The Detection Method of Phenylpropanolamine in Pharmaceuticals by High Performance Liquid Chromatography(HPLC)

by

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DEPARTMENT OF CHEMISTRY

GRADUATE SCHOOL

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Under the Direction of

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1. INTRODUCTION

Phenylpropanolamine(PPA) is an ingredient used in prescription and over-the count(OTC) drug products as a nasal decongestant to relieve stuffy nose or nasal congestion and in OTC weight control drug products to control appetite(Fig. 1).[1, 2]

On may 11, 2000, Food and Drug Administration(FDA, U.S.A) received results of a study conducted by scientists at Yale University School of Medicine. The result of that study showed that the risk of hemorrhagic stroke(bleeding of the brain) was increased in people who were taking phenylpropanolamine. [3]

On October 19, 2000, FDA's Nonprescription Drugs Advisory Committee(NDAC) discussed this report and other information on phenylpropanolamine. NDAC determined that there is an association between phenylpropanolamine and hemorrhagic stroke and recommended that phenylpropanolamine could not be considered

generally recognized as safe for OTC use as a nasal decongestant or for weight control.[2]

Although this risk of hemorrhagic stroke is very low, FDA has significant concerns because of the seriousness of this adverse event(the irreversible outcome) and the inability to predict who is at risk. FDA does not consider the conditions for which phenylpropanolamine is used as justifying the risk of this serious event. FDA agrees with NDAC's recommendation that phenylpropanolamine is considered not safe for OTC use. FDA also has concerns about the safe use of phenylpropanolamine in prescription drug products.

Recently, FDA has been taking steps to remove phenylpropanolamine as an ingredient in OTC and prescription drug products. FDA has notified all manufacturers, repackers, and distributors of any prescription or OTC drug products containing phenylpropanolamine and requested that they discontinue marketing drug products containing phenylpropanolamine.

On August 8, 2001, Korea Food and Drug Administration(KFDA) recommended that consumer stop using drug products containing phenylpropanolamine as an appetite suppressant. Since that time phenylpropanolamine has only been available in OTC and prescription as a nasal decongestant medicines.[4] In November 2001, there were 193 products containing phenylpropanolamine authorized for supply in Korea.

The therapeutic dose has reviewed the report of the US study that

formed the basis for the Food and Drug Administration's action, because there has been no adverse drug reaction report associated with phenylpropanolamine use in Korea.

From 1965 to 1990, 142 adverse drug effects attributed to phenylpropanolamine were reported in the literature. A comprehensive review of these adverse effects revealed that 36% occurred after therapeutic doses and 18 % after unintentional overuse.[5] The recommended therapeutic dose of phenylpropanolamine is one 25 mg immediate-release tablet every 4 to 6 h or one 75 mg sustained-release tablet every 12 to 24 h. Ingestion of a single extra sustained-release dose(150 mg total) or two extra immediate-release doses(75 mg total) significantly elevates systolic and diastolic blood pressure.[6,7] Frequently, consumers take more than the recommended dose of a medication in an effort to increase its efficacy. This is particularly true for over-the-counter preparations, which are commonly considered innocuous because of their ready availability without a prescription. Thus, overuse of phenylpropanolamine will substantially increase the risk of an adverse drug effect. The following information includes only the average doses of phenylpropanolamine in USA(Table 1).

Adverse effects associated with the use of phenylpropanolamine include hypertension, myocardial injury, headache, hypertensive encephalopathy, agitation, psychosis, hemorrhagic and ischemic cerebrovascular accidents, atrial and ventricular brady- and tachydysrhythmias, cardiopulmonary arrest, seizures, bowel ischemia

and infarction, and cerebral arteritis.[5]

The Analysis of this pharmaceuticals containing phenylpropanolamine is of special interest due to its intensive use and the risk of hemorrhagic stroke.

A number of analytical methods have been reported for the determination of phenylpropanolamine mostly based on spectrophotometry,[14] flow-injection technique,[15] gas chromatography,[16~18] and liquid chromatography(HPLC).[19~29]

The resolution of mixtures was accomplished without prior separation or derivatization using partial least-squares(PLS-1) regression analysis of electronic absorption spectral data in the spectrophotometry(Goicoechea et al., 1999)[14]. This method was determined with relatively high accuracy and precision in minor components, but not linearity of the major components. They also compared classical least squares method that have been had unsatisfactory results for the minor components due to several spectral overlapping and/or poor sensitivity towards the minor components. A new flow-injection procedure for the determination of phenylpropanolamine was proposed by Vinas et al., 1998.[15] This method is based on the derivatization reaction of the primary amine group with *O*-phthalaldehyde in the presence of 2-mercaptoethanol using fluorimetric detection.

Analytical Technique to determine phenylpropanolamine in pharmaceuticals have been generally used GC and HPLC method.

Using a nitrogen-phosphorus-flame ionization detector(NP-FID), GC was adopted for the analysis of 34 basic and neutral drugs in urine samples(Soriano et al., 1996)[18]. sample preparation was based on a liquid-liquid extraction with an organic solvent at alkaline pH. A GC-MS method was used for the quantitative analysis of phenylpropanolamine in animal and human hair(Y. Nakahara et al., 1997)[16] and another GC-MS method(Spyridaki et al., 2001)[17] with derivatization(*O*-trimetholsilylated and N-mono-trifluoro-acetylated derivatives) was developed for the confirmation and quantification of phenylpropanolamine in urine and was applied to doping control analysis.

For GC analysis, an extraction procedure is often required to transfer the analyte from aqueous sample to organic solvent and derivatization procedure is also required. But analysis of aqueous sample is possible by HPLC directly. It is very important for HPLC because pharmaceuticals were generally dissolved in water.

