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2008年　2月

博士學位論文

Development and validation of a new HPLC method for the determination of hexoprenaline sulfate and diphenidol hydrochloride in pharmaceutical formulations

朝 鮮 大 學 校　大 學 院

食品医藥學科

鄭　　賢　　我

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指 導 敎 授　崔　厚　均

이 論文을 藥學博士 學位申請論文으로 提出함

2008年　2月　日

朝 鮮 大 學 校　大 學 院

食 品 医 藥 學 科

鄭　　賢　　我

鄭賢我의 博士學位論文을 認准함.

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委　員 釜山食藥廳 硏究官 高榕奭 印

委　員 朝鮮大學校 敎授 崔厚均 印

2007年　12月　日

朝鮮大學校 大學院

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CHAPTER I: Development and validation of a new HPLC method for the determination of hexoprenaline sulfate in pharmaceutical formulations.

CHAPTER II: Development and validation of a HPLC-UV method for the determination of diphenidol hydrochloride in tablets.

(국문초록)

HPLC를 이용한 제제 중 황산헥소프레날린 또는 염산디페니돌의 분석법 개발 및 밸리데이션에 관한 연구

정현아

조선대학교 대학원 식품의약학과

지도 교수 : 최후균

제제 중 황산헥소프레날린을 정량할 수 있는 간단하고 빠른 HPLC 분석법을 개발하고 밸리데이션을 수행하였다. 지금까지 공식적으로 발표된 분석법은 흡광광도법 및 형광광도법을 이용한 것이며 매우 복잡하고 유해시약이 많이 사용되며 시간이 소비되는 방법들이었다. 본 연구에서 개발한 새로운 HPLC법은 고정상으로 C18 Capcellpack MG column (150mm×4.6mm, 5㎛), 이동상으로는 heptanesulfonic acid sodium salt를 0.1% 첨가한 0.025mol/L KH_2PO_4(인산으로 pH를 3.0으로 조정)-아세토니트릴 (90:20, v/v), 유속은 1mL/min이다. UV 검출은 280nm에서, 컬럼온도는 40℃를 유지하였다. 본 방법은 시스템적합성, 특이성, 직선성, 정밀성, 정확성과 같은 밸리데이션 파라미터를 이용하여 그 적정성을 평가하였다. 개발된 방법은 제제 중 황산헥소프레날린의 함량측정에 적용되어 품질관리에 기여할 수 있을 것으로 생각된다.

또한 2장에서는 정제 중 염산디페니돌을 분석할 수 있는 HPLC 방법을 개발하고 밸리데이션과정을 수행한 결과를 기술하였다. HPLC법을 이용한 염산디페니돌의 분석방법에 대해 연구되어온 것은 생물학적시료(혈장)에 대한 것으로서 제제 중 염산디페니돌의 분석법에 관한 것은 흡광광도법이나 모세전기영동법 등 전처리가 복잡하고 사용법이 까다로운 것들이다. 본 방법에서는 고정상으로 C18 Capcellpack MG column (150mm×4.6mm, 5㎛), 이동상으로는 0.3% triethylamine(인산으로 pH를 3.0으로 조

정) ·Acetonitrile (70 : 30)을 사용하였다.217nm에서 UV 검출이 이루어 졌고 컬럼온도는 40℃를 유지하였다. 본 방법 역시 시스템적합성, 특이성, 직선성, 정밀성, 정확성과 같은 밸리데이션 파라미터를 이용하여 그 적정성을 평가하였다. 모든 파라미터들이 적합범위안의 값에 포함되었다.

CHAPTER I

DEVELOPMENT AND VALIDATION OF A NEW HPLC METHOD FOR THE DETERMINATION OF HEXOPRENALINE SULFATE IN PHARMACEUTICAL FORMULATIONS

Abstract

A simple, rapid high performance liquid chromatographic method involving ultraviolet detection was developed for the determination of hexoprenaline sulfate in pharmaceutical dosage forms. Chromatography was conducted on Capcellpak C18 MG (150mm×4.6mm, 5㎛)column using 0.025mol/L KH_2PO_4 solution containing 0.1% of heptanesulfonic acid sodium salt-Acetonitrile(90:20, v/v) adjusted to pH 3.0 with phosphoric acid as mobile phase at a flow rate of 1mL/min and a 280nm detection. Validation parameters as system suitability, specificity, linearity, precision and accuracy were determined. The method can be used for hexoprenaline sulfate assay of hexoprenaline sulfate injections or tablets.

I-1 Introduction

The formula for Hexoprenaline sulfate(HPS)is $C_{22}H_{32}N_2O_6{\cdot}H_2SO_4$ also called Bronalin, Delaprem, Etoscol, Gynipral and Ipradol.[1](Fig.1) Hexoprenaline sulfate is an β-adrenergic agent and used as a long acting bronchodilator for the treatment of bronchospastic disorders.[2,3]

There are few official or analytical methods for the estimation of HPS in pharmaceutical formulation. Traveset et al.(1981) used fluorescence derivatization for the determination of HPS in commercial formulas. The method involves the HPTLC fluorescence quantification of the dansyl derivative of hexoprenaline.[4] Korea Pharmaceutical Codex proposed an UV spectrophotometric assay based on iodometry or a fluorometric assay for the detection of HPS in tablet or injection. These methods are complicated and time consuming compared to a simple HPLC method. [5]

The purpose of the present work is to develop and validate a new and simple liquid chromatography method with UV detection for quantitative analysis of HPS, which can be taken in the form of a tablet or by injection. The proposed method included several advantages including simple sample treatment with sonication, dilution, brief analysis, precision and high recovery. In order to ensure applicability of the study to other relevant areas of research, guidelines set by the International Conference on Harmonization(ICH)[6] were used.

