Tikrit Journal of Pharmaceutical Sciences 2013 9(2)

Spectrophotometric Determination of Procaine Hydrochloride in pharmaceutical preparations Via Oxidative Coupling Reaction

Mouyed Q. Al-Abachi*, Hind S. Al-Ward* and Emad M. Al-Samarrai**
*Department of Chemistry-College of Science-University of Baghdad-Baghgdad-Iraq
**Department of Pharmaceutical Chemistry-College of Pharmacy-University of Tikrit-Sallahdeen-Iraq

Received 13/10/2011 Accepted 15/5/2013

Abstract

A simple, reliable and sensitive spectrophotometric method for the determination of trace amount of procaine hydrochloride in pure and in pharmaceutical preparations (injections) has been developed. The method is based on the oxidative coupling reaction of procaine hydrochloride with phenothiazine and in the presence of ferric nitrate to form a green-water-soluble dye, which is stable and has a maximum absorption at 610 nm. A graph of absorbance versus concentration shows that beer's law is obeyed over the concentration range of 50-2000 µg of procaine hydrochloride in a final volume of 25 ml or (2-80 µg.ml-1) with a molar absorpitivity of 5.51 x 103 L.mol-1.cm-1, a Sandell sensitivity of 4.28 x 10-2 µg.cm-2, a relative error was less than 1.98% and a relative standard deviation was better than 1.72% depending on the concentration. The optimum conditions for full color development are described and the proposed method was applied satisfactorily to pharmaceutical preparations containing procaine Hydrochloride using standard addition method.

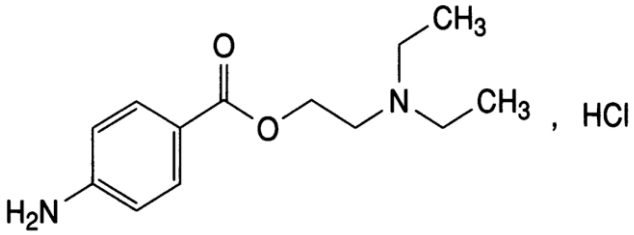
Key words: procaine hydrochloride, determination, spectrophotometry, phenothiazine, ferric nitrate, oxidative coupling reaction.

الملخص

تم تطوير طريقة بسيطة وحساسة لتقدير دواء هيدروكلورايد البروكائين طيفيا " بصورته النقية وفي المستحضرات الصيدلانية (الحقن). اعتمدت الطريقة على تفاعل الازدواج التأكسدي هيدروكلورايد البروكائين مع كاشف الفينوثيازين بوجود نترات الحديديك كعامل مؤكسد لتكوين صبغة خضراء ذائبة في الماء ومستقرة قيست طيفيا" عند الطول الموجي الاعظم 610 نانوميتر . اظهر منحني المعايرة استجابة خطية عند مدى من التراكيز بين 50−2000 مايكروغرام في حجم نهائي 25 مل (اي مايعادل 2−80 مايكروغرام لكل مل) وكانت قيمة معامل الامتصاصية المولارية مساوية ل $5.5 X 10^3$ لتر .مول$^{-1}$.سم$^{-1}$ وبلغت قيمة حساسية ساندل $4.28 X 10^{-2}$ مايكروغرام .سم$^{-2}$ والخطأ النسبي اقل من 1.98% والانحراف القياسي النسبي افضل من 1.72% اعتمادا" على التركيز . وتم دراسة الظروف المثلى لتفاعل وطبقت الطريقة لتقدير الدواء في المستحضرات الصيدلانية بوساطة طريقة الاضافة القياسية.

Introduction:

Procaine is a local anesthetic used alone or with penicillin as an antibacterial drug its chemical name is 2-diethyl aminoethyl-4-aminobenzoate hydrochloride and its formula $C_{13}H_{20}N_2O_2$, with molecular weight of 272.8 the structure of procaine HCl is [1]:



Procaine was first synthesized in 1905, it quickly replaced cocaine because it is easier to synthesize and sterilize, shorter duration of action, non addictive and it has four to six times less toxic than cocaine, procaine, like other local anesthetics such as tetracaine acts as a nerve block, halting the generation and conduction of nerve impulses signal pain, it is also used in obstetrics and some times for relief pain in the lower back and tooth extraction[2].