Of the Analytical Technique to determine phenylpropanolamine, HPLC offer a number of advantages over other techniques. A wide range of column packing materials are available for specific applications, the columns could be reused many times, and most chemical separations of analytes could be achieved at ambient temperature under the anaerobic conditions, which is particularly well suited to the analytes with reactive functional groups. In addition, resolution tends to fall off only slowly with increasing sample size, analysis time is relatively short compared with the other technique, and

retention time of analytes are reproducible under the given conditions.[7, 8]

HPLC equipped with a diode array detector was studied for the simultaneous assay of an over-the-count cold tablet containing phenylpropanolamine(Indrayanto et al., 1995)[29]. The separation of the five components of tablet was achieved using a mixture of acetonitrile-tetrahydrofuran-ion pair solution(7:6:87, V/V/V). A serum samples were analysed for phenylpropanolamine by HPLC method(Rao et al., 1998)[27], equipped with UV spectrophotometric detector that was set at 254nm, and 15cm × 4.6mm(i.d.) octadecyl silane reverse phase column. The mobile phase was acetonitrile-water(10:90) with pH 2.5.

Zaater et al.(1999)[21] also analysed for phenylpropanolamine in human serum using reverse-phase HPLC. The mobile phase was 6.3% acetonitril in 0.025M sodium dihydrogenphosphate(pH 3). For the simultaneous estimation of phenylpropanolamine HCl, guaiphenesin and diphenylpyraline HCl in syrup(Vasudevan et al., 2000)[24], a HPLC method was described. The method was carried out on C_8 column with a mobile phase consisting of acetonitrile-triethylamine(pH 3.5)(35:65, V/V). And Using micellar mobile phase of sodium dodecyl sulfate(SDS), a HPLC procedure was developed for the determination of several phenethylamine (phenylpropanolamine etc.) equipped with C_8 column and UV detection(Gil-Agusti et al., 2000)[26]. Another method was proposed for HPLC determination base on precolumn derivatization with 4-dimethylaminobenzaldehyde(DAB) and elution from C_{18} column with methanol-water and detection by spectrophotometry at 418 nm

(Rind et al., 2001)[22].

In this study, reversed phase high-performance liquid chromatography(RP-HPLC) has been utilized for the separation and the determination of various types of analytes. The term "reversed phase" implies that the mobile phase is more polar than the stationary phase. The separation of analytes is based on the partition between a mobile phase and a hydrophobic stationary phase. The most widely used stationary phase in RP-HPLC consists of octadecyl(C_{18}) group, linked to a silanol surface by covalent bonds. Reversed phase HPLC using silica-based C_{18} or C_8 stationary phase is used in the pharmaceutical industry for quality control and biomedical analysis.[9, 12]

Ultraviolet spectrophotometric detectors are the most widely used detector for HPLC. UV detectors in general can give great selectivity and sensitivity in the analysis of specific compounds, and they are relatively insensitive to changes in temperature or flow-rate. Quantitative analysis by peak height or peak area measurement using a UV photometric detector can be performed with degree of precision by isocratic method, if the mobile phase composition and flow-rate are carefully controlled and standardized.[7, 12]

In the United States Pharmacopoeia(USP)[31], a HPLC method have been used for the quantitative analysis of phenylpropanolamine by internal standard method for type of capsules. The liquid chromatography is equipped with a 254-nm detector and a 4mm × 30cm column that contains packing Phenyl groups chemically bonded to

porous silica particles. The flow rate is about 1.5 mL per minute. The mobile phase consists of mixture of water, methanol, 10% tetramethylammonium hydroxide solution, and phosphoric acid(700 :300:14:3.5). For types of tablet and oral solution, the liquid chromatography is equipped with a 254-nm detector and a 4 mm × 30 cm column that contains packing octadecyl silane chemically bonded to porous silica or ceramic micro-particles. The flow rate is 1.5 mL per minute. The mobile phase consists of mixture of water-solvent A(45:55). (The solvent A : dissolve 1.9 g of sodium-1-hexanesulfonate in 700 mL of water, add 20 mL of 0.25M triethylammonium phosphate(prepared by mixing 500 mL of a solution containing 25.3 g of triethylamine and 500 mL of a solution containing 9.6 g of phosphoric acid), and mix. dilute with water to 1 L, and mix.) And in the Korean Pharmaceutical Codex(second edition)[32], the method for determination of phenylpropanolamine is similar with USP method. There are specific methods to determine phenylpropanolamine for each form of pharmaceuticals. The present work describes a simple and universal method for the determination of variable types of pharmaceuticals containing phenylpropanolamine. A analytical conditions were optimized and the method was applied with useful results to the determination of the phenylpropanolamine in commercial pharmaceuticals.

2. EXPERIMENTAL

2-1. Reagents

Phenylpropanolamine hydrochloride and potassium dihydrogen-phosphate were obtained from Aldrich Chemical Co. (Milwaukee, USA). Methyl alcohol of HPLC grade was obtained from Tedia Co. (Fairfield, Ohio, USA). All other chemicals and reagents were of analytical grade. The Over-the-counter(OTC) medications(one syrup and three capsules) were purchased from a local drugstore. 5% methyl alcohol in 0.025 M potassium dihydrogenphosphate was prepared freshly for each experiment as mobile phase. pH adjusted to 3.0 using 1 M H_3PO_4.

2-2. Instruments

The HPLC system consists of a Waters 515 HPLC solvent delivery system, a variable wavelength detector (Jasco, UV-975) and a Rheodyne 7125 injector with a 20-μL loop. The method was carried out on a Crestpak® C_{18S} (150×4.6 mm i.d.) column(Jasco, Japan) as a stationary phase. Absorbance and pH measurements were carried out with a Hittach UV/Visible Spectrophotometer(Hittach U3210, Japan) and a Metrohm pH Meter(654 pH Meter, Switzerland). Integrator(HP 3394A, USA) was used as a chart record. 0.2 ㎛ Nylon 66 membrane filter (Alltech , USA) was used to remove solvent non-soluble adjuvants in pharmaceuticals. The mobile phase was

degassed using helium gas and the separation was carried out at room temperature.

2-3. Procedure

For the determination of the phenylpropanolamine in pharmaceutical products, capsule forms were weighed after being carefully emptied to obtain the accurate weight of the capsule content. A stock solution was prepared by sonicating one capsule in 0.025M potassium dihydrogenphosphate for 2 hours, filtered through 0.2 ㎛ nylon membranes of 13 mm diameter to remove solvent non-soluble adjuvants, and diluted with 0.025M potassium dihydrogenphosphate in a volumetric flask of the required volume depending on the sample for injection onto the HPLC column.