I-2 Materials and methods

I-2-1 Materials

Hexoprenaline sulfate was a gift from IL-YANG Pharm. (Seoul, South Korea). HPLC grade acetonitrile was purchased from Merck(Darmstadt, Germany), Potassium phosphate monobasic and sodium heptanesulfonate were obtained from Sigma-Aldrich (MO, USA). E-Piradol® inj. and E-Piradol® tablets containing 0.5mg HPS, commercially available in Korea, were purchased through a local pharmacy. 2.5㎍ HPS per mL was contained in injection. HPLC grade deionized water(NanoPure Diamond, Barnstead Thermolyne, USA) was used throughout the analysis. All other chemicals were of reagent grade or above and were used without further purification.

I-2-2 Apparatus and chromatographic conditions

The development of the method and validation work were performed on a Nanospace SI-2 HPLC system(Shiseido, Tokyo, Japan) as well as with a series 3017 PDA detector, series 3001 pump, a series 3023 automatic injector and a series 3004 column oven. Chromatographic separation was carried out at 40℃ with Capcellpak C18 MG (150mm×4.6mm, 5㎛)column from Shiseido(Japan). For the mobile phase, 0.025mol/L KH_2PO_4 solution containing 0.1% of heptanesulfonic acid sodium salt was prepared and then mixed with acetonitrile [90:20(v/v)]. The pH was adjusted to 3.0 with phosphoric acid. The rate of the mobile phase

flow was 1.0mL/min and the injection volume was 10㎕ for tablets and 150㎕ for injections. UV detection was performed at 280nm.

I-2-3 Stock and working standard solutions

HPS stock solution was prepared by adding 50mg of HPS to a 100-mL volumetric flask, dissolving this quantity in mobile phase and filling to the mark with the same solvent. Working standard solutions were prepared by dilution of the HPS stock solution with mobile phase to obtain five different concentrations within the range of interest, in tablet case, 2.5, 5, 10, 25 and 50㎍/mL or in injection case, 1, 1.5, 2, 2.5, 3㎍/mL.

I-2-4 Sample preparation

Twenty tablets, each containing 0.5mg HPS, were accurately weighted and finely powered. A quantity of powder equivalent to 0.5mg HPS was weighed and transferred to a 20mL volumetric flask. Afterwards, about 15mL of the mobile phase was added to the flask, sonicated for 10min, brought to volume with the same solvent and filtered through 0.45㎛ membrane filter(Millipore, Bedford, MA). The first 5mL of the filtrate was rejected.

For the injection assay, 10mL of injection solution was transferred to a 20mL volumetric flask diluted with mobile phase to volume and mixed.

I-2-5 Validation criteria

I-2-5-1 Specificity

Specificity was assessed by comparing the chromatograms obtained from sample of pharmaceutical preparation and standard solution with those obtained from excipients which take part in the commercial tablets or injections and verifying the absence of interferences.

I-2-5-2 System suitability

The system suitability was assessed by six replicate analysis of the drug at a concentration of 2㎍/mL or 25㎍/mL. The acceptance criterion was ≤2% for the %R.S.D. for the peak area and retention times for HPS.

I-2-5-3 Linearity

The calibration curves were constructed with five concentrations raging from 2.5 to 50㎍/mL for tablets or 1 to 3㎍/mL for injections. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method.

I-2-5-4 Accuracy and precision

Method accuracy was tested(% recovery and % R.S.D.) of individual measurements)by analysing samples of HPS at three different levels(50, 100 and 150%) in solutions compressing the drug-matrix used in tablet and formulations. The precision of the assay was studied with respect to repeatability and reproducibility. Repeatability was calculated from six replicate injections of freshly prepared HPS solution in the same equipment at a concentration of 100% of the intended test concentration value on the same day. Intermediate precision was assessed by comparing

the assays on three different days. The acceptance criteria were ≤2% for the %R.S.D. of recovery and 100±2% for the mean recovery(%) of HPS. Reproducibility of the proposed method was tested by analysis of injection formulations in different laboratories. The results of reproducibility was analyzed by ANOVA(Student-Newman-Keuls multiple range test).

I-2-5-5 Stability of the hexoprenaline sulfate solutions

The stability of the hexoprenaline sulfate standard solutions during time was investigated. Solutions at an ambient temperature, a condition protected from light and in a refrigerated condition were reinjected after 24 and then again on the 48th compared with freshly prepared solutions.

I-2-5-6 Robustness

The effects of the changes of operating parameters such as pH of the buffer and column temperature were tested in order to test sensitivity to minor changes in operating conditions. The following chromatographic parameters were varied in order to verify sufficient robustness of the chromatographic separation: column temperature(±2℃), pH of butter(pH±2).

I-3 RESULTS AND DISCUSSION

I-3-1 Optimization of chromatographic conditions

Hexoprenaline sulfate is one of catecholamines. In the literature, many HPLC methods of catecholamines (Epinephrine, Norepinephrine, Dopamine, etc.) have been reported. Of these methods, conventional reversed-phase column, C-8 and C-18, were used. With those columns, typical chromatographic conditions used for mobile phase consists of acidic buffer with methanol as organic solvent and sodium octanesulfonate/ heptanesulfonate as a ion-pairing agent.[7-9] The preliminary experiments were performed as described in the HPLC method above. The effect of composition of the mobile phase on the retention time and peak symmetry of HPS was investigated with C-18 column (150mm×4.0mm, 5㎛ particle size). Several experiments were conducted to optimize chromatographic condition. There was improvement in the stability of peak symmetry and baseline resolution when changing methanol to acetonitrile. Using a mobile phase of acetonitrile-potassium phosphate(pH3.0; 25mM)(90:20,v/v) and 0.1% sodium heptanesulfonate resulted in a well defined analyte peak and appropriate retention.