Several methods have been reported for the determination of procaine HCl include High performance liquid chromatography[3,4], differential-plus voltammetry and electrochemichal analysis [5-8], electrophoresis[9,10], atomic absorption [11], ion association titration [12], gas chromatography [13], fluorimetry [14,15] sequential injection analysis [16,17] colorimetry and spectrophotometric methods [18-27]. In this work a rapid and sensitive method using spectrophotometric detection at 610 nm was proposed for the determination of procaine HCl, the method is based on the oxidative-coupling reaction of procaine HCl with phenothiazine in the presence of ferric nitrate, to form a green water soluble dye. The proposed method has been applied successfully to the determination of procaine HCl in pharmaceutical preparations.

Experimental:

Apparatus:

-all spectral and absorbance measurements were carried out on a Shimadzu UV-visible -260 digital double-beam recording spectrophotometer using 1-cm Silica cell.

Reagents:

All chemicals used were of analytical reagents grade unless other wise stated. Procaine hydrochloride standard material was provided from the state company for drug industries and medical appliances (SDI), Sammara-Iraq.

Procaine stock solution (1000 $\mu g.ml^{-1}$):

A 0.1 gm amount of pure procaine HCl was dissolved in amount of distilled water then the solution was made up to 100 ml in a volumetric flask with same solvent.

Phenothiazine reagent ($5x10^{-3}$ M):

Prepared by dissolving 0.0996 gm of phenothiazine reagent in ethanol and diluted to 100 ml in a volumetric flask with the same solvent.

Ferric nitrate solution ($5x10^{-2}$M):

Prepared by dissolving 5.05 gm of ferric nitrate in 2.5 ml of HNO_3 (1M) and made up to 250 ml volumetric flask with distilled water.

Procedure:

In to a series of 25 ml calibrated flask, transfer increasing volumes of procaine HCl diluted solution (1000 $\mu g.ml^{-1}$) to cover the range of the calibration graph (50-2000 μg in a final volume of 25 ml). add 3 ml of ferric nitrate solution ($5x10^{-2}$M) followed by 1 ml of phenothiazine solution ($5x10^{-3}$M). Shake well and dilute the solution to the mark with distilled water. Allow the reaction mixture to stand for 10 min. at room temperature and measure the absorbance at 610 nm against a reagent blank prepared in the same way but containing no procaine HCl. The colour of the dye formed was stable for more than 90 min. For the optimization of conditions and in all subsequent experiments 1 ml of 1000 $\mu g.ml^{-1}$

of procaine HCl was used in a final volume of 25 ml.

Procedure for Pharmaceutical Preparations:

Four types of injections (different origin) containing Procaine HCl in Procaine Penicillin, these included:

1-Acacaine injections containing (600 mg of Procaine Penicillin)-Acay production.

An accurately weighed portion from mixed three vials powder , equivalent to about 0.241 gm of Procaine HCl , was transferred to a 100 ml volumetric flask and was dissolved and completed to the mark with distilled water to obtained 2410 $\mu g.ml^{-1}$ as a stock solution then transfer 41.5 ml from the stock solution to 100 ml volumetric flask and dilute with distilled water to prepare 1000 $\mu g.ml^{-1}$.

2- Procaine injections containing (300 mg of Procaine Penciliine and 100 mg of benzyl pencilline)-Pakstan production.

3-Procaine injections containing (300 mg of Procaine Penciliine and 100 mg of benzyl pencilline)-Indian production.

For each type of injections, an accurately weighed portion from mixed three vials powder, equivalent to about 0.12 gm of Procaine HCl , was transferred to a 100 ml volumetric flask and was dissolved and completed to the mark with distilled water to obtained 1200 $\mu g.ml^{-1}$ as a stock solution then transfer 83.3 ml from the stock solution to 100 ml volumetric flask and dilute with distilled water to prepare 1000 $\mu g.ml^{-1}$. For the three types of injections 1 ml of procaine HCl solution (1000 $\mu g.ml^{-1}$) was used for the analytical application.

4- Procaine Injections containing (100 mg of Procaine penicillin)- Sammara-Iraq.

Weight 0.04 gm from each vial and dilute to 100 ml with distilled water to prepare 400 $\mu g.ml^{-1}$. and 2.5 ml of procaine HCl solution (400 $\mu g.ml^{-1}$) was used for the analytical application.