1.0 mL of cough/cold syrup was diluted with 0.025M potassium dihydrogenphosphate in a 100 mL volumetric flask, filtered through 0.2 ㎛ nylon membranes of 13 mm diameter, and injected directly onto the HPLC column.

The concentration of the phenylpropanolamine was calculated by referencing to a pre-established calibration curve.

2-3-1. Standard Calibration Curve Method

A Phenylpropanolamine hydrochloride working standard solution in 0.025M potassium dihydrogenphosphate(1mg/mL) was used to provide

calibration standards of 0, 1, 10, 25, 50 and 100 μg/mL. The peak areas of the phenylpropanolamine were calculated and plotted against their concentrations.

2-3-2. Standard Addition Method

1.0 mL of stock solution of pharmaceutical products was pipetted into 10 mL volumetric flasks. Exactly 0.00, 1.00, 2.00, and 3.00 mL of a standard solution containing 100ppm of phenylpropanolamine were added to each. After dilution to volume, they were injected and chromatograms were recorded. The peak areas of the phenylpropanolamine were calculated and plotted against their concentrations.

2-3-3. Wave-length Selection

Absorbance measurement of adequate standard solution containing phenylpropanolamine was carried out against blank solution in wavelength between 185 ~ 300 nm by UV/Visible spectrophotometry. Wavelength was selected high absorption peak against blank solution.

3. RESULTS AND DISCUSSION

3-1. Optimization of Experimental conditions

Preliminary studies including change in mobile phase composition and flow-rate were performed to determine the optimum analytical conditions.

3-1-1. Mobile phase Selection

To improve the resolution of a chromatographic column, the capacity(retention) factor k' is experimentally the most easily manipulated because of the strong dependence of this constant upon the composition of the mobile phase. Normally, in reversed phase separations a solvent mixture of water and polar organic solvent are employed. The capacity factor is then readily manipulated by varying the water concentration. The effect of such manipulations was shown by the chromatograms in figure 2 where the sample was Haben F® produced by the Corea Pharmaceutical Manufacturing Co.(korea). With 20% methanol / 80% buffer solution mixture, k' had a value 2, and all of the analytes were eluted in such a short time(≈7min.) that the separation was quite incomplete(Figure 2-(a)). By increasing the percent buffer solution to 90, elution took place over 15min, which doubled the value of k'(Figure 2-(b)) but that was not great enough for satisfactory resolution. Finally, a 5% methanol / 95% buffer solution mixture

shown in Figure 2-(c) was chosen as the best mobile phase for the separation of the phenylpropanolamine in pharmaceutical productions.

3-1-2. Effect of mobile phase flow rate

Two related terms are widely used as quantitative measures of chromatographic column efficiency: (1) *plate height H* and (2) *plate count plates N*. The two are related by the equation; N = L / H, where L is the length(usually in centimeters) of the column packing. The efficiency of chromatographic columns increases as the plate count becomes greater and as the plate height becomes smaller. [12]

To optimize the mobile phase flow-rate, potassium dihydrogen-phosphate buffer solution and pH were set at 0.025M and 3 respectively. Figure 3 shows the effect of the mobile phase flow-rate in a range of 0.8 to 1.8 mL/min on the plate height for phenylpropanolamine standard solution(10 μg/mL). The plate height reaches its minimum at 1.2 mL/min and increases at higher flow-rate. For later investigations, mobile phase flow-rate was held constant at 1.2 mL/min.

3-1-3. Effect of pH

In the analysis of substances that are ionisable, the pH of the eluent will affect the degree of ionisation of the basic analytes. Moreover, the pH of the eluent will also control the degree of

ionisation of residual silanols present on the surface of the stationary phase. Therefore, the pH of the eluent will influence the ionic interactions between the basic analyte and the stationary phase. It is obvious that eluent pH is a very important parameter in the RPLC analysis of basic analytes and can influence the results obtained.[25] The pH of the mobile phase was chosen to be acidic to enhance to solubility of phenylpropanolamine and to lower its retention factor, because it has a basic character. Buffering the mobile phase is maintaining a constant pH during the analysis, which for ionisable compounds is important to obtain reproducible and robust analysis. The capacity factor of the phenylpropanolamine decreased with decreasing pH. In this study, pH 3 was selected for the analysis of phenylpropanolamine in the pharmaceutical products.

3-1-4. Wave-length Selection

Figure 4 shows absorbance spectrum of phenylpropanolamine. The UV absorbance of the analyte in the mobile phase showed a high absorption peak at 205nm. This was to maximize the absorbance sensitivity to change in concentration.

3-2. Standard Calibration Curve

Under the selected operating condition(mobile phase : 5% methanol

/ 95% water, pH = 3, mobile phase flow rate : 1.2 mL/min, wavelength : 205nm), calibration curve was obtained. Five calibration curves for concentrations from 1 to 100 μg/mL were constructed by linear regression analysis. The mean correlation coefficient, slope and intercept with their corresponding standard deviations were calculated. Calibration was carried out by plotting concentration against the area of the phenylpropanolamine peak. The calibration curve was linear in the range 1～100 μg/mL with the regression equation, $y = 4.41 \times 10^5 x - 0.62 \times 10^5$, where x and y are the injected concentration of PPA and peak area, respectively. The sensitivity of the method was calculated as the slope of the calibration line.

3-3. Limit of detection and quantitation

Most commonly, the method limit of detection(LOD) can be calculated from the calibration curve, area versus concentration, according to LOD = kS_{bl}/m, where the factor k is chosen to be 3, S_{bl} is the standard deviation of the blank measurement, and m is the slope of calibration curve.[12]

The Limit of quantitation(LOQ), defined here as LOQ = $10S_{bl}/m$, was determined on the basis of standard deviation of the blank measurement and the slope. The LOD and LOQ were calculated by means of five determinations. The LOD and LOQ in this work were 4 μg/L and 14μg/L, respectively.