I-3-2 Validation of methods

I-3-2-1 Specificity

Specificity was determined by placebo analysis. Placebos of tablet or injection formulations containing the excipients(HPS excluded) were

prepared for this study. The chromatograms obtained from placebo, samples and HPS standard solution are shown in Fig. 2 and 3. According to these figures, the peak of the HPS was completely separated despite the presence of the peaks of pharmaceutical formulation base components on the chromatogram.

I-3-2-2 System suitability

The % R.S.D. of peak area and retention time were within 2% indication the suitability of the system(Table 1). According to the results presented, the proposed method fulfills requirements within the accepted limits.

I-3-2-3 Accuracy

The data for accuracy were expressed in terms of percentage recoveries of HPS and relative standard deviation in the real samples. These results are summarized in Tables 2 and 3. The recovery data of HPS satisfied the acceptance criteria for the study.

I-3-2-4 Linearity

A linear relationship was evaluated across the range of analytical procedures. Three independent determination were performed at each concentration. Since different amount of HPS are existed in tablet (I) or injection (II), the linearity was performed over the range of 2.5−50㎍/mL

(I) and 1-3㎍/mL(II).

Linear relationships between peak area signal of HPS versus the corresponding drug concentration were observed, as shown by the results presented in Tables 4 and 5. J. ermer et al. [10] asserted that the coefficient of correlation is not suitable as a general acceptance criterion to the linearity performance of an analytical procedure. The relative standard error of slope was used as a parameter with respect to precision of the regression.[10] This parameter should be comparable to the relative standard deviation obtained in precision studies in the given concentration range. The relative standard error of slope were 0.04%(I) and 0.02%(II), that is less than R.S.D. of precision[mean value is about 0.9%(I) and 1.0%(II)].

I-3-2-5 Precision

The repeatability study(n=6) showed a (%)R.S.D. of 0.940% for tablet formulation and 1.088% for injection formulation. The results obtained from these analysis are listed in tables 6 and 7. The data of Table 8 shows that average results of intermediate precision. The results of reproducibility study are listed in Table 9. When results from assay of the same drugs by different laboratories were compared, statistical analysis using ANOVA(Student-Newman-Keuls multiple range test) showed there were no significant difference between the results at 95% confidence level. This results indicated that the equipment used for the study worked correctly for the developed analytical method.

I-3-2-6 Stability of the hexoprenaline sulfate solutions

Stability studies indicated that the samples were stable when kept in an ambient, a condition protected from light and in a refrigerated condition for 48 hours. The results of these stability studies are presented in Table 10, <2% change was noted in potency values.

I-3-2-7 Robustness

The result of the robustness of the assay method is demonstrated in Table 11. Method robustness checked after deliberate alterations of pH and temperature shows that the changes of the operational parameters do not lead to essential changes of the performance of the chromatographic system. The chromatographic parameters are in accordance with established values[11]. Considering the result of modifications in the system suitability parameters, it is concluded that the method conditions are robust.

I-3-2-8 Application of the method to pharmaceutical analysis

Pharmaceutical formulation was presented as tablet(E-Piradol® tablet 0.5mg) and as injection(E-Piradol® inj. 2.5㎍/mL). Each sample was analyzed in triplicate after extracting the drug as mentioned in assay sample preparation of the materials and method section. Table 6 and Table 7 show the composition declared by the manufacturers and those found according to the recommended procedure. The results are in good agreement with the labelled content. Assay results, expressed as the percentage of the label claim, were found to be 97.5% for E-Piradol® tablet and 105.6% for E-Piradol® inj. showing that the content of HPS in the tablet or injection formulation conformed to the content requirements

(90-110%) of the label claim.

I-4 Conclusion

A new reversed-phase liquid chromatographic method for the determination of HPS contents in pharmaceutical formulations was developed. The developed method is simple, rapid and results were obtained confirm suitable accuracy, specificity and precision. Therefore, it is suitable for the routine analysis of HPS determination in tablet and injection formulation.

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Table1 . System suitability study

	Retention time(min)		Area	
	HPS(25㎍/mL)	HPS(2㎍/mL)	HPS(25㎍/mL)	HPS(2㎍/mL)
Injection volume	10㎕	150㎕	10㎕	150㎕
%R.S.D.	0.18	0.07	0.59	1.29

Table 2. Results of accuracy determination by method of standard additions(for tablet formulation)

Accuracy	Theoretical concentration (㎍/mL)	Experimental concentration (㎍/mL)	Recovery (%)	Average (%)	R.S.D. (%)
50%	37.79	37.70	99.77	99.20	0.53
		37.44	99.07		
		37.32	98.75		
100%	27.12	26.99	99.51	99.47	0.48
		26.84	98.97		
		27.10	99.92		
150%	12.48	12.42	99.53	99.81	0.78
		12.38	99.21		
		12.57	100.69		

Table 3. Results of accuracy determination by method of standard additions(for injection formulation)

Accuracy	Theoretical concentration (㎍/mL)	Experimental concentration (㎍/mL)	Recovery (%)	Average (%)	R.S.D. (%)
50%	1.081	1.096	101.40	100.66	1.22
		1.095	101.34		
		1.073	99.24		
100%	1.942	1.931	99.42	99.23	0.16
		1.925	99.15		
		1.925	99.14		
150%	3.309	3.240	97.91	98.76	0.96
		3.262	98.58		
		3.302	99.78		

Table 4. Statistical analysis of calibration curves in the HPLC determination of HPS (for tablet formulation)

Validation parameters	
Concentration range(㎍/mL)	2.5-50
Number of concentration levels	5
Regression equation	
Slope	4.227×10^{-5}
Intercept	-0.0890
STD error	0.04
Correlation coefficient (r^2)	0.999
Residual sum of square	0.00572

Table 5. Statistical analysis of calibration curves in the HPLC determination of HPS (for injection formulation)