Results and discussion

The factors affecting on the sensitivity and stability of the colored product resulting from the oxidative coupling reaction between Procaine HCl and phenothiazine in the presence of ferric nitrate were carefully studied. A typical spectrum for the colored dye formed was measured versus reagent blank which has negligible absorbance at λmax 610 nm (Figure 1).

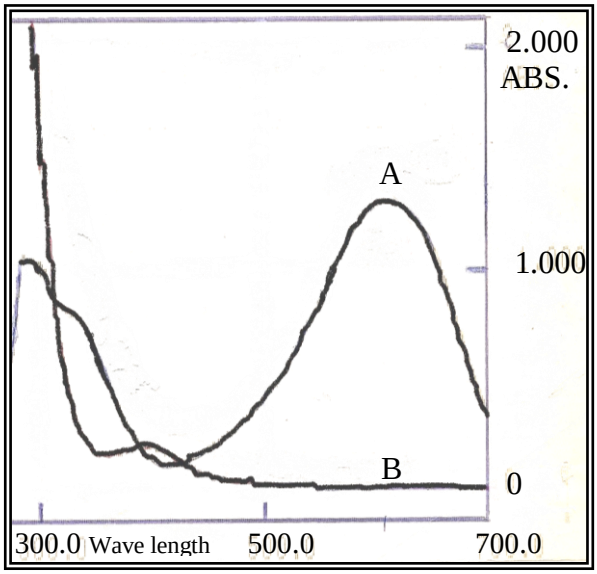


Figure 1:Absorption spectra of the colored dye against reagent blank (A) and blank against distilled water (B).

Study of the optimum reaction conditions:

The effect of various parameters on the absorption intensity of the dye formed were studied and the reaction conditions were optimized.

Effect of reagent concentration:

When various concentration of phenothiazine solution were added to a fixed amount of procain HCl, 1 ml of ($5x10^{-3}$ M) solution was found enough to develop the color to its full intensity and give a minimum blank value and this considered to be optimum for the concentration rang (2-80 µg/ml) of procaine HCl.

Phenothiazine solution in water is colourless but under the conditions described, it become slightly pink after addition of ferric nitrate which is probably due to an oxidation intermediate which subsequently enters the reaction with procaine HCl.

The absorption of the blank solution under the condition used was found to be 0.055.

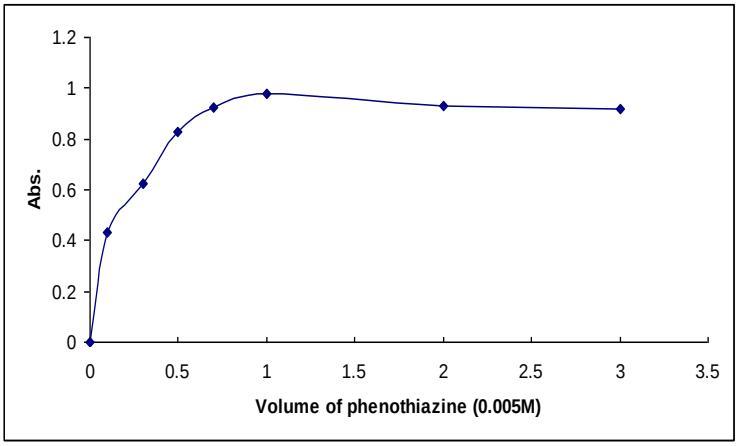


Figure 2: Effect of reagent volume on the dye intensity

Effect of oxidant concentration

The dye formation reached a maximum with about 3 ml volume of ($5x10^{-3}$M) ferric nitrate solution. Since it give a high sensitivity, minimum blank value and to ensure a quantitative determination at the upper limit of the calibration graph.

