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RESEARCH ARTICLES / BİLİMSEL ARAŞTIRMALAR

Dissolution of Reserpine Tablets by High Pressure Liquid Chromatographic with Fluorescence Detector and Fluorescence Spectrophotometric Determination

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Dissolution of Reserpine Tablets by High Pressure Liquid Chromatographic with Fluorescence Detector and Fluorescence Spectrophotometric Determination

Summary : *Reserpine is used as an antihypertensive agent in small doses of 0.10 - 0.25 mg and combined with diüretics.During the quality controls on tablets containing reserpine, we had to choose a very sensitive assay and dissolution rate method due to small doses of reserpine. In our study, we investigated the dissolution rate conditions of the tablets such as composition and volume of dissolution media, and stirring rates. We applied two methods as fluorescence spectrophotometry and high pressure liquid chromatography (HPLC) with fluorescence detection for the determination of reserpine. The results obtained from HPLC method were found more sensitive and suitable than those of fluorescence spectrophotometric method in order to identify dissolution properties of reserpine. The statistical results showed that there was a significant difference between two methods ($p<0.05$).*

Key words: *Reserpine tablets, dissolution rate, HPLC method, fluorescence spectrophotometry*

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Reserpin Tabletlerin Çözünme Hızının Floresans Dedektörlü Yüksek Basınçlı Sıvı Kromatografi ve Floresans Spektrofotometri Yöntemleri ile Tayini

Özet : *Reserpin içeren tabletler, 0.1-0.25mg arasında değişen küçük dozlarda tedavide kullanılmaktadır. Bu nedenle Reserpin içeren tabletlerde yapılan kalite kontrolleri sırasında oldukca duyarlı bir miktar tayini ve çözünme hızı yöntemi tercih edilmelidir. Çalışmamızda Reserpin içeren tabletlerin çözünme hızı koşulları; çözünme ortamı bileşimi, hacmi, karıştırılma hızı gibi faktörler incelenmiş ve etken maddenin miktar tayini için iki yöntem, "Florometrik spektrofotometri ve Florometrik dedektörlü yüksek basınçlı sıvı kromatografisi (HPLC)" uygulanmıştır. Florometrik dedektörlü HPLC yöntemi kullanılarak yapılan miktar tayini analizinden elde edilen sonuçların, florometrik spektrofotometri yönteminden elde edilen sonuçlara göre daha anlamlı olduğu ve Reserpin içeren tabletlerin çözünme hızı analizinde alternatif olarak uygulanabileceği gözlenmiştir.İstatiksel olarak yapılan değerlendirme sonuçları her iki yönteme göre yapılan çözünme hızı verileri arasındaki farkın anlamlı olduğunu ($p<0.05$) göstermiştir.*

Anahtar kelimeler: *Reserpin tablet, çözünme hızı, HPLC yöntemi, florometrik spektrofotometri*

INTRODUCTION

Reserpine is used as an antihypertensive agent in small doses of 0.10 - 0.25 mg and combined with diüretics. The distribution inhomogeneity of small quantities of reserpine in tablet formulations can lead to certain problems in content uniformity, dissolution rate and bioavailability of preparations[1]. In the preparation of solid dosage forms, powders must normally be mixed and processed to ensure homogeneity of drug content and uniform distribution of excipients[2].The distribution of drugs in tablets depends upon mixing criteria. The type of distribution as well as the degree of variation between tablets has been shown to be of importance in content uniformity studies on tablets containing small amouts of potent drugs[3]. When assessing the content uniformity of a solid dosage form containing a small amount of po-

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tent drug, an examination of the type of distribution is also desirable[4]. Therefore, a precise method for assay of tablets containing a small amount of drug should also be established.

Sam et al.[5] used high performance liquid chromatography with fluorescence detector for determination of reserpine in plasma. Suckow et al.[6] developed a liquid chromatographic method coupled with fluorescence detector to achieve sensitive and rapid method for reserpine assay. Yin et al.[7] examined dissolution of reserpine tablets using fluorescence spectrophotometry .

In our study, we aimed to determine dissolution rate conditions and to compare two assay methods, HPLC with fluorescence detection and fluorescence spectrophotometry for reserpine in tablets containing 0.25 mg of reserpine and 50 mg of hygroton[8,9].