Validation parameters	
Concentration range (㎍/mL)	1-3
Number of concentration levels	5
Regression equation	
Slope	2.919×10^{-6}
Intercept	0.0755
STD error	0.02
Correlation coefficient (r^2)	0.999
Residual sum of square	0.00132

Table 6. Application of the procedure to the tablet formulation

Pharmaceutical formulation	Declared composition	Found (mg/tablet)	Average recovery(%)	%R.S.D.
E-Piradol® tablet	0.5mg/tablet	0.495	97.5%	0.940
		0.490		
		0.489		
		0.483		
		0.484		
		0.484		

Table 7. Application of the procedure to the injection formulation

Pharmaceutical formulation	Declared composition	Found (mg/tablet)	Average recovery(%)	%R.S.D.
E-Piradol® inj.	2.5㎍/mL	2.609	105.6%	1.088
		2.611		
		2.627		
		2.648		
		2.667		
		2.677		

Table 8. Intermediate precision data for HPS

Spike level	Inter-day, recovery(%)±R.S.D.(%)			Inter-day mean±R.S.D.(%)
	Day 1	Day 2	Day 3	
50%	99.2±0.5	99.0±0.4	99.6±0.5	99.3±0.3
100%	99.5±0.5	99.0±0.2	100.2±0.8	99.5±0.6
150%	99.8±0.8	100.2±0.9	100.4±0.4	100.1±0.3

Table9. Inter-laboratory method validation of hexoprenaline sulfate.

	Laboratory 1	Laboratory 2
1	105.90	104.39
2	105.47	104.46
3	107.29	105.10
4	105.50	105.94
5	108.03	106.71
Mean	106.43	105.32
p-value	0.1404	
SEM* (n=10)	0.3720	

* Standard errors of mean

Table 10. Stability test of HPS solutions(24.95㎍/mL)

	Ambient	Protected from light	Refrigerated conditions
24h			
Mean	100.44	102.29	100.98
%CV	0.92	4.28	0.71
48h			
Mean	98.25	98.62	98.99
%CV	0.31	0.85	0.95

Each value is the result of triplicate analysis

Table 11. Results for robustness test study

Parameter	Variations	k'	$N(\times 10^3)$ [a]	Asymmetry factor
Column temperature	38℃	3.83	48.7	0.99
	40℃	3.63	50.2	0.98
	42℃	3.52	47.2	0.97
Mobile phase pH	2.8	2.61	47.4	0.95
	3.0	3.63	50.2	0.98
	3.2	3.08	50.2	0.98

[a]Theoretical plates per meter

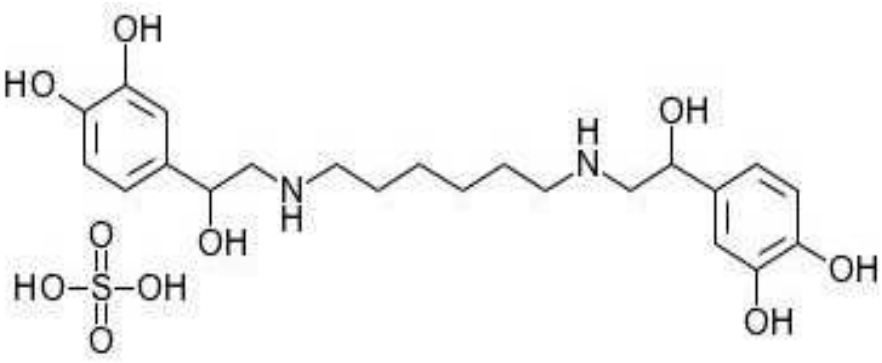
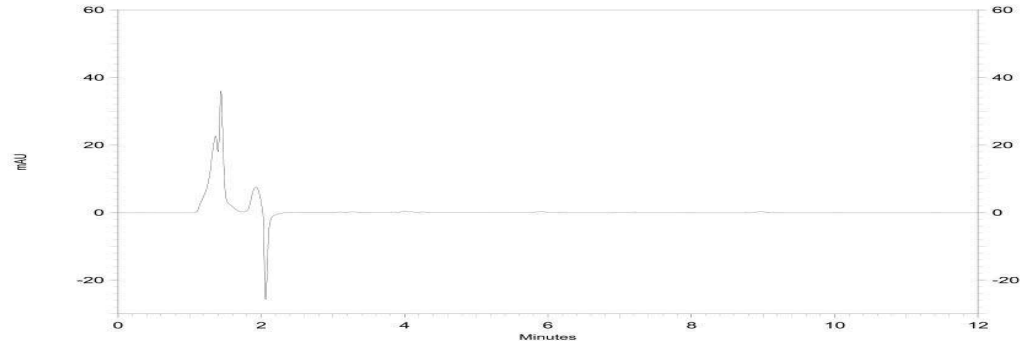
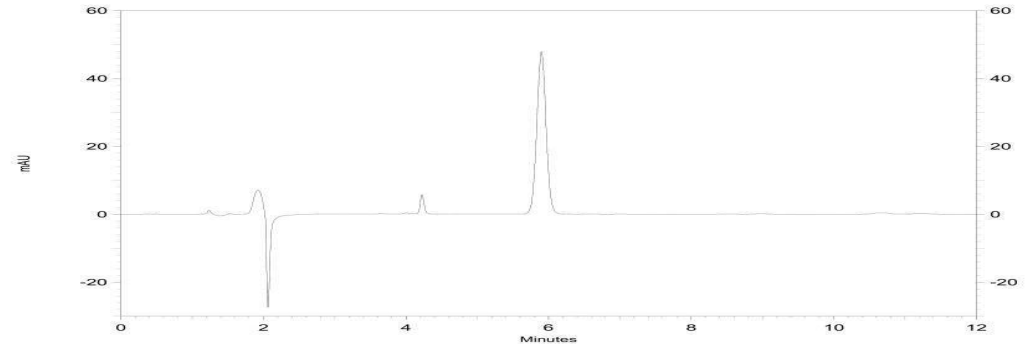


