Aliquots of 2 mL of stock solution and aliquots of 4 mL of sample solution were added to a 100 mL volumetric flask, made up to volume with distilled water. The exact volume of 40 and 70 mL of this solution were transferred to a 100 mL volumetric flask and diluted with distilled water. The final concentrations of three test solutions were 17.7, 31, 44.4 ㎍/mL. These studies were performed in triplicate. Recovery was calculated as actual concentration and analyzed by ANOVA(Student-Newman-Keuls multiple range test).

5)Precision : Precision of the method was studied with respect to repeatability. It was assayed at the concentration 46 ㎍/mL of piracetam standard solution under the same operating conditions. The relevant standard deviation (R.S.D) value and was calculated in six replicate measurements.

6)Quantitation limits : The quantitation limit was evaluated based on the standard deviation of the response and the slope. The quantitation

limit (QL) equation was expressed as: QL= 10 × δ/S. The slope S and the standard deviation of the response δ were estimated from the data of calibration curve.

Fenoterol hydrbromide tablets

1)Specificity : Specificity for HPLC analysis was studied by comparing the photodiode array (PDA) of standard solution and dissolution solution of fenoterol hydrobromide at the 100% dissolution concentration.

2)Stability : Fenoterol hydrobromide stability for HPLC analysis was evaluated in each dissolution media. The solutions (2.8 ㎍/mL) were kept at an ambient temperature during the period of the test for at least 3 h, investigating the chromatogram of drug and degradation products in the base line.

3)Linearity : The standard calibration curves of fenoterol hydrobromide were determined with five concentrations in the range of 0.66 - 3.3 ㎍/mL(0.66, 1.32, 1.98, 2.64, 3.3 ㎍/mL). The stock solution was prepared by dissolving 33 mg of fenoterol hydrobromide in a 100 mL distilled water in a 100 mL volumetric flask. Standard calibration solutions were prepared by dilution of the fenoterol hydrobromide stock solution with distilled water. Each solution was prepared in triplicate and the linearity was evaluated by linear regression analysis, which was calculated by the least square regression method.

4)Accuracy : The method accuracy was at the three levels of fenoterol hydrobromide in the fenoterol hydrobromide stock solution and sample solution. Fenoterol hydrobromide stock solution was prepared by dissolving 28 mg of drug in a 100 mL distilled water in a 100 mL volumetric flask. Sample solution was prepared by adding 1 tablet of fenoterol hydrobromide of Reference C-1 in a 900 mL distilled water.

They were mixed and diluted to final concentrations of 1.68, 2.23, 2.79 ㎍/mL. Recovery studies were performed in triplicate and analyzed by ANOVA(Student-Newman-Keuls multiple range test).

5)Precision : Precision of the method was assayed at the concentration 2.8 ㎍/mL of piracetam standard solution under the same operating conditions. The R.S.D value was calculated in six replicate measurements.

6)Quantitation limit : The quantitation limit was also evaluated based on the standard deviation of the response and the slope.

Sobrerol capsules

1)Specificity : Specificity was studied by comparing the photodiode array (PDA) of standard solution and dissolution solution of sobrerol at the 100% dissolution concentration.

2)Stability : Stability of sobrerol in each dissolution medium was evaluated using standard and sample solutions. The solutions (110 ㎍/mL) were kept at an ambient temperature during the period of the test for at least 3 h, investigating the chromatogram of drug and degradation products in the base line.

3)Linearity : A stock solution was prepared by dissolving 100 mg of sobrerol in 100 mL of pH 6.8 dissolution medium. Five standard solutions in the range of 5-130(5, 30, 70, 110, 130) ㎍/mL were prepared by appropriate dilution of stock solution with pH 6.8 dissolution medium. Each solution was prepared in triplicate and the peak area versus concentration data was evaluated by the linear least square regression analysis.

4)Accuracy : Accuracy was accomplished by adding known amounts of sobrerol standard to drug products. A stock solution containing 1 mg/mL of drug was prepared in pH 6.8 dissolution medium. Sample solution was

prepared by adding 1 capsule of sobrerol of Reference E-1 in a 900 mL pH 6.8 dissolution medium. They were mixed for final concentrations which were 11.3, 62.3, 113.3 ㎍/mL and made up to volume with pH 6.8 dissolution medium. These studies were performed in triplicate and analyzed by ANOVA(Student-Newman-Keuls multiple range test).

5)Precision : Sobrerol standard solution at the concentration 110 ㎍/mL prepared with pH 6.8 dissolution medium was assayed for precision. The R.S.D value was calculated as the average of six replicate measurements.

6)Quantitation limits : The quantitation limit was also evaluated based on the standard deviation of the response and the slope.

Dilazep hydrochloride tablets

1)Specificity : Specificity was studied by comparing the photodiode array (PDA) of standard solution and dissolution solution of dilazep hydrochloride at the 100% dissolution concentration.

2)Stability : For the investigation of standard and sample solution stability, the solutions (56 ㎍/mL) were kept at an ambient temperature for 3 h. The change of the chromatograms and degradation products were evaluated with time.

3)Linearity : The calibration curves were performed with six concentrations from 1.75 to 70 ㎍/mL by diluting a stock solution (700 ㎍/mL) with pH 1.2 dissolution medium. The assay was studied in triplicate and the linearity was evaluated by the linear least square regression analysis.

4)Accuracy : Accuracy was assessed with three concentration levels, 5.6, 30.7 and 55.7 ㎍/mL, as the percentage of recovery. A stock solution containing 0.35 mg/mL of drug was prepared in pH 1.2 dissolution medium. Sample solution was prepared by adding 1 tablet of

Reference G-1 in a 900 mL pH 1.2 dissolution medium. They were mixed to theoretical concentrations in three replicates and the recovery(%) was calculated by the assay of them.

5)Precision : Precision test was assessed using dilazep hydrochloride standard solution (55.3 ㎍/mL) in pH 1.2 dissolution medium. The R.S.D value was calculated by six replicate injections.

6)Quantitation limits : The quantitation limit was also evaluated based on the standard deviation of the response and the slope.

3. Results and Discussion

3-1. Assay of tablets

To set the dissolution specification of generic drug, it is the first stage to select the lot that is appropriate for the test of the comparative testing. According to the guidance in KP, it should be selected the lot that the difference between the content of samples and its labeled amount is not more than 5% and difference between the content of reference and test is not more than 5%. The samples of piracetam tablets, fenoterol hydrobromide tablets, sobrerol capsules and dilazep hydrochloride tablets used in this study were suitable for the standard of contents in KPC which should be labeled 95.0~105.0%. Also, the differences between the contents of reference and test were not more than 5% (data not shown).

3-2. Setting the dissolution specification of Piracetam Tablets

1) Dissolution profiles and Sample analysis

The preliminary experiments to study the dissolution profiles for four different medium (pH 1.2, 4.0, 6.8 and distilled water) were evaluated. The test was performed at 37 ℃ using KP apparatus II at 50 rpm with 900 mL of each dissolution medium. The samples were withdrawn at predetermined time intervals and equivalent amount of the medium was added into the vessel to keep the sink condition. It is typical to study samples up to 2 hours of the test at appropriate intervals with 6 test specimen. Using the described chromatographic condition in the method above, the dissolution solutions were analyzed. For piracetam compound, the different mobile phase depending on the dissolution media

was used to obtain sharp and symmetrical peak with good baseline. In the dissolution results of reference A-1, the amounts dissolved in all medium were over 85% within 60 min (Figure 2). In the guideline of KP, it is recommended that the test may be terminated at a point when the final dissolution rate reaches not less than 85%. Drug solubility to maintain the sink condition is important property to be considered when selecting dissolution medium. The sink condition is defined as the volume of medium at least greater than three times that required to form a saturated solution of a drug substance[31,32]. In these respects, distilled water was determined as the medium where drug is freely soluble. And the convenience of experiment preparation and environmental friendship is also considered. Since the rotation speed is sufficient to release the drug using 50 rpm, no other speed was tested. From these results, conditions for the final dissolution test was determined as presented in section 2-3-2.

2) Development and Validation of the HPLC method

To verify the HPLC method for analyzing the dissolution samples of the drug product, validation parameters in the following method were studied: specificity, stability, linearity, accuracy, precision and quantitation limit. The validation parameters met the acceptance criteria in KP "guidelines of validation of analytical procedures of pharmaceuticals" and drug solution dissolved in the distilled water was applied on the basis of above results.

The specificity was determined by comparing the photodiode array (PDA) of standard solution and drug product solution. Piracetam was well separated and its retention time was 4.68 min. Figure 3 represents that no interfering peaks induced by tablet excipients were found in the chromatogram.

To evaluate the stability of sample and standard solution in each medium, the variation of chromatogram and retention time was investigated. There was no significant change in the HPLC data obtained. The results demonstrated that they were stable for dissolution condition in the period tested (data not shown).

The linearity was studied in the concentration range of 5.3 ~ 53 ㎍/mL, which involves the concentration range of first sampling time to 120% of labeled amount. Three independent determination were performed at each concentration and R.S.D. value for the slope and Y-intercept of the calibration curve was calculated. The data is presented in Table 7. The mean values of slope and Y-intercept were 30960.29 and 7915.66, with correlation coefficients over 0.999. These results show there was a good correlation between the peak area and drugs concentration.

The accuracy was expressed by percentage recoveries of known amounts of piracetam. That was tested in the three different levels of drug, where the high concentration was 100% dissolution value and the intermediate concentration was mid-point of high and low concentration. The acceptance criteria of recoveries in dissolution tests are recommended from 98.0 to 102.0%. In case of piracetam, three solutions with 17.7, 31, 44.4 ㎍/mL were prepared. The recovery percentages for those were ranged from 98.7 to 101.6% as presented in Table 8, confirmed the accuracy of the corroborating the accuracy of the method. Also, the data were validated by means of statistical analysis using ANOVA(Stuendt-Newman-Keuls multiple range test), which showed no significant difference between the results ($P<0.05$).

Precision was examined by analyzing the solution representing 100% of the target concentration of the method (46 piracetam ㎍/mL). That was evaluated by performing six replicate injections of the sample in the same HPLC conditions. The R.S.D.% of experimental concentration

calculated by theoretical concentration and standard purity was 0.6% lower than 1% (Table 9).

The quantitation limit is used as the parameter of quantitation assay for low levels of compounds in sample matrices which can be determined with suitable precision and accuracy. In these studies, that was found to be 1.400 ㎍/mL based on the standard deviation of the response and the slope.