MATERIALS and METHODS

Materials

Reserpine and commercial tablets containing reserpine and hygroton were supplied from Ciba-Geigy. Acetic acid, methanol, n-propyl alcohol, chloroform, hydrochloric acid, sulfuric acid, sodium nitrite were obtained from Merck.

Methods

1- Determination by fluorescence spectrophotometry
Fluorescence spectrophotometry stated in USP XXII was used for assay of reserpine[9]. Stock solutions of 1 mg/mL of reserpine were prepared in mixture of phosphate buffer (pH 8.0) and n-propyl alcohol (3:2), and treated according to USP XXII. The fluorescences of the solutions were measured in fluorescence spectrophotometer (Spex Nova) arranged to deliver activation radiation at 405 nm and to measure the resultant fluorescence at the emission wavelength of about 500 nm.

1.1. Dissolution test

Procedure was performed according to monograph of "Reserpine and Chlorothiazide Tablets"in USP XXII[9]. Dissolution test was carried out at 37.0 ± 0.5°C using the USP paddle method (apparatus II) at 75 rpm. A 900 mL volume of phosphate buffer (pH 8.0) and n-propyl alcohol (3:2) was used as the dissolution medium. At the suitable time intervals for a period of 60 min, 5 mL aliquot portion of the dissolution medium was withdrawn. The samples were treated according to USP XXII and their fluorescences were measured at the same conditions above. The amount of reserpine dissolved was calculated and evaluated. (n=3)

2. Determination by HPLC

HPLC (Schimadzu RF 55) was used to determine amount of reserpine dissolved. The applied conditions in this procedure were as follows :

- Detection : By fluorometry
- Detection wavelength : 280 nm (excitation), 340 nm (emission)
- Column : Nucleosil C18 CN
- Column temperature : Room temperature (15 - 25°C)
- Mobile phase : Methanol - water - acetic acid (63:36:1)
- Flow rate : 1 ml/min
- Injection volume : 10 µl
- Retention time : 2.6 min
- Chromatography time : 5 ml

2.1. Standard curve of reserpine

Stock solutions of 40 µg/ml of reserpine were prepared in mobil phase and further diluted to 0.2 - 1.4 µg/ml. The solutions (10 µl) were injected on column. The peak areas of reserpine were plotted against concentrations. The linear regression analysis was applied to these data.(n=3)

2.2. Dissolution test

The dissolution test of reserpine tablets was performed at 37.0 ± 0.5°C using the USP paddle method at stirring rates of 50 and 100 rpm. 250 mL and 500 mL volumes of mobile phase (pH 3.5) were used as the dissolution medium. At suitable time intervals for a period of 60 min, 1 mL of samples were withdrawn from dissolution medium. 20 µL of these samples were injected on column and then detected by fluorometry. The amount of reserpine dissolved was calculated from the calibration equation above by the area of peak in the chromatograms.

RESULTS and DISCUSSION

According to USP XXII test method , the values of the percentage of reserpine dissolved versus time were shown in Figure 1. Dissolution data of reserpine was

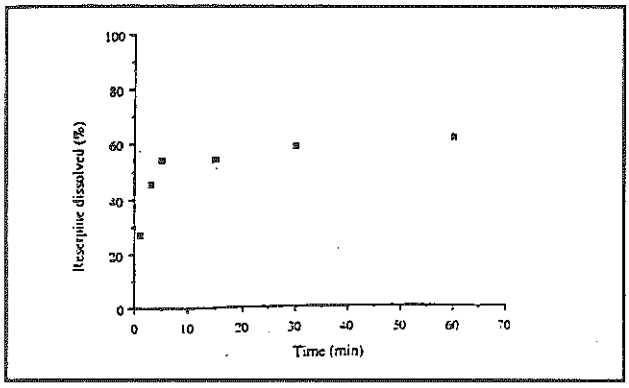

Figure 1. The dissolution profile of reserpine tablets according to the USP XXII test method (n=3)

applied to the equations of zero order, first order, RRSBW, Hixson-Crowell[10-12].Dissolution rate constants (k) and determination coefficients (r^2) were calculated, and presented in Table 1. According to the kinetic examinations, obtained r^2 values were very low, therefore no kinetic harmony could be assessed (Table 1).