3-4. Applications to commercial products

The method was applied for the determination of PPA in pharmaceutical products. Table 2～5 lists the results obtained from each pharmaceutical preparation commercially available by calibration curve method. Each analytical sample was injected by quintuplicate into HPLC system and the concentration was calculated by interpolating the area obtained in the calibration curve. As the release requirement for the dosage form is ± 10 % of nominal label claim,[31] the result with Haben F® was significantly different from the stated content of the formulation. This may be due to the type of excipients used or the processes involved in the manufacturing of these products. Phenylpropanolamine hydrochloride should contain not less than 10 percent and more than 10 percent of the labeled amount of phenylpropanolamine hydrochloride in USP.[31] Therefore, the results obtained indicate that the commercial formulation for PPA is in good agreement with the label claims.

To compare calibration curve method, standard addition method also carried out. The result obtained from each pharmaceutical preparation by standard addition method is shown in Table 6～9. The results also is in good agreement with the label claims but the result with Haben F® was significantly different.

4. CONCLUSION

A simple HPLC method has been developed for the determination of phenylpropanolamine(PPA) in the commercial preparations from four different manufacturers without previous preparation of the sample. Under the optimal operating condition(mobile phase : 5% methanol / 95% buffer solution, pH = 3, mobile phase flow rate : 1.2 mL/min, wavelength : 205nm), analytical performances of the system evaluated, including linearity, accuracy, precision, and limit of detection and quantitation from the consecutive measurements. Run times were 18min with spectrophotometric detection. The limit of detection and quantitation in this work were 4μg/L and 14μg/L, respectively. These results indicate that the described HPLC method could be useful for routine analytical and quality control assays of dosage forms of PPA.

In conclusion, the analytical offers the advantage that the sample preparation is very simple.

Table 1. Recommended Therapeutic Dosage of Phenylpropanolamine

Use	Dosage Form	Recommended Therapeutic Dosage	Limited Therapeutic Dosage
Appetite control	Oral dosage forms (capsules and tablets)	25 mg three times / day	75mg / day
	long-acting dosage forms (extended-release capsules and tablets)	75 mg once / day	75mg / day
Stuffy nose	Oral dosage forms (capsules and tablets)	25 mg / 4 hours	150 mg / day
	long-acting dosage forms (extended-release capsules and tablets)	75 mg / 12 hours	150 mg / day

Table 2. Analysis of Phenylpropanolamine(PPA) in Sinoca®
by Standard Calibration Curve Method.

Sample No.	Labelled Amount	Amount taken for analysis (mg/L)	Amount obtained (mg/L)
1	166.7 mg/100mL	16.67	16.45
2		16.67	16.88
3		16.67	17.22
4		16.67	16.55
5		16.67	17.43
Mean			16.91
Standard Deviation			0.42

Table 3. Analysis of Phenylpropanolamine(PPA) in Mabrin® by Standard Calibration Curve Method.

Sample No.	Labelled Amount	Amount taken for analysis	Sample Weight	Amount obtained (mg/L)
1	30.0 mg/capsule	30.0 mg/L	0.274	28.0
2			0.290	30.4
3			0.284	30.2
4			0.278	29.0
5			0.284	29.5
Mean			0.282	29.4
Standard Deviation			0.006	1.0

Table 4. Analysis of Phenylpropanolamine(PPA) in Contac600® by Standard Calibration Curve Method.

Sample No.	Labelled Amount	Amount taken for analysis	Sample Weight	Amount obtained (mg/L)
1	40.0 mg/capsule	40.0 mg/L	0.362	38.3
2			0.370	42.8
3			0.372	43.5
4			0.360	40.4
5			0.359	40.0
Mean			0.365	41.0
Standard Deviation			0.006	2.1

Table 5. Analysis of Phenylpropanolamine(PPA) in Haben F® by Standard Calibration Curve Method.

Sample No.	Labelled Amount	Amount taken for analysis	Sample Weight	Amount obtained (mg/L)
1	6.25 mg/capsule	62.5 mg/L	0.355	48.3
2			0.361	47.5
3			0.358	48.1
4			0.354	45.0
5			0.358	46.4
Mean			0.357	47.1
Standard Deviation			0.003	1.4

Table 6. Analysis of Phenylpropanolamine(PPA) in Sinoca® by Standard Addition Method.

Sample No.	Labelled Amount	Amount taken for analysis (mg/L)	Amount obtained (mg/L)
1	166.7 mg/100mL	16.67	15.98
2		16.67	15.63
3		16.67	16.96
4		16.67	17.05
5		16.67	15.67
Mean			16.26
Standard Deviation			0.70

Table 7. Analysis of Phenylpropanolamine(PPA) in Mabrin® by Standard Addition Method.

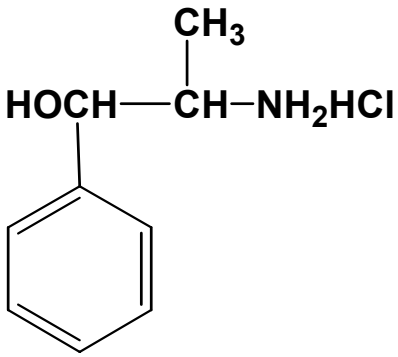
Sample No.	Labelled Amount	Amount taken for analysis	Sample Weight	Amount obtained (mg/L)
1	30.0 mg/capsule	30.0 mg/L	0.274	28.2
2			0.290	28.3
3			0.284	30.1
4			0.278	27.3
5			0.284	27.9
Mean			0.282	28.4
Standard Deviation			0.006	1.1

Table 8. Analysis of Phenylpropanolamine(PPA) in Contac600® by Standard Addition Method.

Sample No.	Labelled Amount	Amount taken for analysis	Sample Weight	Amount obtained (mg/L)
1	40.0 mg/capsule	40.0 mg/L	0.362	37.4
2			0.370	39.6
3			0.372	40.4
4			0.360	37.0
5			0.359	39.3
Mean			0.365	38.7
Standard Deviation			0.006	1.5

Table 9. Analysis of Phenylpropanolamine(PPA) in Haben F® by Standard Addition Method.