Fig. 1. Chemical structure of hexoprenaline sulfate



(a)



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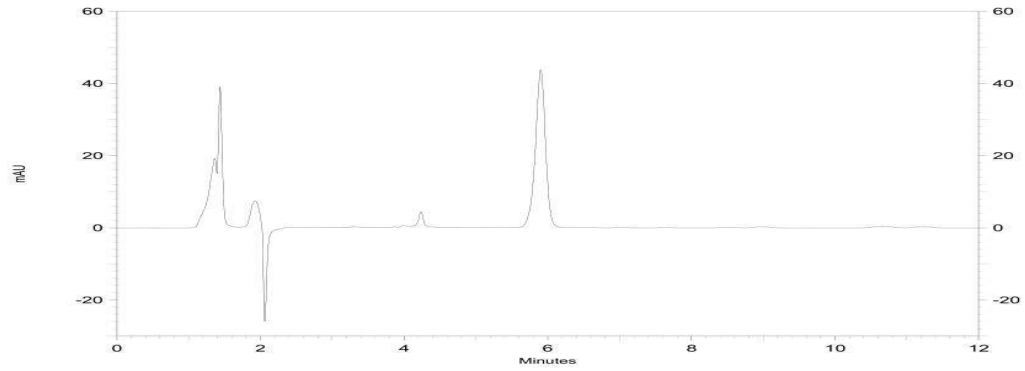
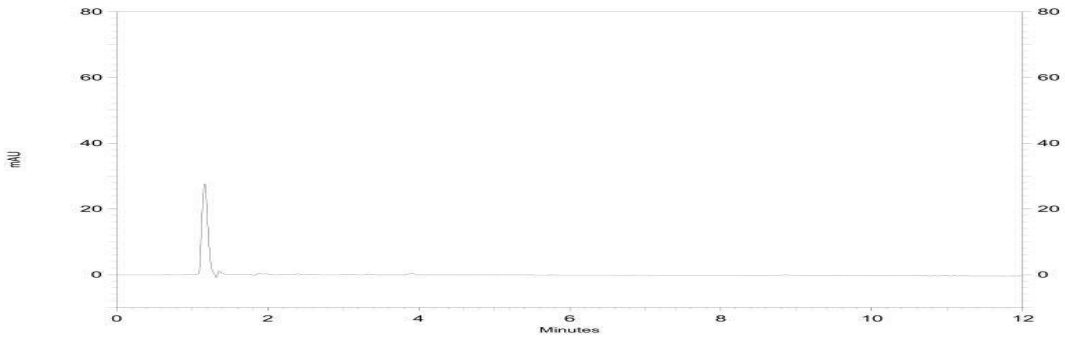
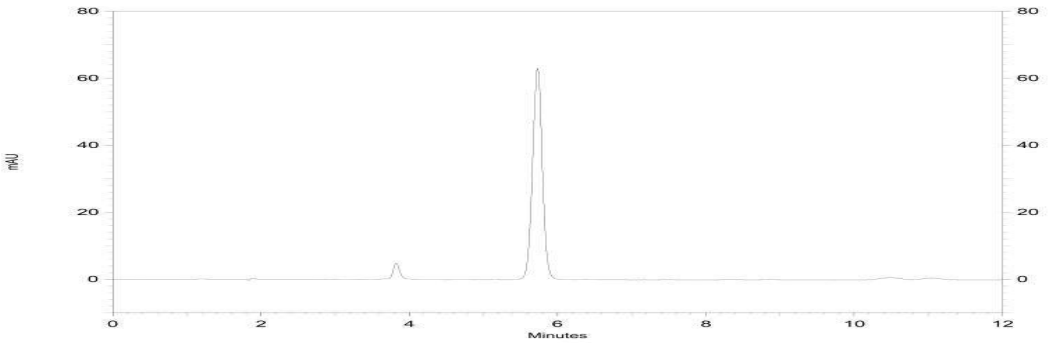


(c)

Fig. 2. Chromatograms obtained from the analysis of (a) excipients; (b) HPS standard solution(25㎍/mL) and (c) HPS extracted from tablets(25㎍/mL)



(a)



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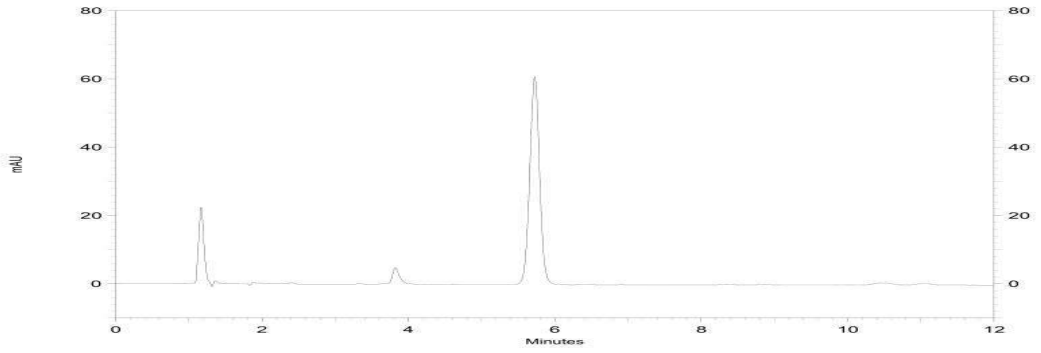

(c)

Fig. 3. Chromatograms obtained from the analysis of (a) excipients; (b) HPS standard solution(2㎍/mL) and (c) HPS extracted from injections(2㎍/mL)

CHAPTER II

DEVELOPMENT AND VALIDATION OF A HPLC-UV METHOD FOR THE DETERMINATION OF DIPHENIDOL HYDROCHLORIDE IN TABLETS

Abstract

This study describes the validation of an isocratic HPLC method for the assay of diphenidol hydrochloride in tablet. The method employs Capcellpak C18 MG (150mm×4.6mm, 5㎛)column with a mobile phase of acetonitrile/0.3% triethylamine adjusted to pH 3.0 by phosphoric acid 30:70, v/v) and UV detection at 217nm. A linear response(r>0.999) was observed in the range of 25-75㎍/mL. The degree of linearity of the calibration curves and the percent recoveries of diphenidol hydrochloride were determined. The method was found to be simple, specific, precise and accurate. The method was applied for the quality control of commercial diphenidol hydrochloride tablets to quantify the drug.