The overall validation results of the HPLC method were summarized in Table 10. These results demonstrated that the proposed method is suitable to analyze the dissolution samples in the distilled water medium. Moreover, since same HPLC analyzing method was applied to assay of drug contents and dissolution samples, it will be able to provide convenience for quality control of piracetam tablets.

3) In vitro dissolution studies

The accomplishment of dissolution profile is recommended as an assurance the quality of solid dosage forms by the pharmaceutical industry and the establishment of in vitro/in vivo correlation. Based on the dissolution condition determined by preliminary tests, final tests to set dissolution specification of drug were evaluated. The dissolution tests for reference drug A-1, A-2, A-3 lots with each 12 specimens per lot were studied. The experiments were performed using 50 rpm and 900 mL of distilled water at the conditions mentioned in Table 2. The dissolution of piracetam after 30 min reached 94.9± 6.2(%) in drug A-1, 96.8± 4.4(%) in drug A-2 and 98.5± 3.3(%) in drug A-3 as shown in Figure 4-1. The average released amounts for three lots of piracetam tablets were 96.7± 4.6(%) and the dissolution rate reached at an almost plateau in the graph after 30 min. The results from the cross test performed by another laboratory were similar with our dissolution

profiles(Figure 4-2). From these data, the specification of piracetam dissolution rate was determined as 'not less than 80 % in 30 min' which was the value of about 10 % less than the average rate of dissolution. The single point specification can be set for the dissolution test of the conventional release dosage forms in which not less than 70 ~ 85 % of the drug is dissolved within 60 minutes. The other product having different tablet formulations was tested based on the proposed dissolution specification. Except for reference drug, only 1 product in the form of piracetam tablets is distributed in domestic market. The average dissolution amounts (n=6) in test-B drug were found to be 97.8% at 30 min. This result was satisfactory to the expected dissolution criteria.

3-3. Setting the dissolution specification of Fenoterol Hydrobromide Tablets

1) Dissolution profiles and Sample analysis

In order to study the general considerations for the dissolution testings, the preliminary experiments were performed. The dissolution profiles for each medium (pH 1.2, 4.0, 6.8 and distilled water) using paddles were studied. In case of tablets, KP apparatus 2 with paddles rotating at 50 rpm would be preferred in the preliminary test. The dissolution solutions were analyzed using newly developed HPLC method instead of UV spectrophotometry method established in assay tests of KPC. Figure 5 shows that there was no significant effect of characteristics of dissolution medium on the dissolution rate of fenoterol hydrobromide. As expected for a highly soluble compound, the dissolution was rapid and essentially complete within 10 min under all of these test conditions. Although drug products contain the highly soluble drug substances, setting the dissolution specification is

required for narrow therapeutic range drugs [33]. Based on these results, distilled water as the medium and paddles with 50 rpm as the apparatus were determined to be suitable in the final dissolution testing.

2) Development and Validation of the HPLC method

In this study, the HPLC method with PDA detector was selected because of its ability to separate fenoterol hydrobromide from the tablet excipients comparing with UV spectrophotometry method in assay tests of KPC. The HPLC method used to analyze the dissolution samples was validated with validation parameters. Of course, experimental solutions were prepared with distilled water.

The specificity was examined by analyzing the standard solution and tablet solution containing excipients mixture. The photodiode array (PDA) of those showed same lambda max around 277 nm, indicating that no interferences were observed (Figure 6). The chromatographic run time of 5.3 min was sufficient for sample analysis in a short period of time.

The dissolution sample solution prepared with each medium was stored at ambient conditions and assayed after 1, 2, 3 h against a freshly prepared standard solution. Solutions were stable without significant change in the chromatogram and retention time of HPLC data (data not shown).

Linearity of the method was confirmed by preparing standard curves for the analytical range of 0.66~3.3 ㎍/mL. The linearity range assayed was narrow, since the commercial marketing products of fenoterol hydrobromide are 2.5 mg/tablet. The regression line and calibration curve were obtained as the fenoterol hydrobromide concentration (㎍/mL) and peak area as presented in Table 11. The response for drug was linear and the correlation coefficients were >0.999 at all three

curves. These data indicate that the developed method confirmed the good linearity for dissolution samples.

The accuracy was evaluated by the recoveries of fenoterol hydrobromide at three different levels, 60, 80, and 100% of the nominal assay concentration of drug. Because, the dissolution amounts at first sampling time were over 60% as studied in preliminary experiments. Table 12 summarizes the accuracy results at the concentration 1.68, 2.23, 2.79 ㎍/mL, expressed as recovery percentage and standard deviation. The average recoveries ranged from 98.9 to 101.7% for drug, considered to be acceptable criteria. And, the statistical value obtained form ANOVA(Stuendt-Newman-Keuls multiple range test) showed good reproducibiliy of study results($P<0.05$).

Repeatability is a measure of the precision under the same operating conditions over a short interval of time and it is also known as intra assay precision. The results obtained by analyzing 2.8 ㎍/mL fenoterol hydrobromide solution were presented in Table 5. The data show the good precision of the method with R.S.D.% lower than 1%.

The quantitation limit of the method was evaluated to be 0.411 ㎍/mL by the standard deviation of the response and the slope. This value is sufficient to demonstrate that dissolution amounts could be reliably quantified.

The results of validation parameters for the proposed HPLC method were summarized in Table 14. It was shown that these can be successfully applied to the analysis of fenoterol hydrobromide of tablet dosage forms in the dissolution tests.

3) In vitro dissolution studies

Based on the preliminary studies and method validation, the dissolution profiles for reference drug were investigated with three

lots: C-1, C-2, C-3 lots. The test conditions were presented in Table 3. Average data from 12 tablets were used to present each dissolution profiles. As shown in Figure 7-1, average dissolution amounts of each tablets were 95.7± 3.0(%), 97.5± 5.6(%), 98.6± 7.9(%) at 10 min. They maintained similar dissolution profiles by the end of 45 min time point. To verify our results, the cross tests were also performed by another laboratory in same condition. Although released amounts at 10 min were a little low, no significant difference was observed between dissolution profiles (Figure 7-2). According to these data, the specification of dissolution rate in fenoterol hydrobromide tablets was chosen as 'not less than 80% in 15 min' which was the value of about 10% less than the average rate of dissolution. The proposed specification was investigated whether be consistent with other products. The dissolution rate of test-D drug was also fast and reached 92.1% of fenoterol hydrobromide after 15 min.

3-4. Setting the dissolution specification of Sobrerol Capsules

1) Dissolution profiles and Sample analysis

To select the dissolution medium and paddle speed, the tests were performed using 900 mL of dissolution medium pre-heated at 37 ± 0.5 ° C. Basket is usually used as apparatus for capsules or pharmaceutical forms that tend to float in the dissolution medium. However, sobrerol capsules in the medium were disintegrated within 2 min and the paddle as apparatus was used in these studies. In the chromatogram of standard and sample solutions in pH 1.2, main peak of drug was split into two peaks that shown same spectrum. Moreover, the side peak was increased with time (data not shown). Therefore, the dissolution profiles of reference E-1 in pH 4.0, pH 6.8 and distilled water were evaluated

using newly developed HPLC analysis method. At 50 rpm, the dissolution rate of sobrerol was too slow as shown Figure 8, which indicate that stirring speed is not satisfactory. Especially, in the distilled water, dissolved amounts of drug maintained below 70% constantly. When rotation speed was increased to 100 rpm in pH 6.8 dissolution medium, the rate was fast and approximately 90% of sobrerol was dissolved in 60 min. In these respects, pH 6.8 was determined as the medium and later dissolution studies were performed with paddles at 100 rpm. The conditions for the final dissolution test were summarized in Table 4.

2) Development and validation of the HPLC method

To develop HPLC method instead of GC method in KPC monograph, the appropriate wavelength of sobrerol was investigated. The maximum absorbance for drug occurred at about 200 nm. However, most of the chemical compound has absorbance at 200 nm and specificity of an analytical procedure could be influenced by excipients in dosage formulation. Therefore, UV detection wavelength for the drug was determined as 210 nm. On the basis of preliminary experiments results, sobrerol solution in pH 6.8 phosphate buffer was used in validation studies. .

Figure 9 demonstrated the specificity of method, showing that same retention time and chromatogram for standard solution and drug product solution were obtained. No interference peak was also observed.

Stability of sobrerol in the dissolution medium was evaluated using standard and sample. Except for pH 1.2 buffer solution, drug was found to be stable under dissolution test conditions for 72 hr. The peak area and retention time were not changed from the initial value and no degradation products were observed in any of the chromatograms (data not shown).

Calibration plots were constructed by plotting the area of the main peak versus the concentration of drug, covering concentration range 5 ~ 130 ㎍/mL. The linearity in the range could be determined as the range of calibration points showing good correlation (r^2>0.999). All of three determinations showed good linearity as presented Table 15. The slope and intercept obtained was 3780.82 and -139.74. These data indicate that the method is linear for dissolution test.

The accuracy was established by the recovery of known amounts of drug to product solution. Test solutions were prepared at three different levels, 11.33, 62.33 and 113.33 ㎍/mL. Table 16 presented the accuracy results, determined as percentage recovery and statistical analysis using ANOVA(Stuendt-Newman-Keuls multiple range test). The mean recovery(%) was 100.32 ± 1.02, demonstrating the accuracy of the method. The data showed no significant difference between the results (P<0.05).

The injection precision of the method was evaluated by performing six replicate injections of the standard solution. The peak area and experimental concentration R.S.D.% was below 1% (as shown in Table 17), in accordance with acceptance criteria.

The quantitation limit is the lowest amount of analyte in sample that can be determined with acceptable precision and accuracy under same experimental conditions. The value for presented method was found to be 2.149 ㎍/mL and could be used for analyzing dissolution sample of sobrerol in the capsule formulation.

Table 18 summarizes the validation results of the method, showing that the method is specific, linear, accurate and precise. In the final tests to set the dissolution specification, newly developed HPLC method was used.

3) In vitro dissolution studies

Based on the screening studies, pH 6.8 phosphate buffer was selected as the dissolution medium and paddle rotating at 100 rpm as an apparatus. The capsules of reference drug E-1, E-2, E-3 lots with each 12 specimens per lot were tested. The dissolution profiles of all three lots were found to be similar(Figure 10-1). The percentage of drug released for all three different products were >80% in 60 min. The dissolved amounts of each lots reached 91.6± 6.9(%) in drug E-1, 90.9± 5.2(%) in drug E-2 and 86.2± 9.4(%) in drug E-3. The increase of rotation speed from 50 to 100 rpm could be sufficient to release sobrerol capsules. The profile and amount dissolved in the cross test performed by another laboratory were almost same with our dissolution results (Figure 10-2). Therefore, the specification of dissolution rate in the sobrerol capsules was suggested as 'not less than 70 % in 60 min' which was the value of about 10 % less than the average rate of dissolution. The other product was also tested whether be acceptable to proposed dissolution specification. The dissolution rate of test-F drug was more fast and the average amount dissolved (n=6) was evaluated to be 103.4% at 60 min.