Sample No.	Labelled Amount	Amount taken for analysis	Sample Weight	Amount obtained (mg/L)
1	6.25 mg/capsule	62.5 mg/L	0.355	43.2
2			0.361	46.8
3			0.358	49.6
4			0.354	41.4
5			0.358	44.9
Mean			0.357	45.2
Standard Deviation			0.003	3.2



α-[Aminoethyl]benzenemethanol hydrochloride

Figure 1. Chemical Structure of the Phenylpropanolamine

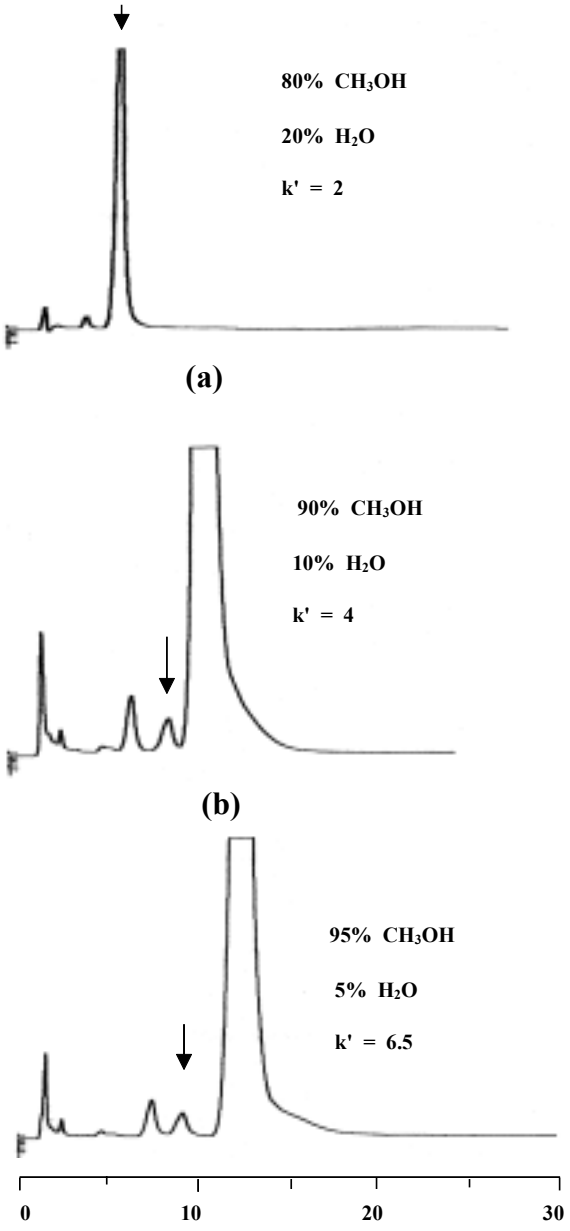


Figure 2. Systematic approach to the separation of Phenylpropanolamine in Over-The-Counter (Haben F®)