Ⅱ-1 Introduction

Diphenidol hydrochloride(DPN)(Fig. 1) is used to relieve or prevent nausea, vomiting and dizziness caused by certain medical problems.[1,2]

Only a few analytical methods for the determination of the DPN in bulk materials and pharmaceutical formulations have been developed, including capillary electrophoresis[3], amperometric assay[4] and UV spectrophotometric assay[5]. No chromatographic methods have been reported until date for the analysis of DPN except for a pharmacokinetic study.[2]

This study describes the development of a sensitive, selective, and rapid RP-HPLC method with UV detection for the determination of DPN in tablet formulation. This work also describes the validation parameters stated by KFDA guidance and ICH guidelines, to achieve an analytical method with acceptable characteristics of suitability, reliability and feasibility [6,7]. The proposed method has been validated and applied for DPN tablets containing 25mg active substance.

Ⅱ-2 Materials and methods

Ⅱ-2-1 Materials

Diphenidol hydrochloride was obtained from IL-SUNG Pharm. (Seoul, South Korea). Cephadol® tablets containing 25mg diphenidol hydrochloride were purchased from a local pharmacy. All reagents and solvents were of analytical and HPLC grade and included triethylamine, acetonitrile(Merck, Darmstadt, Germany) and phosphoric acid(Junsei, Japan). Double distilled water was used during the entire HPLC procedure.

Ⅱ-2-2 Instrumentation and analytical conditions

The Nanospace SI-2 HPLC system (Shiseido, Tokyo, Japan) consisted of a Series 3001 pump equipped with an automatic Series 3023 injector, a Series 3017 PDA detector and Series 3004 column oven. The analytical column, Capcellpak C18 MG (150mm×4.6mm, 5㎛, Shiseido, Tokyo, Japan) was operated at 40℃. Isocratic elution with acetonitrile/0.3% triethylamine adjusted to pH 3.0 by phosphoric acid 30:70, v/v) was used at a flow rate of 1.0mL/min. The mobile phase was freshly prepared, filtered through a 0.2㎛ membrane filter(Millipore, Ireland) using a glass vacuum filtration apparatus and degassed by sonicating for 5min before use. The injection volume was 10㎕ and UV detection was performed at 217nm.

Ⅱ-2-3 Stock and working standard solutions

Stock standard solutions of 0.25mg/mL of DPN were prepared freshly by accurately weighing approximately 25mg of DPN into a 100mL volumetric flask and making up to volume with mobile phase. These solutions were further diluted with mobile phase in the concentration range of 25, 37.5, 50, 62.5 and 75㎍/mL of DPN covering 50-150% of the intended test concentration of 50㎍/mL for the pharmaceutical formulation. The calibration curve was plotted with five concentrations of 25-75㎍/mL working standard solutions.

Ⅱ-2-4 Sample preparation

Twenty tablets (labelled "concentration 25mg diphenidol hydrochloride") were weighed and their mean mass was determined. After homogenizing the tablets using a grinder, an accurately weighed portion of the pooled sample equivalent to 25mg DPN was quantitatively transferred into a 100mL volumetric flask with about 90mL of mobile phase. The solution was sonicated for 15min, and brought to volume with mobile phase. A 5mL aliquot was transferred into a 25mL volumetric flask and diluted to volume using mobile phase. The theoretical DPN concentration after dilution was 50㎍/mL. An aliquot of this solution was filtered through a 0.45㎛ membrane filter (Millipore, Bedford, MA) prior to the injection into the HPLC system.

Ⅱ-2-5 Validation Procedure

The objective of method validation is to demonstrate that the method

is suitable for its intended purpose as stated in the KFDA guidances. The method was validated for system suitability, specificity, linearity, accuracy and precision.

In order to determine the specificity of the method, the following solutions were injected : solution containing placebo sample without DPN, a standard solution and a solution sample preparation.

A system suitability test was performed by six replicate injections of the standard solution at a concentration of 50㎍/mL verifying %R.S.D. of peak retention time and peak area.

The calibration curves were constructed with five concentrations from 25 to 75㎍/mL. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method.

Precision of the assay was determined by repeatability and reproducibility. Repeatability refers to the use of the analytical procedure within a laboratory over a short period of time that was evaluated by assaying samples during the same day. Repeatability test was performed by six repeated analysis of tablet formulations. Reproducibility expresses the precision between laboratories as in collaborative studies. The proposed method was investigated by analysis of tablet formulation in different laboratories.

The accuracy of the method was performed over the range of 50%, 100% and 150% of the label claim. A minimum of three samples at each concentration level were prepared and injected.

For the assessment of sample solution stability, the DPN standard solutions(25.15㎍/mL) were stored for 72hours under an ambient condition, a condition protected from light and a refrigerated condition and

injected versus fresh standard preparations.

For the robustness experiment, two different buffer solutions were prepared. The pH of one solution was adjusted to 2.8, while the pH of the other was adjusted to 3.2(this range is ±0.2 pH units form nominal). The effect of variance of temperature on peak parameters was also studied.