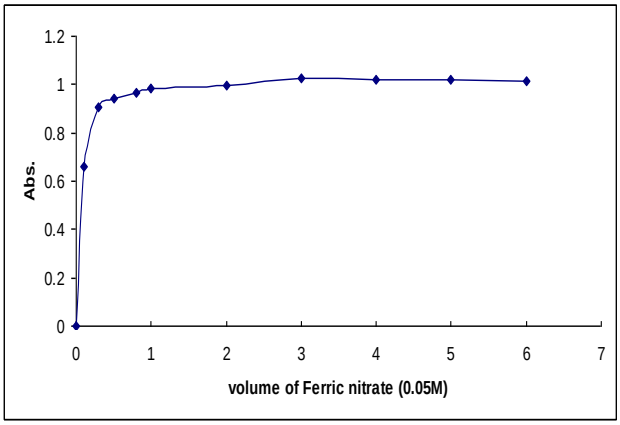


Figure 3: Effect of oxidant volume on dye intensity

Effect of reaction time

The color intensity reached a maximum after the drug procaine HCl had been reacted with phenothiazine and ferric nitrate for 10 min. Therefore, 10 min development time was selected as optimum in the general procedure. The color obtained was stable for at least 90 mins.

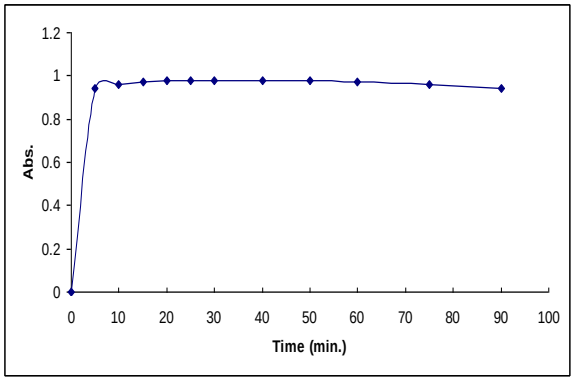


Figure 2: Effect of time on stability of dye

Effect of order of addition

To obtain optimum results the order of addition of reagents should be followed as given under the procedure, otherwise a loss in color intensity and stability was observed.

Effect of temperature

The effect of temperature on the color intensity of the dye was studied. In practice a high value of absorbance was observed when the color was developed at room temperature ($25C^{o}$), but a loss in intensity and stability was observed when the calibrated flask was placed in an ice bath at ($5\text{-}10C^{o}$) or in a water bath at ($45C^{o}$), therefore it is recommended that the color reaction should be carried out at room temperature ($25C^{o}$).

Accuracy and precision

To determine the accuracy and precision of the method, procaine HCl was determined at three different concentrations. The results obtained are shown in (Table 1). It indicates that a satisfactory precision and accuracy could be obtained with the proposed method.

Table 1: Accuracy and precision of the proposed method

Amount of procaine HCl µg.ml		Recovery*%	Relative standard deviation%	Error%
Present	Found			
20	20.40	101.98	1.720	1.980
40	39.99	99.98	0.480	-0.019
60	60.75	101.26	0.286	1.260

- for five determinations

Calibration Graph

Employing the condition described under procedure, a linear calibration graph (Figure 2), for procaine HCl was obtained which shows that Beer's law was obeyed over the concentration rang of 50-2000 µg in a final volume of 25 ml, (2-80 ppm), with a correlation coefficient of (0.9993) and an intercept of 0.0125. The conditional molar absorptivity of the green dye formed with reference to procaine HCl was found to be $5.510x10^{3}L.mol^{-1}.cm^{-1}$ and a sandell sensitivity of $4.280x10^{-2}$ $\mu g.cm^{-2}$.