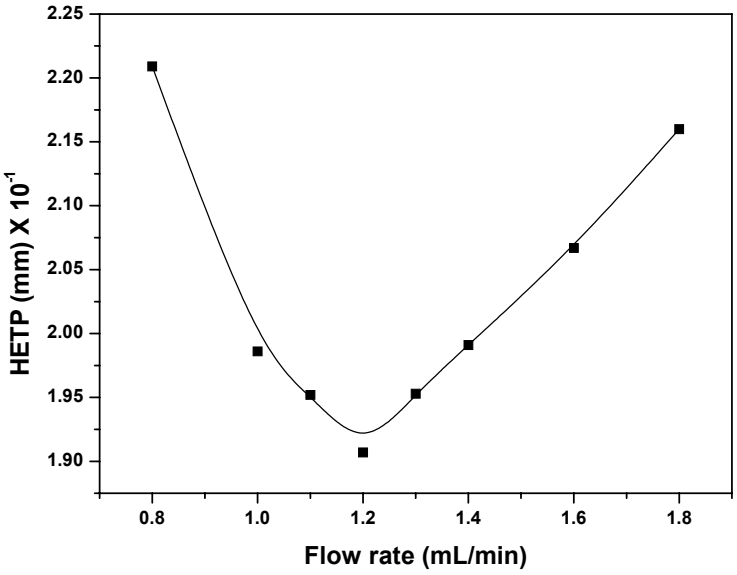


Figure 3. Effect of mobile phase flow rate

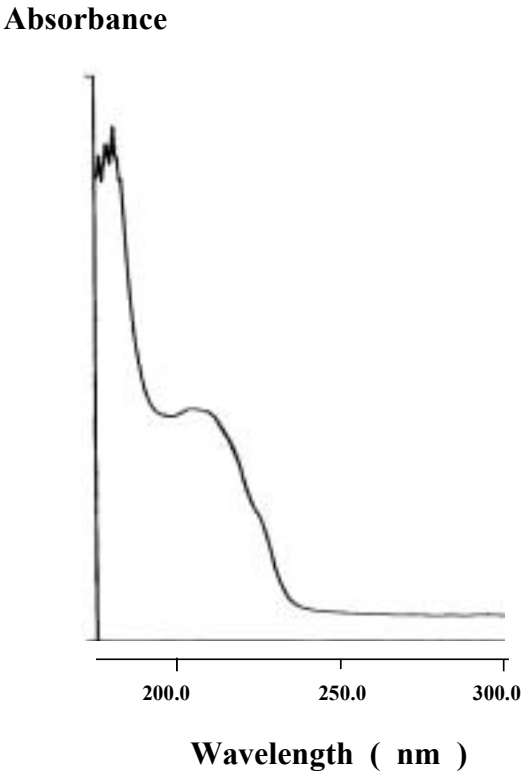


Figure 4. Absorbance Spectrum of Phenylpropanolamine

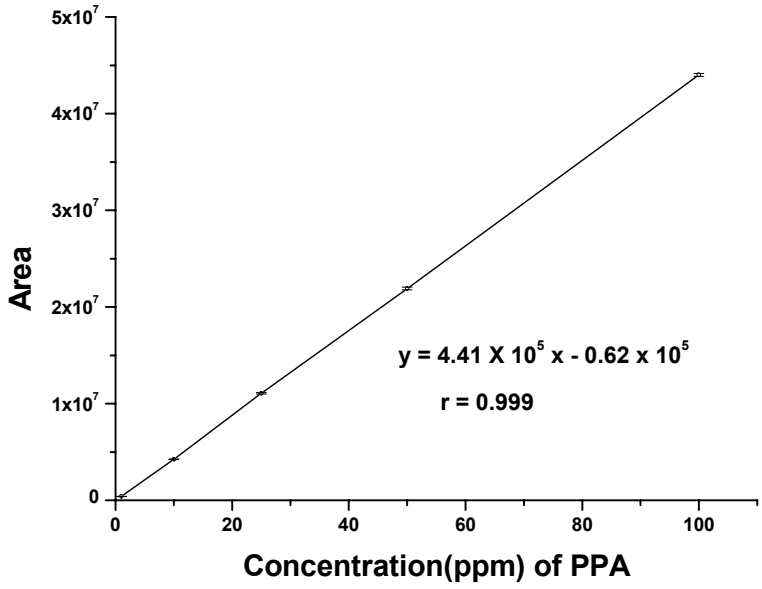


Figure 5. Calibration Curve of Standard Phenylpropanolamine

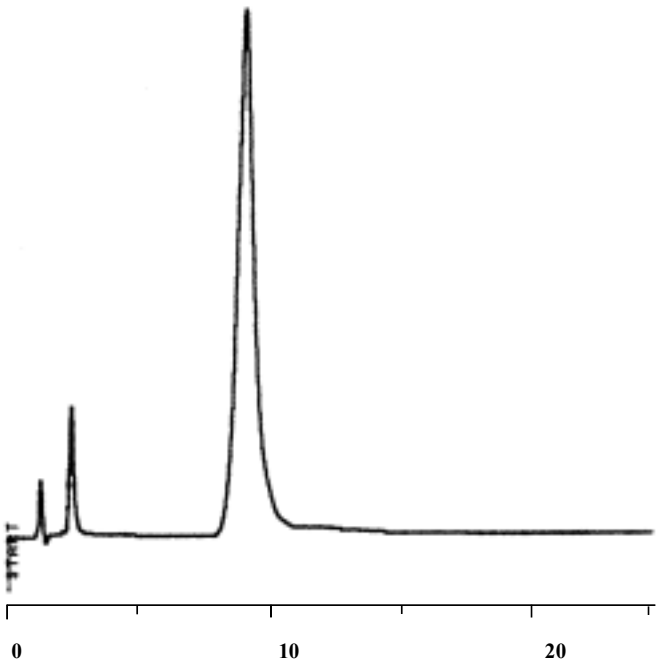


Figure 6. Chromatogram of PPA in Contac600®

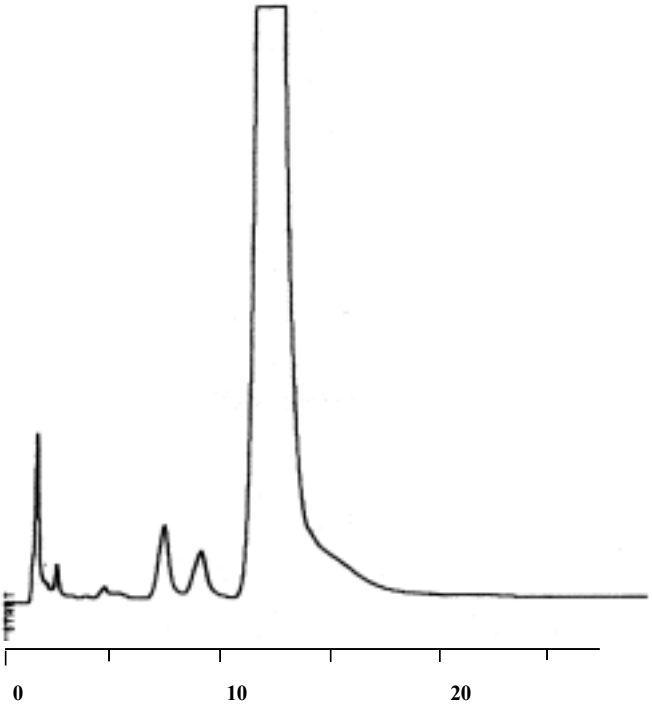


Figure 7. Chromatogram of PPA in Haben F®

Figure 8. Chromatogram of PPA in Mabrin®

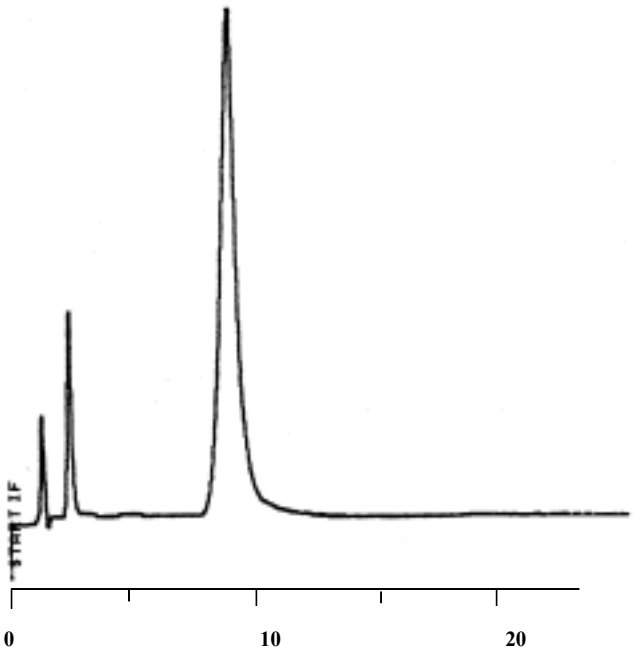


Figure 9. Chromatogram of PPA in Sinoca®

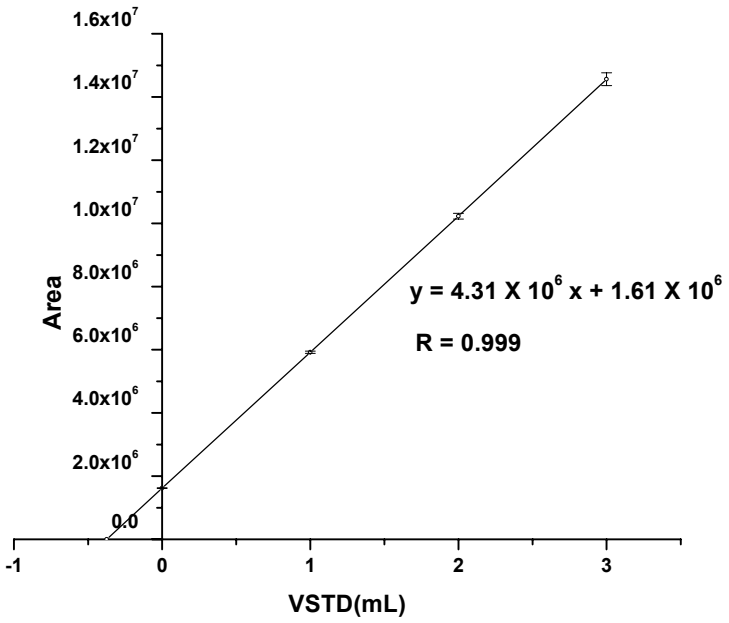


Figure 10. Standard addition curve of PPA in Contac600®

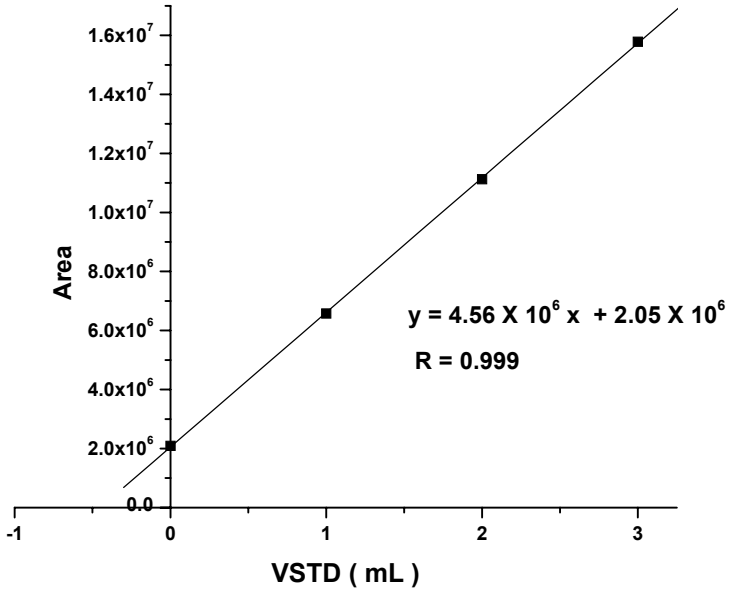