3-5. Setting the dissolution specification of Dilazep Hydrochloride Tablets

1) Dissolution profiles and Sample analysis

The assay of dilazep hydrochloride tablets in the monograph of KP is UV spectrophotometry method and filter material is not specified. However, drug contents of commercial products were different depending on the filter material in the assay test. In this respect, five types of hydrophilic syringe filters were evaluated whether drug dissolved in the dissolution medium is adsorb to filter or not. The test solutions

(3.4㎍/mL) in each dissolution medium were prepared in volumetric flasks and the final solution was analyzed without filtration and filtered with the filters listed above. The pH 6.8 dissolution medium was excluded due to instability of drug. The filtrates were analyzed by proposed HPLC method. Table 19 shows that peak area value(%) was dramatically decreased after filtered using hydrophilic PVDF filter(generally used in dissolution tests). In case of nylon filter, the value was rather increased in buffer medium and the chromatogram of filtrate in distilled water was not detected, indicating that most of drug were adsorbed into the filter. For a filter to be acceptable for use, the results of the filtered portions are within 98-102% of the original concentrations of the unfiltered solution and the centrifuged sample solution [34]. These results suggest that 0.45㎛ hydrophilic PTFE syringe filter(Advantec Toyo, Tokyo, Japan) could be suitable in the dissolution tests. The drug contents of reference G-1, 2, 3 and test H were also analyzed after filtering with hydrophilic PTFE syringe filter and those results were in accordance with the specification. The preliminary experiments to study the dissolution profiles for four different medium (pH 1.2, 4.0, 6.8 and distilled water) were also evaluated after filtering with that.

The dissolution test conditions were studied based on the preliminary test in the 4 types of dissolution medium and the paddle method at 50rpm was applied preferentially. The dissolution profile in the pH 6.8 was not tested, since a change of main drug was observed, resulting in increased impurity peak in HPLC chromatogram with the passage of time (data not shown). Figure 11 presented that the dissolution rate of reference G-1 was very slow, which could be resulted from its preparation, film-coated tablets. When the distilled water was used, only about 80% of dilazep hydrochloride was released in

120 min. At pH 1.2 and pH 4.0, the amount of drug was relatively quickly dissolved but reached only more than 80% of the labeled claim in 90 min. In the initial stage, the dissolution rate was slightly fast in pH 4.0, but pH 1.2 buffer was determined as the dissolution medium. Because the highest solubility was obtained in the filter suitability test above mentioned and also ensured sink conditions. To get the satisfactory dissolution of the drug in pH 1.2 medium, further studies were performed by increasing the rotation to 100 rpm. In those conditions, the dissolution rate of >80% at 60 min was observed. There was no increase in the dissolution rate at 150 rpm. And, the rotation speed may be occasionally set at 100 rpm, but not less than 150 rpm is not recommended in setting the dissolution specification. Based on the screening studies with medium and stirring speed, the final dissolution conditions were determined as Table 5.

2) Development and validation of the HPLC method

The HPLC method used to analyze the dissolution samples was also validated.

The specificity was demonstrated that there was no another peak, generated by excipients, in the retention time of main drug (Figure 12). And sharp and symmetrical peak was obtained with good baseline resolution.

The sample and standard solution in each medium was investigated that chromatogram and peak area would be changed. The degradation product was observed in the chromatogram of dilazep hydrochloride solutions in pH 6.8 buffer after 10 min and this interference peak was increased with time. Therefore, pH 6.8 dissolution medium was excluded in the tests. The solutions in other medium were stable for the course of experiments, at least 36 hr (data not shown).

The linearity was evaluated between analyte concentration and area

of the chromatographic peaks. All three independent determination showed good linearity with good correlation coefficient (r^2 > 0.999). The calibration range was from 1.75 to 70 ㎍/mL. The mean slope and intercept obtained was 74864.2 and -33825.8 as shown Table 20.

The accuracy of the method was studied at three concentration levels, 5.57, 30.65 and 55.73 ㎍/mL. As indicated in Table 21, the average recoveries ranged from 99.4 to 101.8%. These results satisfied the acceptance criteria (98.0~102.0%) and showed no significant difference between the results (P<0.05).

Precision was examined at 100% level of dissolved amounts (55.3 ㎍/mL). The results presented in Table 22 and R.S.D.% value was 0.6% lower than 1%.

The quantitation limit of the method was calculated to be 0.216 ㎍/mL by the standard deviation of the response and the slope.

The validation results of the HPLC method were summarized in Table 23. The developed method has been proven suitable for the determination of dilazep hydrochloride samples obtained in the in vitro dissolution studies.

3) In vitro dissolution studies

According to the dissolution conditions determined by preliminary tests, dissolution profiles of reference drug G-1, G-2, G-3 lots with each 12 specimens per lot were studied. The tablets were tested in 900 mL of pH 1.2 buffer using a paddle at 100 rpm. The dissolution profiles were presented in Figure 13-1 and showed the similar dissolution rate between the drug lots. At 60 min, mean released amounts reached > 80% of the drug contents. However, the results was quite variable. The S.D. (%) value in the dissolved amounts was below 5% at the first time point (5 min) and above 10% after 45 min. These results imply that the dissolution control to ensure lot to lot uniformity in the quality is

required. A difference in dissolution characteristics could lead to a difference in characteristics of final products. The dissolution profiles obtained in the cross test were also similar with our results (Figure 13-2). Based on the results of final and cross tests, the specification of dissolution rate for dilazep hudrochloride tablets was determined as 'not less than 75 % in 60 min' considering the dissolution amounts and standard deviation in those. A different tablet formulation which was produced by different company was tested with same conditions. The dissolution rate of test-H drug was more fast and 101.8% of the labeled claim was dissolved in 60 min. This was appropriate to meet the proposed dissolution specification.

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4. Conclusion

For the establishment of dissolution specification, the dissolution testing methods of piracetam tablets, verapamil hydrobromide tablets, sobrerol capsules and dilazep hydrochloride tablets were developed. The preliminary studies were conducted to select the dissolution apparatus, rotation speed and dissolution medium for the tests. Based on the dissolution conditions determined by the tests, the dissolution profiles of each drug products were investigated and dissolution specifications were determined for ensuring good quality in pharmaceutical industries. A simple and efficient HPLC method was applied to the analysis the dissolution samples of each drug pharmaceuticals. The methods were validated by parameters such as specificity, stability, linearity, accuracy and quantitation limit, which was acceptable to KP guideline. These results suggest that the proposed specification and analysis method could be utilized in the revised version of KPC.

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Table 1. Piracetam Tablets, Fenoterol Hydrobromide Tablets, Sobrerol Capsules and Dilazep Hydrochloride Tablets.

Drug	Product Name	Sample No.	Dosage Form	Labeled Amount
Piracetam Tablets	Reference A	A -1	Film coated tablets	800 mg/ Tab.
		A -2		
		A -3		
	Test B	B		
Fenoterol Hydrobromide Tablets	Reference C	C -1	Uncoated tablets	2.5 mg/Tab.
		C -2		
		C -3		
	Test D	D		
Sobrerol Capsules	Reference E	E -1	Soft Capsules	100 mg/ Cap.
		E -2		
		E -3		
	Test F	F		
Dilazep hydrochloride tablets	Reference G	G -1	Film coated tablets	50 mg/ Tab.
		G -2		
		G -3		
	Test H	H		

Table 2. Conditions of dissolution test for Piracetam Tablets

Apparatus	KP General Tests, Dissolution Test Apparatus II (Paddle)
Dissolution Medium	Water
Medium Volume	900 mL
Sampling time	5, 10, 15, 30, 45, 60 min
Temp.	37 ± 0.5 ℃
Rotation Speed	50 rpm

Table 3. Conditions of dissolution test for Fenoterol Hydrobromide Tablets

Apparatus	KP General Tests, Dissolution Test Apparatus II (Paddle)
Dissolution Medium	Water
Medium Volume	900 mL
Sampling time	5, 10, 15, 30, 45 min
Temp.	37 ± 0.5 ℃
Rotation Speed	50 rpm

Table 4. Conditions of dissolution test for Sobrerol Capsules

Apparatus	KP General Tests, Dissolution Test Apparatus II(Paddle)
Dissolution Medium	pH 6.8
Medium Volume	900 mL
Sampling time	5, 10, 15, 30, 45, 60, 90, 120 min
Temp.	37 ± 0.5 ℃
Rotation Speed	100 rpm

Table 5. Conditions of dissolution test for Dilazep Hydrochloride Tablets

Apparatus	KP General Tests, Dissolution Test Apparatus II (Paddle)
Dissolution Medium	pH 1.2
Medium Volume	900 mL
Sampling time	5, 10, 15, 30, 45, 60, 90, 120 min
Temp.	37 ± 0.5 ℃
Rotation Speed	100 rpm

Table 6. Mobile phase of Piracetam Tablets in HPLC methods

.

Mobile phase	Dissolution medium, pH 1.2 - 0.01 % $HClO_4$: Acetonitrile (90 : 10) Dissolution medium, pH 4.0 and pH 6.8 - pH 5.8 phosphate buffer solution : Acetonitrile (90 : 10) Dissolution medium, distilled water - 10% methanol pH 6.5 solution adjusted with 0.001mol/L $(NH_4)_2HPO_4$

Table 7. Results of regression analysis of the linearity data of Piracetam.

Standard solution	Concentration (μg/mL)	Actual* Concentration (μg/mL)	Peak Area	Regression Equation
I	5.31	5.31	160924	y=31074.5x + 10313 r^2 = 0.9996
II	15.93	15.93	519867	
III	26.55	26.55	843288	
IV	37.17	37.17	1161376	
V	53.1	53.1	1656261	
I	5.33	5.33	168368	y=30733.1x + 10523.4 r^2 = 0.9999
II	15.99	15.99	504440	
III	26.65	26.65	839158	
IV	37.31	37.31	1153242	
V	53.3	53.3	1646396	
I	5.32	5.32	159390	y=31073.3x + 2910.6 r^2 = 0.9998
II	15.96	15.96	500599	
III	26.6	26.6	843711	
IV	37.24	37.24	1158716	
V	53.2	53.2	1650194	

Mean of Slope	30960.29	y=30960.29x + 7915.66 (y:peak area x:concentration)
Mean of Intercept	7915.66	
SD of Slope	196.79	
SD of Intercept	4335.75	

*Actual concentration(μg/mL) = Concentration(μg/mL) × standard purity(%/100)

Table 8. Results of accuracy determination of Piracetam.