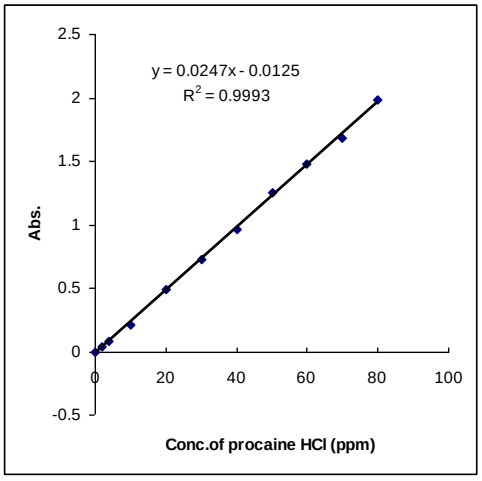
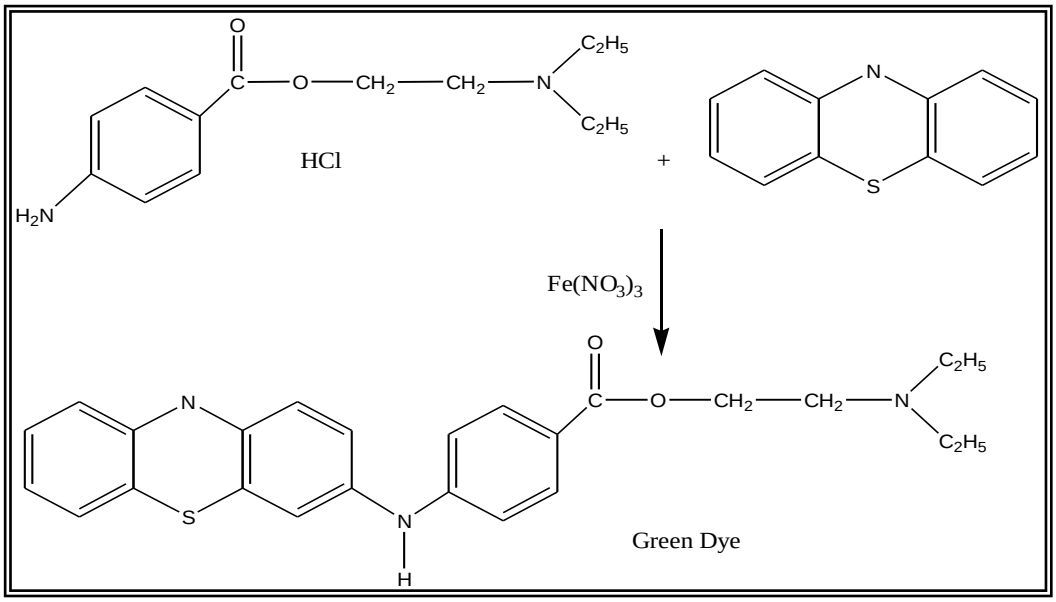


Figure 2: a calibration graph of procaine HCl

Structure of the dye

The formed dye was soluble in water and the stoicheiometry of the reaction between procaine HCl and phenothiazion was investigated using the Job's method. The results obtained (figure3) shows that a (1:1) product is formed between the drug and the reagent at 610 nm , therefore the formation of the dye probably occurs as follows:



Scheme 1: reaction sequence

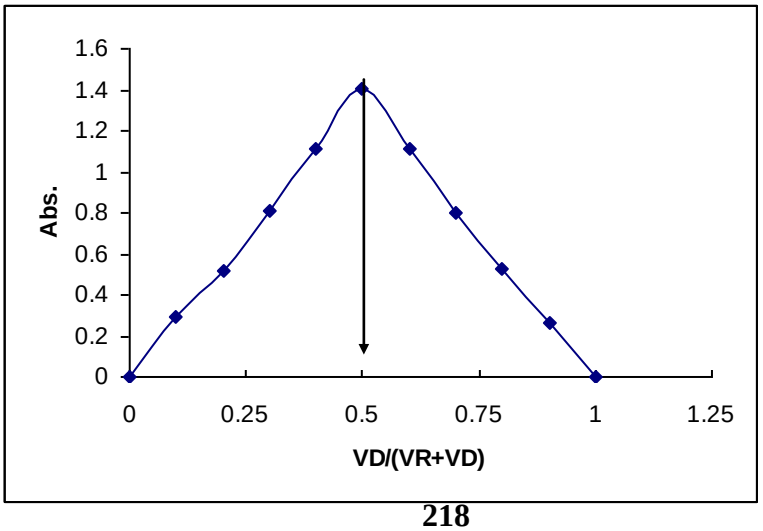


Figure 3: Continuous variation plot

The apparent stability constant was calculated by comparing the absorbance of a solution containing stoicheiometric amount of procaine HCl and phenothiazine and fixed amount of oxidant with that of solution containing five-fold excess of phenothiazine reagent and fixed amount of oxidant. The average conditional stability constant of the dye in water under the described experimental condition is ($4.32x10^6$ $L.mol^{-1}$) as shown in (Table 2).