Figure 11. Standard addition curve of PPA in Haben F®

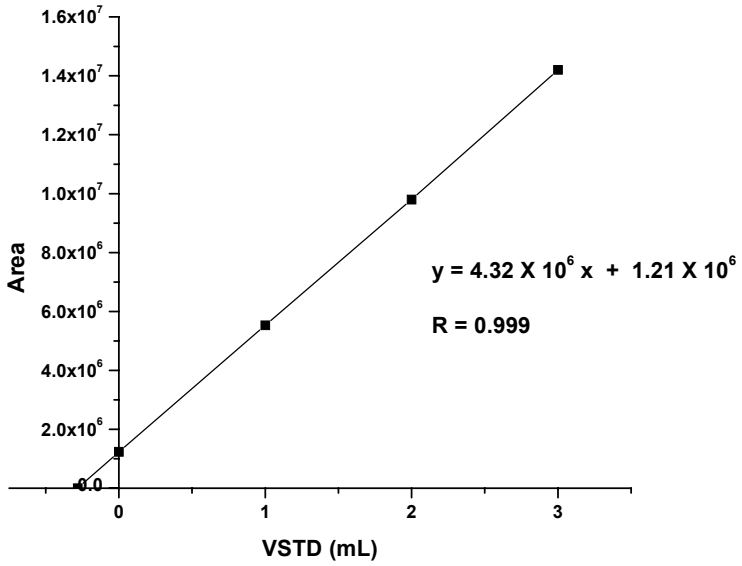


Figure 12. Standard addition curve of PPA in Marbrin®

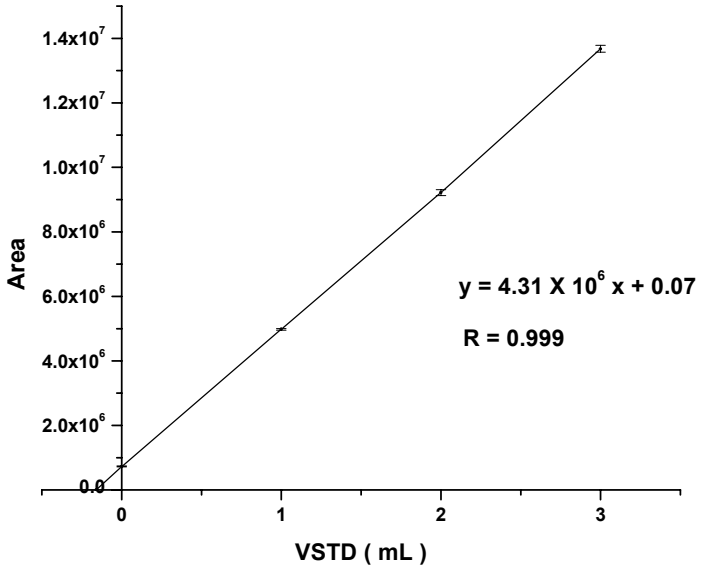


Figure 13. Standard addition curve of PPA in Sinoca®

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ABSTRACT

The Detection Method of Phenylpropanolamine in Pharmaceuticals by High Performance Liquid Chromatography(HPLC)

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고성능 액체 크로마토그래피를 이용하여 일반 의약품(감기약, 체중 조절제) 속에 함유된 phenylpropanolamine을 검출하는 방법에 대한 연구를 수행하였다. Phenylpropanolamine은 최근 출혈성 뇌졸중을 유발하는 약물로 Food and Drugs Administration(FDA)에 의해 그 사용이 중지된 약물 중 하나로, 현재 국내에서는 체중 조절제로서 사용이 중지된 상태이다.