Standard solution	Theoretical concentration(㎍/mL)	Peak Area	Experimental concentration(㎍/mL)	Recovery(%)
1-Ⅰ	17.77	560061	17.83	100.4
2-Ⅰ	31.09	983889	31.52	101.4
3-Ⅰ	44.42	1404981	45.12	101.6
1-Ⅱ	17.77	559511	17.82	100.3
2-Ⅱ	31.09	975377	31.25	100.5
3-Ⅱ	44.42	1388578	44.59	100.4
1-Ⅲ	17.77	550778	17.53	98.7
2-Ⅲ	31.09	984690	31.55	101.5
3-Ⅲ	44.42	1391877	44.70	100.6

Average Recovery(%)	100.59
Average R.S.D(%)	0.888
Sample Size	9
95% Confidence Interval	99.90 ~ 101.27

Table 9. Precision data for Piracetam.

Theoretical concentration (㎍/mL)	Number of replicates	Experimental concentration (㎍/mL)	Average	S.D	R.S.D(%)
46.2	1	46.5	46.6	0.277	0.595
	2	46.2			
	3	46.4			
	4	46.9			
	5	46.9			
	6	46.7			

Table 10. Summarize of validation studies of Piracetam.

solvent (dissolution medium)	Validation Parameters	Criteria	Results
Distilled water	specificity	lack of interference chromatographic peak in drug	Not detected
	linearity	r^2>0.999	r^2>0.999
	accuracy (n = 9)	Recovery(%) 98.0-102.0%	100.59 ± 0.888
	precision (n = 6)	R.S.D < 1 %	R.S.D = 0.60%
	quantitation limit	-	1.400 ㎍/mL

Table 11. Results of regression analysis of the linearity data of Fenoterol Hydrobromide.

Standard solution	Concentration (㎍/mL)	Actual* Concentration (㎍/mL)	Peak Area	Regression Equation
I	0.66	0.66	133966	y=222694.8x - 19533.2 r^2 = 0.9993
II	1.32	1.32	266990	
III	1.98	1.98	421943	
IV	2.64	2.64	563520	
V	3.3	3.3	720594	
I	0.66	0.66	133805	y=209324.5x - 1960 r^2 = 0.9998
II	1.32	1.32	273892	
III	1.98	1.98	418304	
IV	2.64	2.64	549980	
V	3.3	3.3	686532	
I	0.66	0.66	133192	y=213229.2x - 12297.3 r^2 = 0.9995
II	1.32	1.32	265709	
III	1.98	1.98	403305	
IV	2.64	2.64	555148	
V	3.3	3.3	692129	

Mean of Slope	215082.88	y=215082.88x - 11263.50 (y:peak area x:concentration)
Mean of Intercept	-11263.50	
SD of Slope	6875.19	
SD of Intercept	8832.09	

*Actual concentration(㎍/mL) = Concentration(㎍/mL) × standard purity(%/100)

Table 12. Results of accuracy determination of Fenoterol Hydrobromide.

Standard solution	Theoretical concentration(㎍/mL)	Peak Area	Experimental concentration(㎍/mL)	Recovery(%)
1-Ⅰ	1.68	347856	1.67	99.4
2-Ⅰ	2.23	468790	2.23	100.1
3-Ⅰ	2.79	582453	2.76	98.9
1-Ⅱ	1.68	349062	1.68	99.7
2-Ⅱ	2.23	475124	2.26	101.4
3-Ⅱ	2.79	599001	2.84	101.7
1-Ⅲ	1.68	353583	1.70	101.0
2-Ⅲ	2.23	468933	2.23	100.1
3-Ⅲ	2.79	585319	2.77	99.4

Average Recovery(%)	100.19
Average R.S.D(%)	0.962
Sample Size	9
95% Confidence Interval	99.45 ~ 100.93

Table 13. Precision data for Fenoterol Hydrobromide.

Theoretical concentration (㎍/mL)	Number of replicates	Experimental concentration (㎍/mL)	Average	S.D	R.S.D(%)
2.8	1	2.74	2.75	0.017	0.628
	2	2.77			
	3	2.74			
	4	2.76			
	5	2.73			
	6	2.74			

Table 14. Summarize of validation studies of Fenoterol Hydrobromide.

solvent (dissolution medium)	Validation Parameters	Criteria	Results
Distilled water	specificity	lack of interference chromatographic peak in drug	Not detected
	linearity	r^2>0.999	r^2>0.999
	accuracy (n = 9)	Recovery(%) 98.0-102.0%	100.19 ± 0.962
	precision (n = 6)	R.S.D < 1 %	R.S.D = 0.63%
	quantitation limit	-	0.411 ㎍/mL

Table 15. Results of regression analysis of the linearity data of Sobrerol Capsules.

Standard solution	Concentration (㎍/mL)	Actual* Concentration (㎍/mL)	Peak Area	Regression Equation
I	5	5	18781	y = 377.5x - 346.9 r^2 = 0.9998
II	30	30	114109	
III	70	70	260350	
IV	110	110	419217	
V	130	130	489054	
I	5	5	18867	y = 3765.5x - 756.18 r^2 = 0.9991
II	30	30	113088	
III	70	70	269887	
IV	110	110	405961	
V	130	130	495070	
I	5	5	18858	y = 3799.5x - 828.5 r^2 = 1.0000
II	30	30	111781	
III	70	70	266489	
IV	110	110	415607	
V	130	130	493934	

Mean of Slope	3780.82	y=3780.82x - 139.74 (y:peak area x:concentration)
Mean of Intercept	-139.74	
SD of Slope	17.22	
SD of Intercept	812.40	

*Actual concentration(㎍/mL) = Concentration(㎍/mL) × standard purity(%/100)

Table 16. Results of accuracy determination of Sobrerol Capsules.

Standard solution	Theoretical concentration(㎍/mL)	Peak Area	Experimental concentration(㎍/mL)	Recovery(%)
1-Ⅰ	11.33	42883	11.38	100.4
2-Ⅰ	62.33	238945	63.24	101.5
3-Ⅰ	113.33	428601	113.40	100.0
1-Ⅱ	11.33	43090	11.43	100.9
2-Ⅱ	62.33	236524	62.60	100.4
3-Ⅱ	113.33	432492	114.43	101.0
1-Ⅲ	11.33	42392	11.25	99.3
2-Ⅲ	62.33	238149	63.03	101.1
3-Ⅲ	113.33	420616	111.29	98.2

Average Recovery(%)	100.32
Average R.S.D(%)	1.02
Sample Size	9
95% Confidence Interval	99.53-101.10

Table 17. Precision data for Sobrerol Capsules.

Theoretical concentration (㎍/mL)	Number of replicates	Experimental concentration (㎍/mL)	Average	S.D	R.S.D(%)
110	1	109.2	109.4	0.683	0.625
	2	108.8			
	3	109.9			
	4	110.5			
	5	108.8			
	6	109.3			

Table 18. Summarize of validation studies of Sobrerol Capsules.

solvent (dissolution medium)	Validation Parameters	Criteria	Results
pH 6.8	specificity	lack of interference chromatographic peak in drug	Not detected
	linearity	r^2>0.999	r^2>0.999
	accuracy (n = 9)	Recovery(%) 98.0-102.0%	100.3 ± 1.02
	precision (n = 6)	R.S.D < 1 %	R.S.D = 0.6%
	quantitation limit	-	2.149 ㎍/mL

Table 19. Filter adsorption results of dilazep hydrochloride

dissolution medium	diameter(mm): 25 pore size (㎛): 0.45						
	filter material	centrifugation	PVDF	PTFE	mixed celluose acetate	celluose acetate	nylon
	peak area value (%)						
pH 1.2		100	32.9	98.7	79.7	79.7	107.3
pH 4.0		100	82.3	98.3	46.9	46.9	103.0
distilled water		100	59.0	101.4	32.6	32.6	0

* peak area value(%) : peak area after filtering / peak area after centrifugation(100)× 100

Table 20. Results of regression analysis of the linearity data of Dilazep Hydrochloride Tablets.

Standard solution	Concentration (㎍/mL)	Actual* Concentration (㎍/mL)	Peak Area	Regression Equation
I	1.75	1.70	86413	
II	3.5	3.4	215060	
III	17.5	17.0	1247352	y=74701.55x - 31999.17
IV	35	34.1	2506053	r^2 = 0.9995
V	56	54.5	4120655	
VI	70	68.2	5001995	
I	1.75	1.70	89062	
II	3.5	3.4	218781	
III	17.5	17.0	1250924	y=74863.33x - 34417.24
IV	35	34.1	2487273	r^2 = 0.9996
V	56	54.5	4119732	
VI	70	68.2	5026201	
I	1.75	1.70	89142	
II	3.5	3.4	219923	
III	17.5	17.0	1248803	y=75027.77x - 35060.85
IV	35	34.1	2496368	r^2 = 0.9997
V	56	54.5	4125797	
VI	70	68.2	5037509	

Mean of Slope	74864.2	
Mean of Intercept	-33825.8	y=74864.2x - 33825.6
SD of Slope	163.1	(y:peak area x:concentration)
SD of Intercept	1614.3	

*Actual concentration(㎍/mL) = Concentration(㎍/mL) × standard purity(%/100)

Table 21. Results of accuracy determination of Dilazep Hydrochloride Tablets.

Standard solution	Theoretical concentration(㎍/mL)	Peak Area	Experimental concentration(㎍/mL)	Recovery(%)
1-Ⅰ	5.57	381394	5.55	99.5
2-Ⅰ	30.65	2278983	30.89	100.8
3-Ⅰ	55.73	4122192	55.51	99.6
1-Ⅱ	5.57	380970	5.54	99.4
2-Ⅱ	30.65	2260904	30.65	100.0
3-Ⅱ	55.73	4134810	55.68	99.9
1-Ⅲ	5.57	385225	5.60	100.4
2-Ⅲ	30.65	2301245	31.19	101.8
3-Ⅲ	55.73	4166388	56.10	100.7

Average Recovery(%)	100.23
Average R.S.D(%)	0.757
Sample Size	9
95% Confidence Interval	99.65 ~ 100.81

Table 22. Precision data for Dilazep Hydrochloride Tablets.