Ⅱ-3 RESULTS AND DISCUSSION

Ⅱ-3-1 Method development and Optimization

Preliminary experiments were performed on the basis of the HPLC method described by Hernández et al.[2] In order to investigate the appropriate wavelength for DPN, solution of DPN in mobile phase were scanned by UV spectrophotometer in the range 200-400nm. The maximum absorbance for tested substances occurred at about 200nm and ant at 217nm at a weak shoulder peak ; larger peak areas were observed at 200nm. However the peak shape and symmetry were found to be better when the responses were recorded at 217nm.

II-3-2 Method validation

II-3-2-1 System suitability

The % R.S.D. of mean(n=6) peak area and retention time were 0.34% and 0.24% respectively. These parameters tested met the acceptance criteria(Table 1).

II-3-2-2 Specificity

Fig. 2 shows chromatogram of a blank placebo sample and drug-spiked sample. No interference between drug and excipients was depicted at this wavelength. The chromatographic run time of 11 min. was

sufficient for sample analysis that allows analysis of numerous samples in a short period of time.

II-3-2-3 Linearity

Five points calibration graphs were constructed covering a concentration range 25−75㎍/mL. The regression line was calculated as Y=A+BX, where X was the DPN concentration(㎍/mL) and Y was the response(peak area expressed as AU). The calibration curve was obtained using the linear least squares regression procedure. Characteristic parameters for regression equation confirmed the good linearity of the method developed. (Table 2)

II-3-2-4 Accuracy and precision

The accuracy was evaluated by the recovery of DPN at three different levels. Table 3 summarizes the accuracy results, expressed as percentage recovery and relative standard deviation(%R.S.D.). The method showed good recovery.

The results obtained for repeatability studies are presented in Table 4 Method precision has a %R.S.D. below 0.8% which comply with the acceptance criteria proposed(%R.S.D. ; not more 2.0%)

Table 5 shows the reproducibility study results. The statistical values were obtained from ANOVA(Student−Newman−Keuls multiple range test). There were no significant difference between results(p>0.05). These results show that the method is rugged and precise.

II-3-2-5 Stability of the DPN solutions

The results of stability of DPN are presented in Table 6. Solutions were stable for 72h, during which time results did not decrease below 99%. This indicates that DPN is stable in standard solutions for at least three days at an ambient temperature, a condition protected from light and in a refrigerated condition.

II-3-2-6 Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters, and provides an indication of its reliability during normal usage[8]. The results are shown in Table 7. Moderate mobile phase pH and temperature changes were shown to have virtually no effect on the chromatography; almost no change in peak shape or column efficiency was noted.

II-3-2-7 Assay of tablets

The method developed in the present study was applied for determination of DPN in tablets. Fig 2 shows an HPLC chromatogram of DPN in tablets. None of the tablet ingredients interfere with the analyte peak. Table 4 shows the composition declared by the manufacturer, and those found in accordance with the recommended procedure. The recoveries obtained agreed with the declared composition.

Ⅱ-4 Conclusion

The present method differs only slightly from those reported previously in terms of chromatographic conditions.[2] The HPLC methods previously reported have been developed for determination of DPN in biological fluids. In the present study, a simple validated HPLC method was developed for the analysis of DPN in tablet formulations. The rapid run time of 11min allows the analysis a number of samples with less mobile phase that proves to be cost-effective. Hence, this HPLC-UV method can be used for the routine drug analyses.

II-5 References

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Table1 . System suitability study

	Diphenidol hydrochloride	
	Retention time(min)	Peak area
Mean(n=6)	5.78	3741453
%R.S.D.	0.24	0.34

Table 2. Results of regression analysis of the linearity data of DPN

Validation parameters	
Concentration range(㎍/mL)	25-75
Number of concentration levels	5
Regression equation	
Slope	8.079×10^{-6}
Intercept	-5.913
STD error	0.88
Correlation coefficient(r^2)	0.999
Residual sum of square	2.336

Table 3. Accuracy study

Level(㎍/mL)	Amout recovered(㎍/mL)	Recovery(%)	Mean(%)	%R.S.D.
50%(28.53)	28.46	99.75	100.27	0.45
	28.69	100.57		
	28.67	100.49		
100%(48.21)	48.14	99.86	99.63	0.30
	48.08	99.73		
	47.87	99.29		
150%(74.42)	73.31	98.51	99.19	0.63
	74.23	99.74		
	73.92	99.32		

Table 4. Summary of Repeatability data

Pharmaceutical formulation	Declared composition	Found (mg/tablet)	Found(%)	%R.S.D.
Cephadol® tablet	25mg/tablet	24.47	97.8%	0.80
		24.47		
		24.11		
		24.39		
		24.60		
		24.67		

Table5. Inter-laboratory method validation of Diphenidol HCl.

	Laboratory 1	Laboratory 2
1	96.84	96.90
2	97.10	97.89
3	96.38	96.46
4	96.30	97.59
5	97.07	97.83
Mean	96.73	97.33
p-value	0.1062	
SEM* (n=10)	0.1835	

*Standard errors of mean

Table 6. Stability test of DPN solutions(25.15㎍/mL)

	Ambient temp.	Protected from light	Refrigerated conditions
72h			
Mean	101.79	99.24	99.67
%CV	1.57	1.63	1.57

Each value is the result of triplicate analysis

Table 7. Results for robustness test study

Parameter	Varinations	κ	$N(\times 10^3)$ [a]	Asymmetry factor
Column temperature	38℃	4.47	62.0	1.29
	40℃	4.39	62.7	1.30
	42℃	4.32	64.1	1.28
Mobile phase pH	2.8	4.62	63.5	1.29
	3.0	4.39	62.7	1.30
	3.2	4.29	62.5	1.29