Table 2. The stability constant

Conc. of Drug($\mu g.ml^{-1}$)	C (M) $x10^{-5}$	A_s*	A_m*	A	K ($L.mol^{-1}$) $x10^5$
4	1.69	0.109	0.119	0.084	7.67
20	8.46	0.407	0.432	0.057	3.33
40	16.92	0.957	1.010	0.052	2.03

*** for five determinations**

Analytical Applications

Four types of injections (different origin) containing procaine HCl as a Procaine Penicillin have been analyzed by the proposed method and the results obtained are summarized in (Table 3)

Table (3): analytical applications

Injections	Concentration of Bromhexine HCl $\mu g.ml^{-1}$		Error%	Recovery %	R.S.D%*
	Present	Found*			
Acacaine injections 600 mg of Procaine Penicillin)-Acay production	40	46.29	+15.75	115.75	1.65
Procaine injections (300 mg of Procaine Penciliine, 100 mg of benzyl pencilline)-Pakstan production.	40	49.00	+22.50	122.50	1.03
Procaine injections (300 mg of Procaine Penciliine, 100 mg of benzyl pencilline)-Indian production.	40	43.05	+7.62	107.62	0.98
Procaine Injections 100 mg of Procaine pencilline) - Sammara-Iraq.	40	44.00	+10.06	110.06	0.79

*for three vials

The results obtained (table 3) indicates a high recovery values in compression with British pharmacopoeia method using the usual calibration method. Therefore, the standard addition method[28] was applied to determine the procaine HCl in various injections samples and a good recovery was obtained as shown in (Table 4).

Table (4): comparison between the recoveries of calibration method and standard addition method and british pharmacopia method.

Injections	**Concentration of Bromhexine HCl $\mu g.ml^{-1}$**		**Recovery%**		
	Present	Found by standard addition method	Calibration method	Standard addition method	British pharmacopeia method

Acacaine injections 600 mg of Procaine Penicillin)-Acay production	40	39.46	115.75	98.65	99.45
Procaine injections (300 mg of Procaine Penciliine 100 mg of benzyl pencilline)-Pakstan production.	40	40.40	122.50	101.00	101.09
Procaine injections (300 mg of Procaine Penciliine 100 mg of benzyl pencilline)-Indian production.	40	41.20	107.62	103.00	99.90
Procaine Injections 100 mg of Procaine Benzylpencilline)-Sammara-Iraq.	40	40.00	110.06	100.00	101.00

Evaluation of the proposed method

For evaluating the competence and the success of the proposed method, the results obtained were compared with those by British pharmacopeia method (standard method)[(1)] , the same pharmaceutical preparations for Procaine HCl were analyzed by the standard addition method. The results obtained were statistically compared, using the Student t-test and variance ratio F-test at 95% confidence level [(29)], the calculated t- and F-values did no exceed the theoretical values, which indicate that there is no significant difference between the methods in the determination of Procaine HCl in pharmaceutical preparations (table 5).

Table (5): The comparison of the proposed method with standard method using t- and F-statistical tests

The pharmaceutical preparations for 40 µg.ml^{-1}	Standard addition method		British pharmacopeia method	
	Rec.%	$(Xi-Xi^{-})^2_1$	Rec.%	$(Xi-Xi^{-})^2_1$
Pure procaine HCl	100.00	0.280	100.92	0.202
Acacaine injections Acai Production	98.65	3.534	99.45	1.040
Procaine injections)-Pakstan production.	101.00	0.220	101.09	0.384
Procaine injections Indian production.	103.00	6.1009	99.90	0.324
Procaine Injections Sammara-Iraq.	100.00	0.280	101.00	0.280
	$(Xi^{-})_1$= 100.53	$\sum(Xi-Xi^{-})^2_1$= 10.414	$(Xi^{-})_2$= 100.47	$\sum(Xi-Xi^{-})^2_2$= 2.23

$F_{calculated} = S_1^2 / S_2^2 = 2.603/0.557 = 4.673$

$F_{theoretical} = 6.39$ $F_{theoretical} > F_{calculated}$, at 95% confidence level**,**

$T_{calculated} = 0.075$, $T_{theoretical} = 2.776$

$T_{theoretical} > T_{calculated}$; at 95% confidence level**.**

Conclusion

A simple, accurate and sensitive spectrophotometric method has been proposed for the determination of trace amount of Procaine HCl in aqueous solution based on its oxidative coupling reaction in the presence of ferric nitrate at room temperature, the proposed method has some advantages like the fast determination of the drug on its pure form and in pharmaceutical preparations also it did not require temperature control, solvent extraction and expensive reagents and solvents.

The wide linear rang that obeyed Beer' low of the proposed method gave a good application for the pharmaceutical preparation .

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