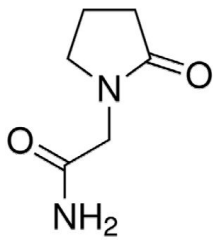
Theoretical concentration (㎍/mL)	Number of replicates	Experimental concentration (㎍/mL)	Average	S.D	R.S.D(%)
55.3	1	55.4	55.8	0.341	0.611
	2	55.6			
	3	56.2			
	4	55.5			
	5	56.1			
	6	56.1			

Table 23. Summarize of validation studies of Dilazep Hydrochloride Tablets.

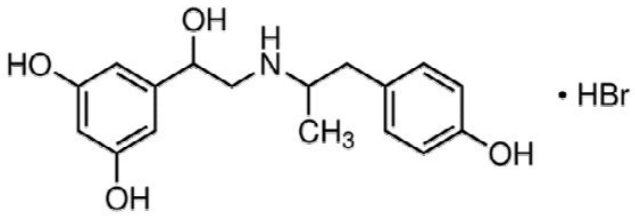
solvent (dissolution medium)	Validation Parameters	Criteria	Results
pH 1.2	specificity	lack of interference chromatographic peak in drug	Not detected
	linearity	r^2>0.999	r^2>0.999
	accuracy (n = 9)	Recovery(%) 98.0-102.0%	100.2 ± 0.76
	precision (n = 6)	R.S.D < 1 %	R.S.D = 0.6%
	quantitation limit	-	0.216 ㎍/mL

Figure 1-(a) Structure of Piracetam, (b) Structure of Fenoterol Hydrobromide (c) Structure of Sobrerol (d) Structure of Dilazep hydrochloride.

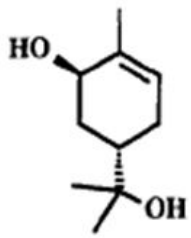
(a)



(b)



(c)



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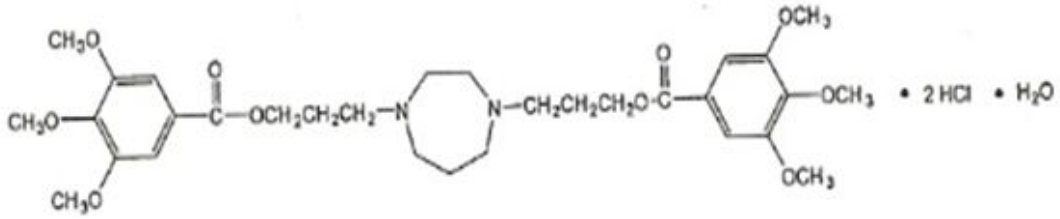


Figure 2. Dissolution profiles of reference drug of Piracetam tablets A-1.

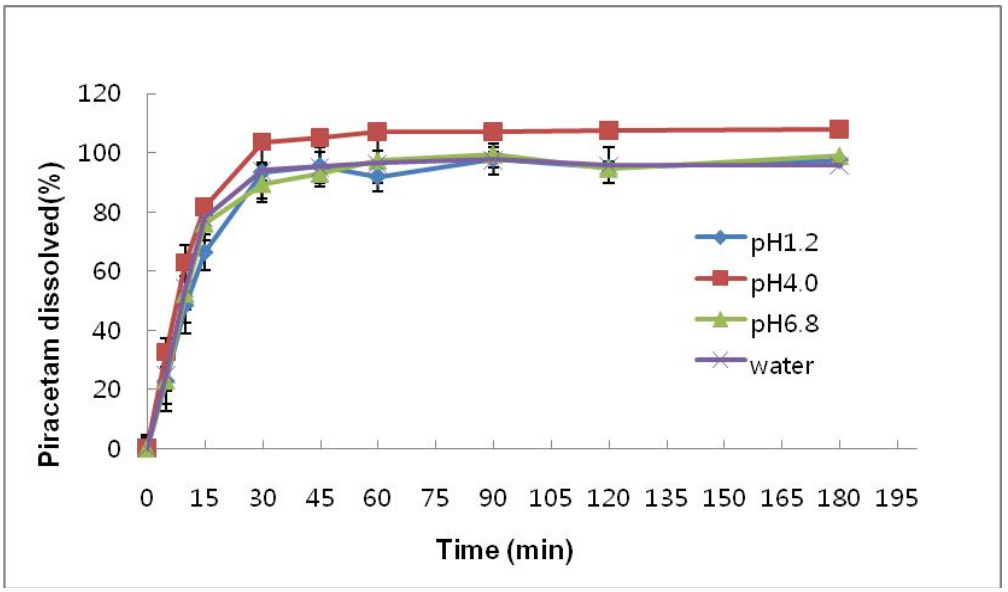
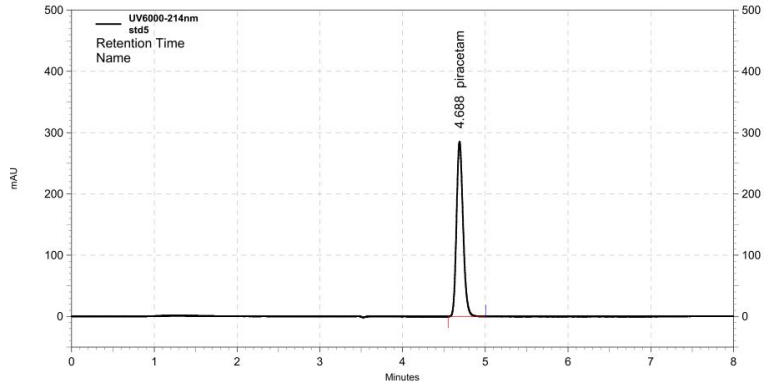
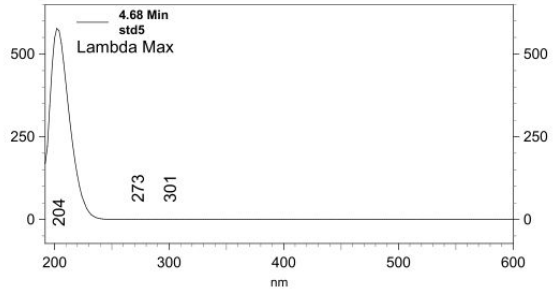


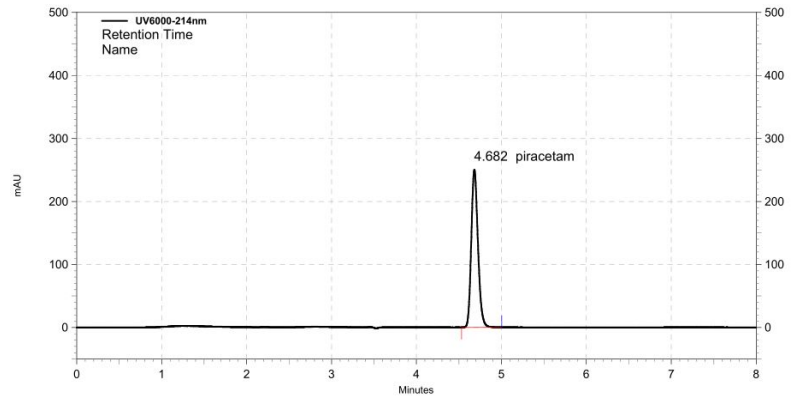
Figure 3. (a) Chromatogram and PDA scan of standard solution; Piracetam, (b) Chromatogram and PDA scan of sample solution; Piracetam

(a)





(b)



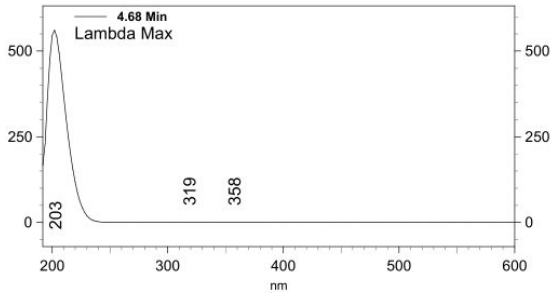

4.68 Min
Lambda Max
500
250
0
203
319
358
200
300
400
500
600
nm

Figure 4-1. Mean dissolution profiles of reference drug of Piracetam tablets A-1, A-2 and A-3(n=12).

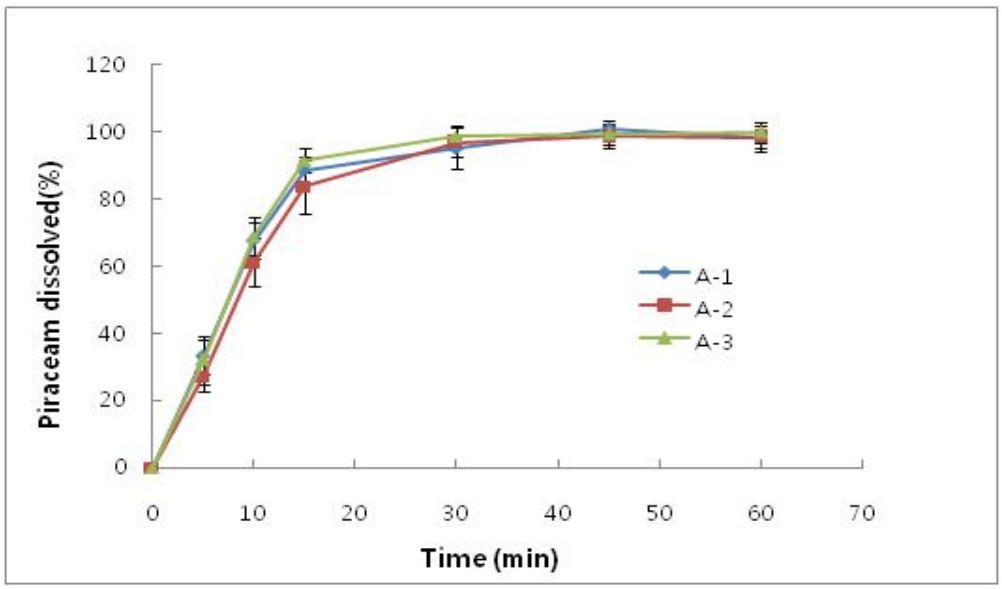


Figure 4-2. Cross test results: Mean dissolution profiles of reference drug of Piracetam tablets A-1, A-2 and A-3(n=12).