이동상은 Methanol과 0.25mM potassium dihydrogenphosphate을 5:95의 부피비로 취하여 혼합액을 잘 섞은 후, 헬륨기체로 약 10분 동안 탈기시킨 후 사용하였으며, C_{18} 역상 컬럼을 이용하여 성공적으로 분리를 이루었다. 최적화된 분석 조건에서 최소검출한계는 4μg/L이었으며, 최소검량한계는 14μg/L이었다.

최적 조건에서 검정선법과 표준물 첨가법에 의한 방법으로 일반 의약품 속의 phenylpropanolamine을 정량하였으며 그 결과 상품에 표시된 양과 허용 오차 내에서의 일치됨을 알 수 있었다. 따라서 본 방법이 여러 가지 형태의 일반 의약품 속에 함유된 phenylpropanolamine을 검출할 수 있는 방법으로 유용함을 알 수 있었다.

ACKNOWLEDGMENTS

무언가 이루리라는 기대감과 부푼 희망으로 항상 새롭게 시작한다는 생각으로 지금껏 뛰어 왔지만, 돌이켜보니 이룬 것보다는 못한 것들에 대한 아쉬움으로 가득합니다. 힘들고 어려운 순간도 있었지만 많은 사랑하는 사람들의 도움으로 이 자리에 설 수 있었습니다. 음으로 양으로 도와 주신 많은 사람들을 떠올리며 이렇게 감사의 말을 전할려고 합니다. 지금껏 아낌없는 가르침과 따뜻한 충고와 조언으로 이끌어주신 유영재 교수님께 가슴깊이 감사를 드립니다. 항상 자신감을 심어주신 이용일 교수님, 이렇게 지면을 빌어 감사의 말씀 올립니다. 그리고, 대학생활 및 대학원 생활을 하면서 물심양면 많은 도움을 주신 백건호 교수님, 너그러움과 자상함을 가르쳐 주신 이창순 교수님, 지금은 미국에서 열심히 연구 중이신 마음 좋으신 신동수 교수님, 늘 따뜻한 눈으로 지켜봐 주신 이민주 교수님, 넉넉한 웃음과 많은 가르침을 주신 안철진 교수님, 작은 곳까지 가슴을 열어주신 원태진 교수님께 다시 한번 감사를 드립니다.

실험실에서 많은 시간을 함께 나누면서 누님처럼 마음을 열어 따뜻한 조언을 아끼지 않으셨던 김미경 박사님, 늘 곁에서 지켜봐주신 김원규 선생님, 정말 고마움을 어떻게 표현해야 할 지 모를 형이자, 어떤 때는 친구처럼 도움을 주신 강신봉 님, 항상 최선을 다하는 모습을 보여주신 미국에서 열심히 학업 중인 김재국 님, 다정히 신혼을 꿈꾸고 있는 임재민 님과 박성하 님, 넉넉한 웃음이 좋았던 최종수 님, 열심히 사시고 계실 김영욱 님, 서울에서 더 나은 미래를 준비 중이신 조효현 님, 따뜻한 말 한마디가 더 아쉬웠던 김영주 님, 깊고 넓은 마음을 가졌던 이선영 님께 감사드립니다.

무엇보다 오늘까지 생활을 같이 하면서 알게 모르게 너무나 의지했던 친구 변기환 님께 감사드리고, 조용히 지켜봐 주신 마음이 따뜻한 조영수 님께도 고마움을 전합니다. 주고 싶은게 너무 많았지만 주지 못했던 이정은 님, 힘들지만 웃음을 잃지 않던 나진희 님, 아쉬움이 많이 남는 김상득 님, 실험실의 귀염둥이 황현성, 권재열, 김혜경, 황인정 님들, 같이 웃으며 이야기 나누던 이원배, 이수경, 김승건, 이태희 님들, 따뜻한 충고와 조언을 주시던 최승철 님, 삶에 대해 이야기 나누었던 하진렬 님, 이끌어 주시던 박주희 님, 최경민 님 그리고, 송상용 님께 감사합니다.

늘 격려를 아끼지 않던 소중한 친구들이 있어 내 삶은 더욱 윤택할 수 있었습니다. 고추친구인 이건목 님, 애 아빠가 되신 김대은 님, 열심히 삶을 살고 있는 노재욱 님, 맑은 웃음이 생각나는 손선동 님, 항상 어른스러움을 보여주신 이인제 님, 열심히 공부 중인 진정훈 님, 님들아 고맙다. 큰형처럼 믿음을

심어주었던 김지언 님, 멀리 떨어져 있어도 내 생각할 김쌍호 님과 진정미 님, 너무나 많은 관심과 따뜻한 사랑을 주었던 김태훈 님, 믿음직스러운 김손권 님, 가슴이 넓은 배춘한 님, 바다 이유정 님, 그리고 많은 사랑하는 친구님들에게 감사드립니다.

너무나 큰 도움을 주시고 따뜻한 마음 주신 큰 자형 김덕수 님, 어떻게 갚아야 할 지 모를 만큼 큰 사랑 주신 큰 누님 한정숙 님, 멀리서 늘 지켜봐주시는 작은 자형 남상국 님, 가슴을 어루만져 주시던 작은 누님 한정미 님, 마음이 따뜻한 막내 자형 박찬우 님, 작은 곳까지 신경 써 주시던 막내 누님 한명숙 님, 거친 바다에 큰 배처럼 언제나 믿음을 주시던 형 한상헌 님, 주신 사랑이 너무너무 고마운 형수 양연화 님에게 감사드립니다.

관심어린 눈으로 마음을 주신 당숙님과 당숙모님, 큰 디딤돌이 되어주신 사촌형님 한기원 님, 한기태 님, 한기술 님께 감사드리며, 재종 형님 한기덕 님, 한기범 님, 조카 한정아 님, 한정석 님께 고마움을 전합니다.

언제나 마음 속에 가장 큰 자리를 잡고 계시는 어머님께 존경과 사랑으로 이 조그마한 결실을 바칩니다. 장한 아들이 되도록 열심히 살겠습니다. 그리고, 지금껏 지켜봐 주신 모습 그대로 저를 지켜봐 주십시오. 항상 최선을 다하는 모습으로 제게 주어진 길을 가겠습니다.