[a]Theoretical plates per meter

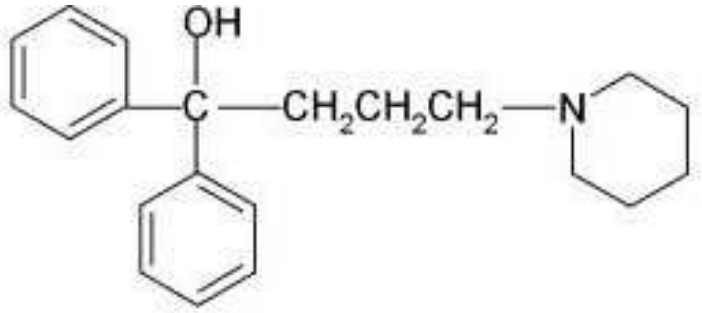
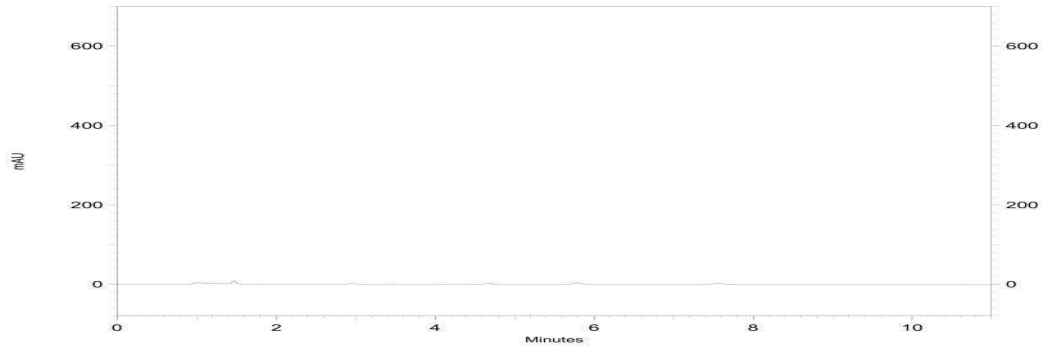


Fig. 1. Structure of Diphenidol



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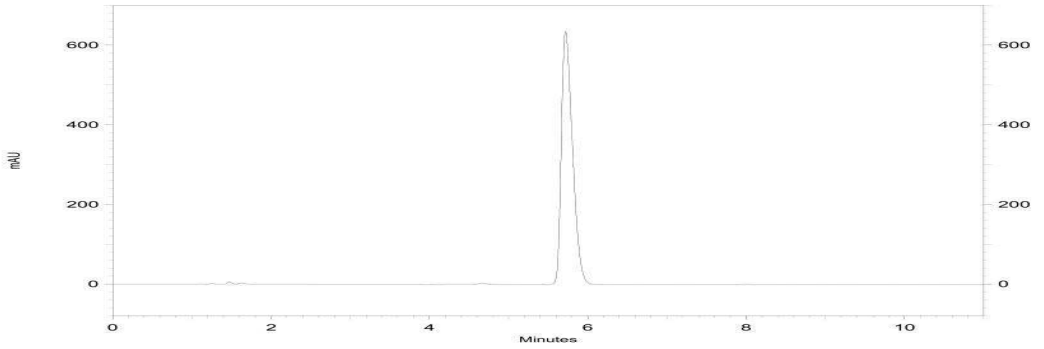

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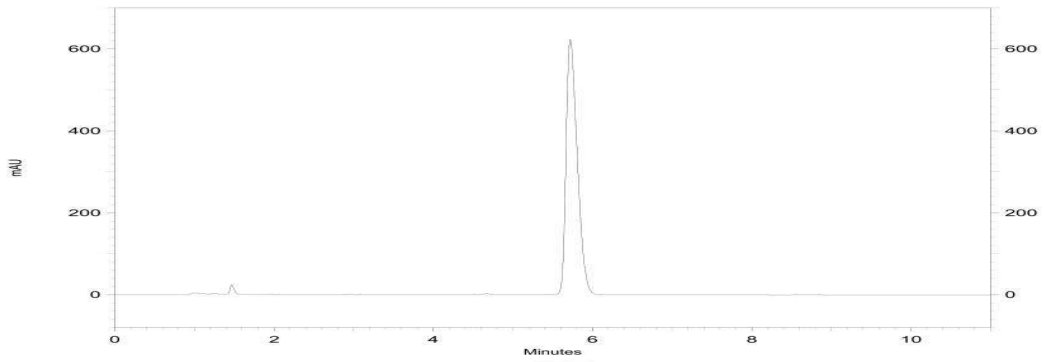

(c)

Fig. 2. Chromatograms obtained from the analysis of (a) excipients; (b) DPN standard solution(50㎍/mL) and (c) DPN extracted from tablets(50㎍/mL)

저작물 이용 허락서

학 과	식품의약학과	학 번	20057542	과 정	박사
성 명	한글: 정현아 한문 : 鄭賢我 영문 : CHEONG,HYUN-AH				
주 소	광주광역시 북구 오룡동 1110-5번지				
연락처	E-MAIL : pharm.cha@gmail.com				
논문제목	한글 : HPLC를 이용한 제제 중 황산헥소프레날린 또는 염산디페니돌의 분석법 개발 및 밸리데이션에 관한 연구 영어 : Development and validation if a new HPLC method for determination of hexoprenaline sulfate and diphedidol hydrochloride in pharmaceutical formulations				

본인이 저작한 위의 저작물에 대하여 다음과 같은 조건아래 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 편집·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 또는 출판을 허락을 하였을 경우에는 1개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물의 이용허락 이후 해당 저작물로 인하여 발생하는 타인에 의한 권리 침해에 대하여 일체의 법적 책임을 지지 않음
7. 소속대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의 전송·출력을 허락함.

동의여부 : 동의(○) 반대( )

2007년 12월 31 일

저작자: 정 현 아 (서명 또는 인)

조선대학교 총장 귀하