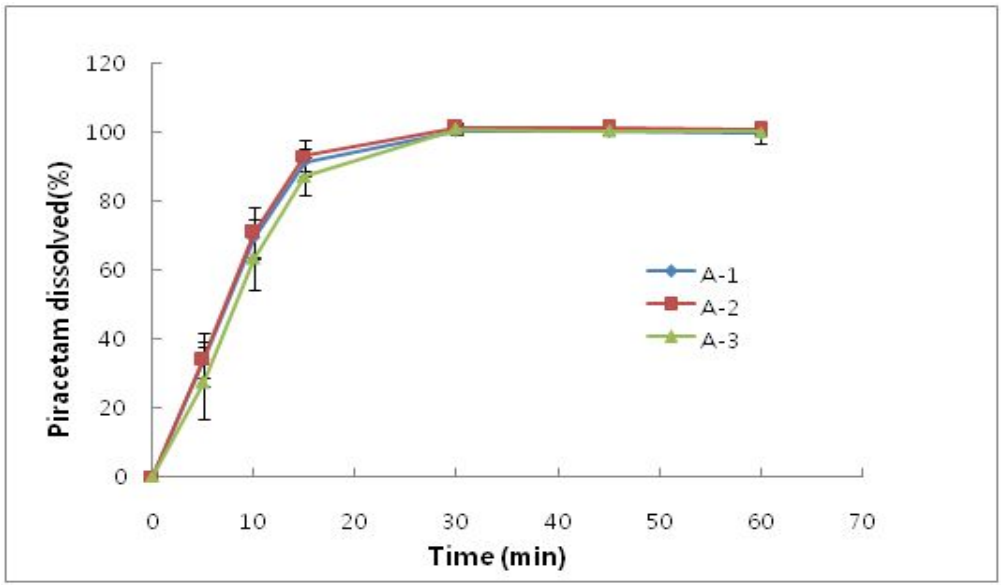


Figure 5. Dissolution profiles of reference drug of Fenoterol Hydrobromide(HBr) tablets C-1.

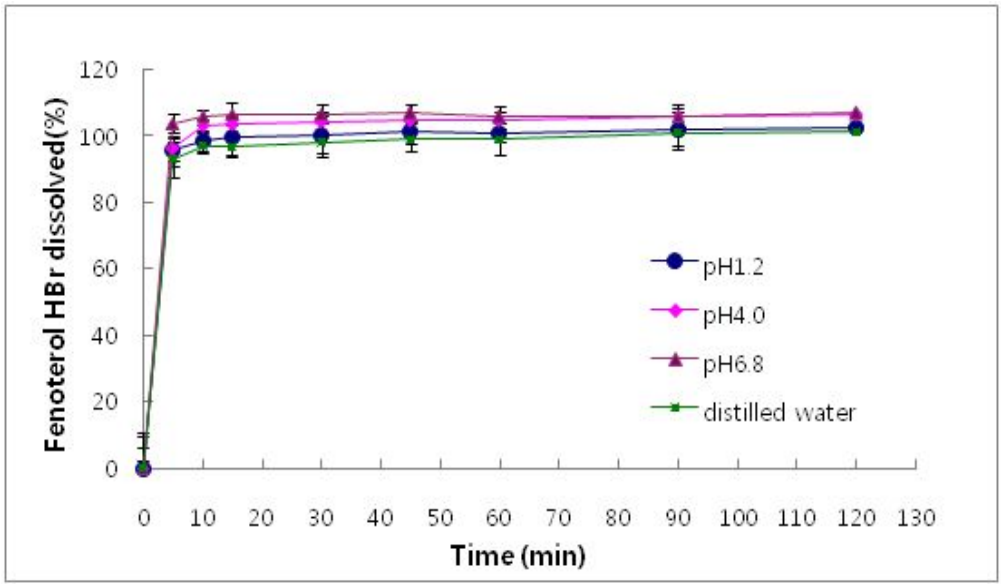
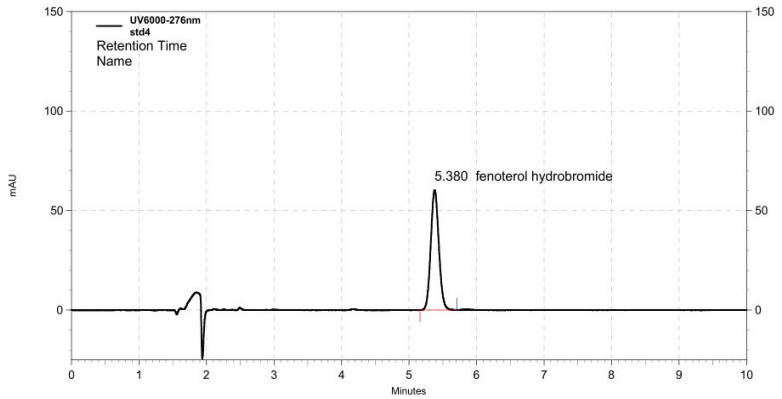
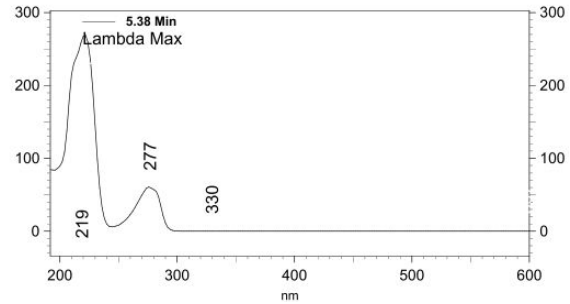


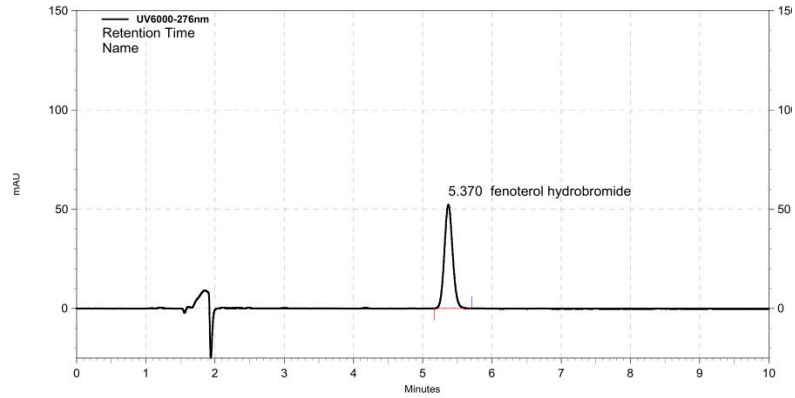
Figure 6. (a) Chromatogram and PDA scan of standard solution; Fenoterol Hydrobromide, (b) Chromatogram and PDA scan of sample solution; Fenoterol Hydrobromide

(a)





(b)



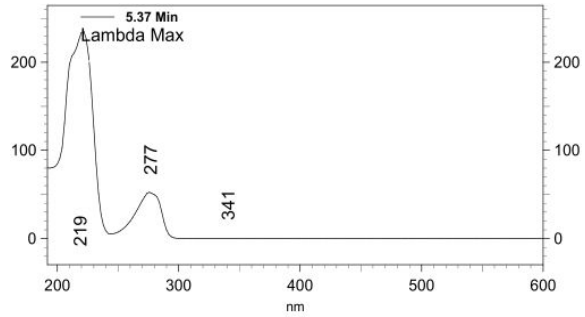

5.37 Min
Lambda Max
200
100
0
219
277
341
200
300
400
500
600
nm

Figure 7-1. Mean dissolution profiles of reference drug of Fenoterol Hydrobromide(HBr) tablets C-1, C-2 and C-3(n=12).

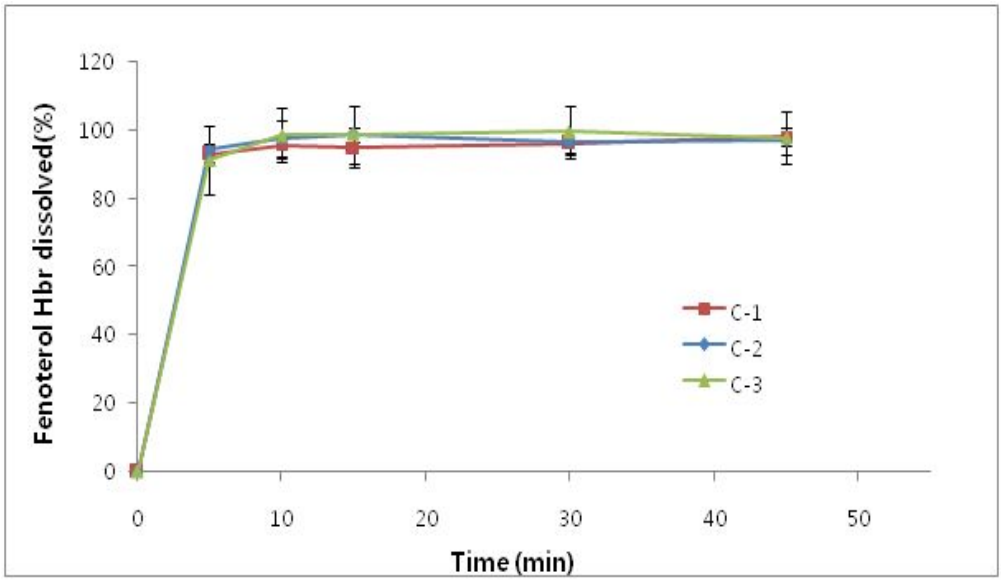


Figure 7-2. Cross test results: Mean dissolution profiles of reference drug of Fenoterol Hydrobromide(HBr) tablets C-1, C-2 and C-3(n=12).

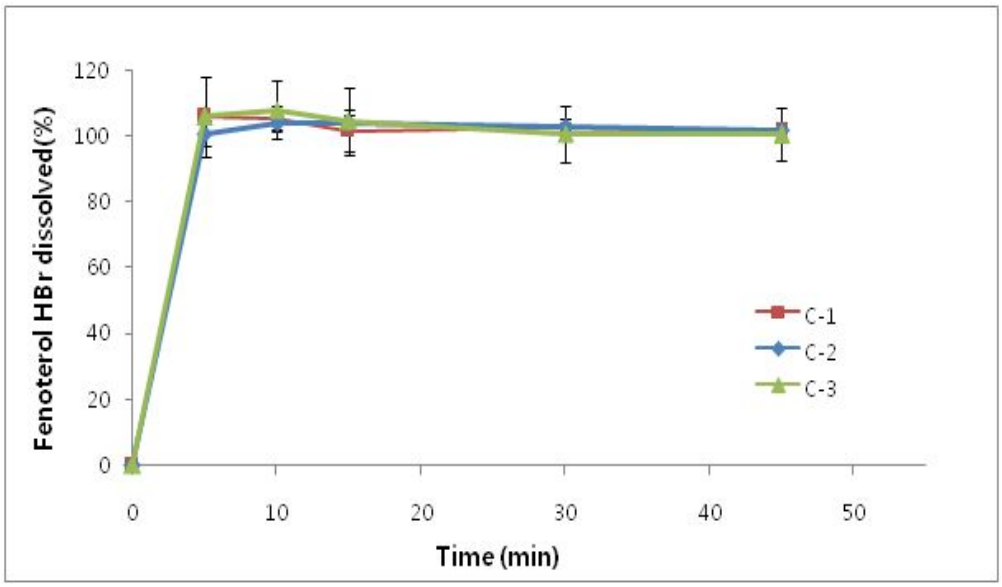


Figure 8. Dissolution profiles of reference drug of Sobrerol Capsules E-1.

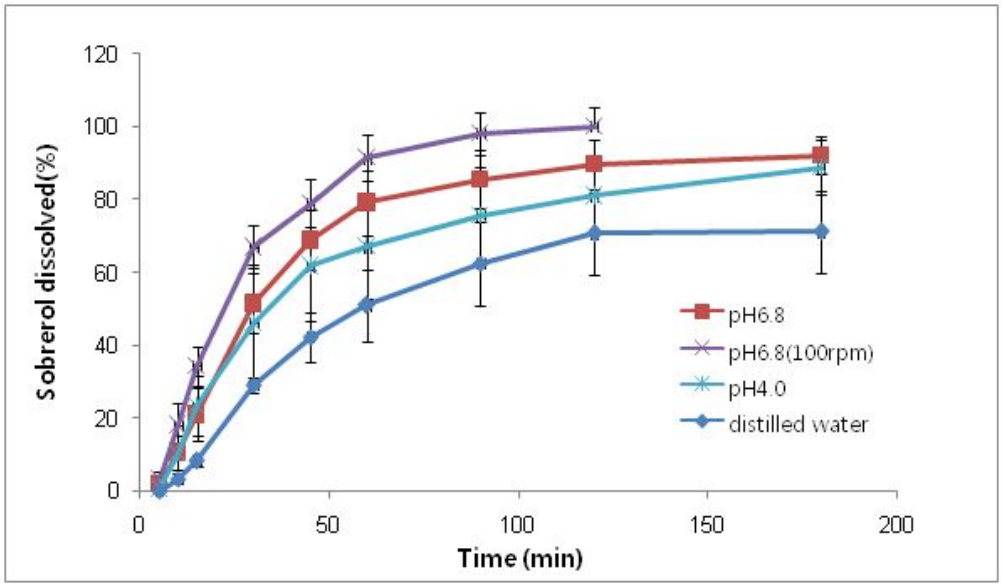
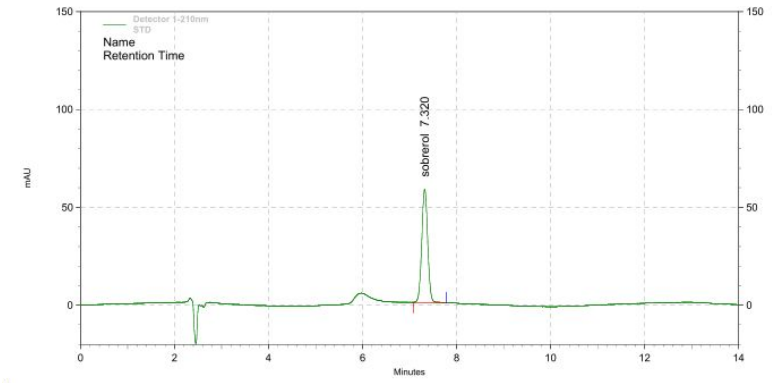
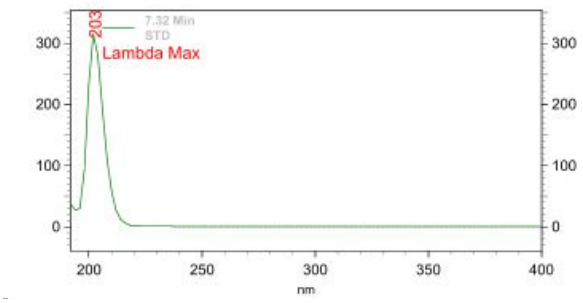


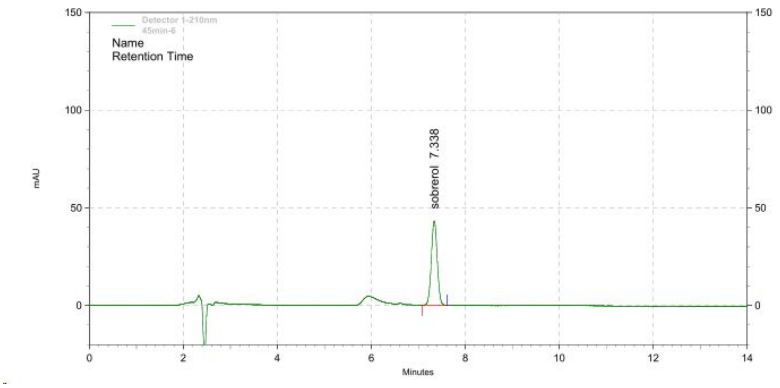
Figure 9. (a) Chromatogram and PDA scan of standard solution; Sobrerol, (b) Chromatogram and PDA scan of sample solution; Sobrerol

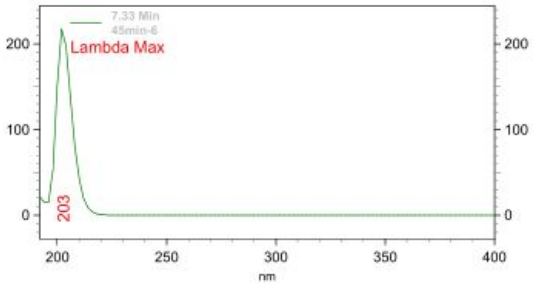
(a)





(b)




7.33 Min
45min-6
Lambda Max
203
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100
0
200
250
300
350
400
nm

Figure 10-1. Mean dissolution profiles of reference drug of Sobrerol Capsules E-1, E-2 and E-3(n=12).

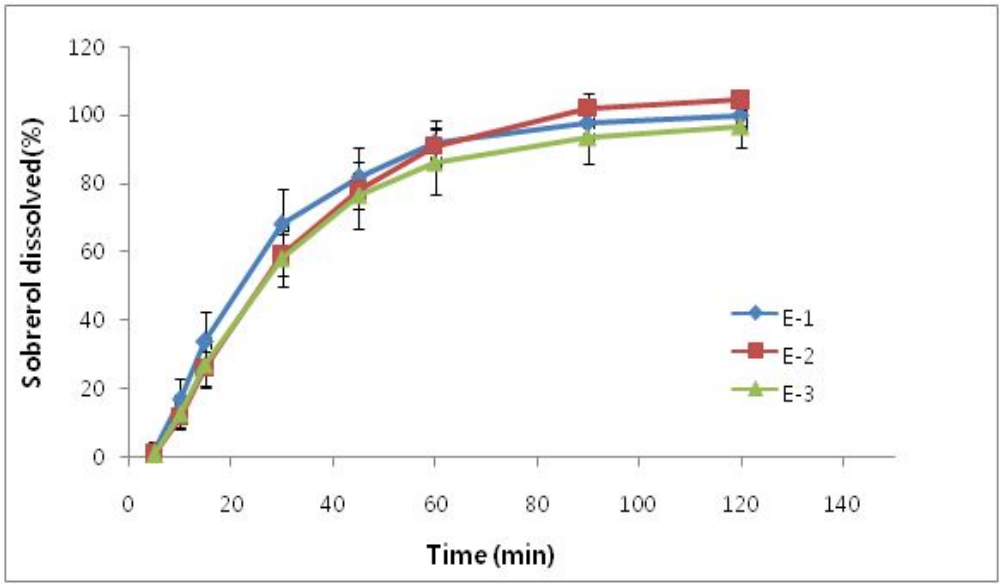


Figure 10-2. Cross test results: Mean dissolution profiles of reference drug of Sobrerol Capsules E-1, E-2 and E-3(n=12).

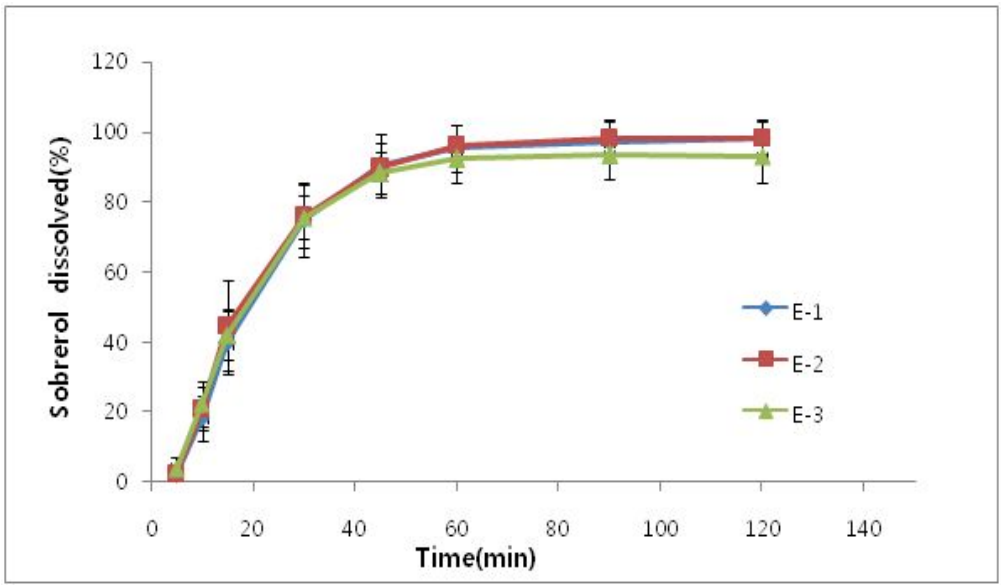


Figure 11. Dissolution profiles of reference drug of Dilazep Hydrochloride Tablets G-1.

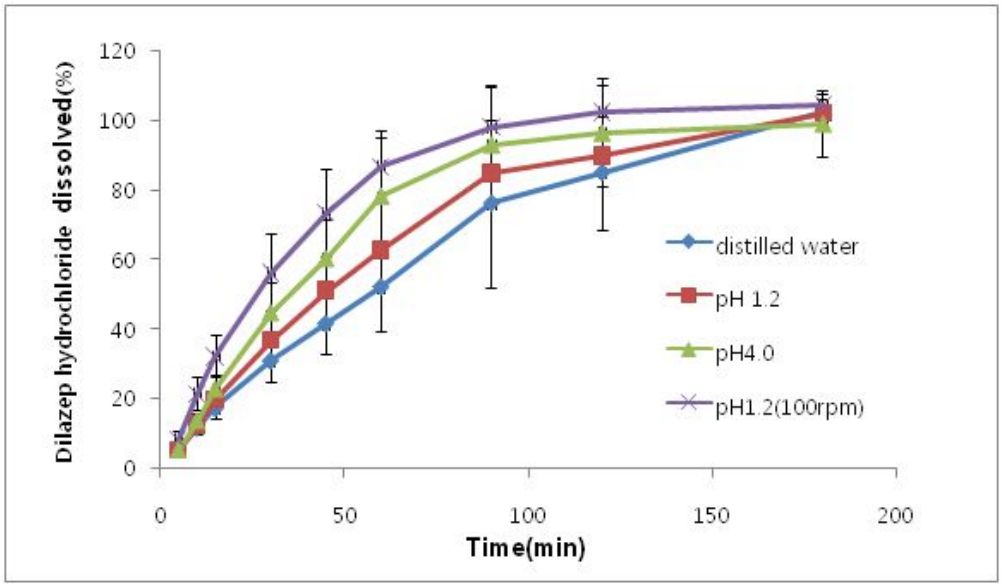
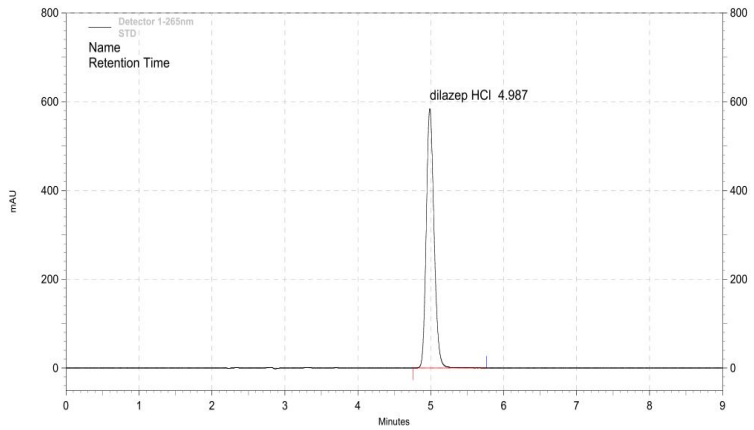
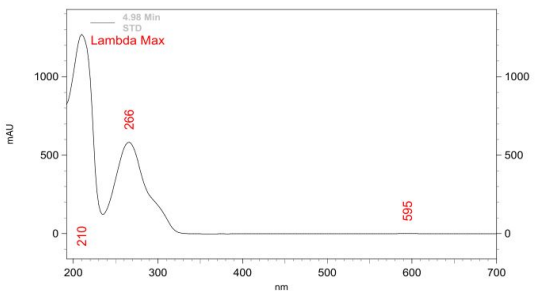


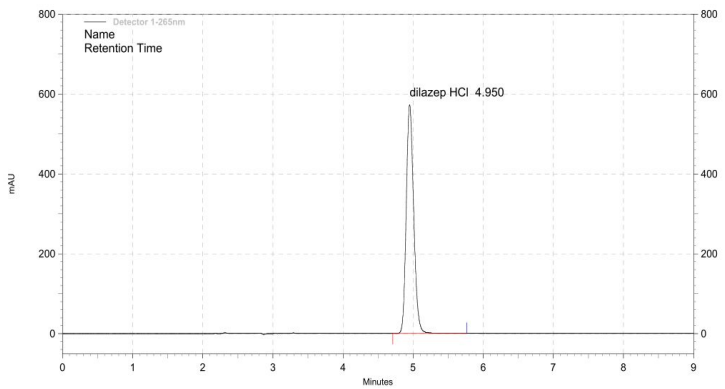
Figure 12. (a) Chromatogram and PDA scan of standard solution; Dilazep Hydrochloride, (b) Chromatogram and PDA scan of sample solution; Dilazep Hydrochloride

(a)





(b)



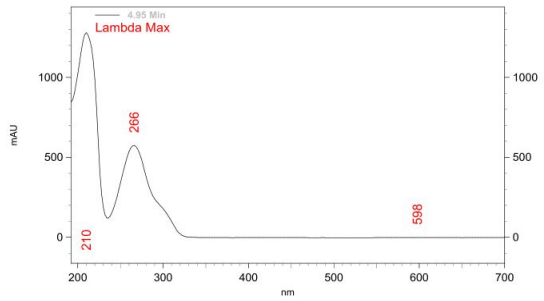

4.95 Min
Lambda Max
1000
500
0
mAU
266
210
598
200
300
400
500
600
700
nm

Figure 13-1. Mean dissolution profiles of reference drug of Dilazep Hydrochloride Tablets G-1, G-2 and G-3(n=12).

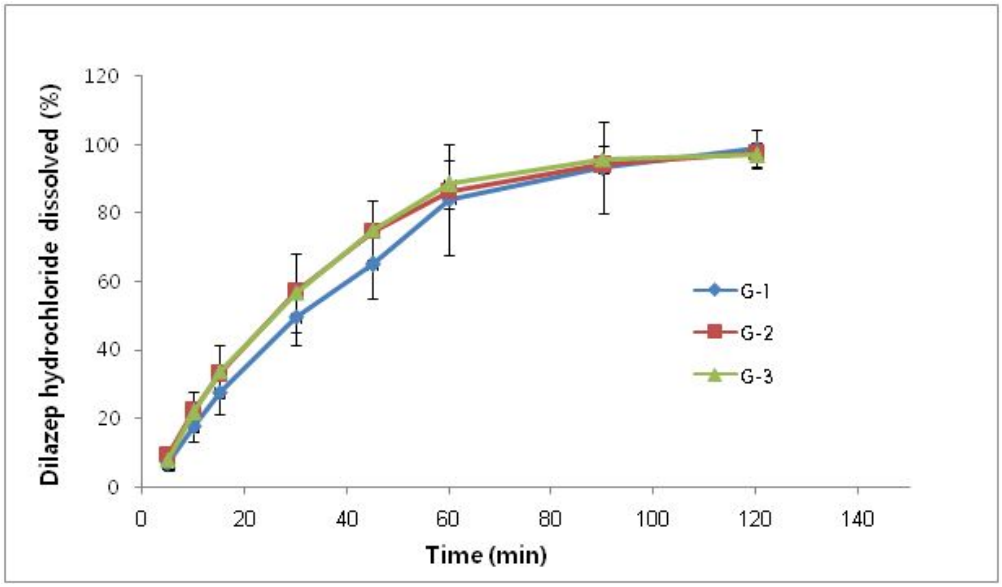
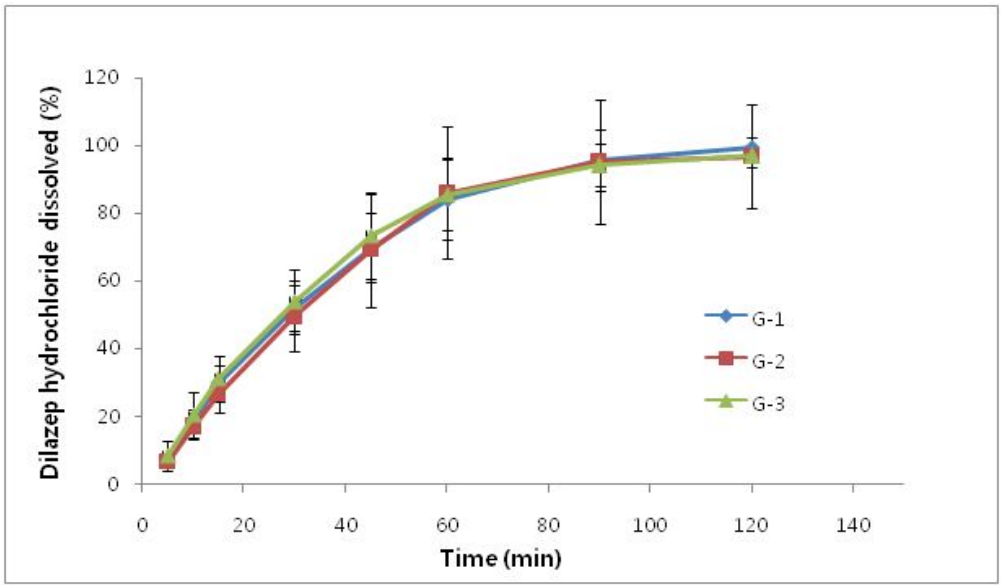


Figure 13-2. Cross test results: Mean dissolution profiles of reference drug of Dilazep Hydrochloride Tablets G-1, G-2 and G-3(n=12).



Acknowledgements

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논문제목	한글: 고시수재 의약품 중 용출규격 미설정 제제의 용출규격 설정에 관한 연구				
	영문: Development of Dissolution testing method for Piracetam Tablets, Fenoterol Hydrobromide Tablets, Sobrerol capsules and Dilazep Hydrochloride Tablets in Korean Pharmaceutical Codex				

본인이 저작한 위의 저작물에 대하여 다음과 같은 조건 아래 조선대학교가 저작물을 이용할 수 있도록 허락하고 동의합니다.

- 다 음 -

1. 저작물의 DB구축 및 인터넷을 포함한 정보통신망에의 공개를 위한 저작물의 복제, 기억장치에의 저장, 전송 등을 허락함.
2. 위의 목적을 위하여 필요한 범위 내에서의 편집과 형식상의 변경을 허락함. 다만, 저작물의 내용변경은 금지함.
3. 배포·전송된 저작물의 영리적 목적을 위한 복제, 저장, 전송 등은 금지함.
4. 저작물에 대한 이용기간은 5년으로 하고, 기간종료 3개월 이내에 별도의 의사 표시가 없을 경우에는 저작물의 이용기간을 계속 연장함.
5. 해당 저작물의 저작권을 타인에게 양도하거나 출판을 허락을 하였을 경우에는 1개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물 이용의 허락 이후 해당 저작물로 인하여 발생하는 타인에 의한 권리 침해에 대하여 일체의 법적 책임을 지지 않음.
7. 소속 대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의 전송·출력을 허락함.

동의여부 : 동의(○) 반대( )

2013년 2월

저작자: 김 은 정 (인)

조선대학교 총장 귀하