Table 1. The kinetic evaluation of data obtained from dissolution of reserpine tablets according to the USP XXII test method

Kinetics	k*	r^{2**}	τd^{***}	β^{****}
Zero order	0.382 ($\mu g.min^{-1}$)	0.479	-	-
First order	8.23×10^{-3} (min^{-1})	0.384	-	-
Hixson-Crowell	0.0127 % $\mu g^{1/3} min^{-1}$	0.540	-	-
RRSBW	-	0.800	2.45	0.237

k* : Dissolution rate constant

r^{2**} : Determination coefficient

τ_d^{***} : Time at which 63.2 % of reserpine is dissolved

β^{****} : Shape coefficient

The amount of reserpine dissolved at the end of 60 minutes, was approximately 60 % (Figure 1). It was concluded that the amount of reserpine could not be determined precisely by using fluorescence spectrophotometric method.

Hence, HPLC method coupled with fluorescence detection was developed to measure reserpine concentrations and adapted to the USP XXII paddle method. A sample chromatogram of reserpine was shown in Figure 2.

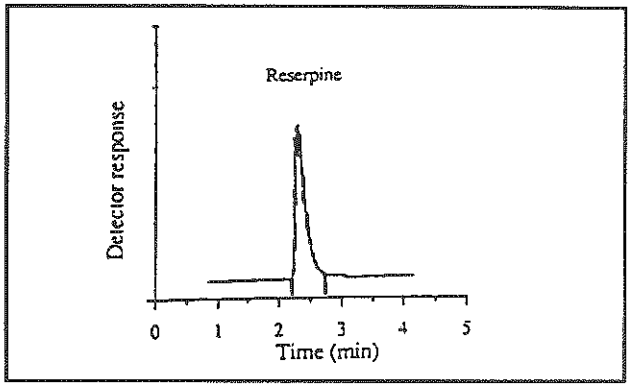

Figure 2. Sample chromatogram of reserpine solution containing 0.2 µg/ml (Chromatographic conditions as described in text)

It is stated in the USP XXII that not less than 75 % of the labelled amount of reserpine should be dissolved in 60 minutes. In the HPLC method, we used the mobile phase as the dissolution medium. Dissolution test was carried out at two different stirring rates using two different volumes of dissolution medium in order to meet the USP statement and to produce an acceptable dissolution profile for reserpine. At the different dissolution conditions, the percentage of reserpine dissolved versus time was shown in Figure 3 and these values were also given in Table 2.

Table 2. The data obtained from dissolution tests performed at the different conditions for reserpine tablets (n=3).

Time	Reserpine dissolved (%)			
min	50 rpm 500 mL	50 rpm 250 mL	100 rpm 500 mL	100 rpm 250 mL
5	59.7	51.9	89.4	88.9
10	70.5	61.8	97.7	100.0
15	75.1	67.5	99.3	100.0
30	79.1	72.6	99.9	99.9
45	90.5	76.0	100.0	100.0
60	97.1	71.1	-	-
75	100.0	74.2	-	-

Reserpine dissolved rapidly in the dissolution test performed at 100 rpm using 250 mL volume of dissolution media and all amount of drug in the tablets dissolved in ten minutes. When the stirring rate was decreased to 50 rpm, the dissolution rate of drug was slower than the dissolution at 100 rpm. Amount of reserpine dissolved was 74.2 % in 75 minutes. The vol-

ume of dissolution medium elevated to 500 mL in order to enhance dissolved reserpine amount at the same stirring rate. As it was seen in Table 2, at the end of 75 minutes, all amount of drug was dissolved. The amount of reserpine dissolved (97.1 %) in 60 minutes was not less than 75 %. According to the data obtained at 100 rpm using 500 mL volume of dissolution medium, the dissolution of reserpine was found to be faster (Table 2).

The results showed that the dissolution test carried out at the stirring rate of 50 rpm using 500 mL volume of mobile phase as the dissolution medium, gave the most consistent dissolution results. The best dissolution profile of reserpine was observed at same conditions (Figure 3).

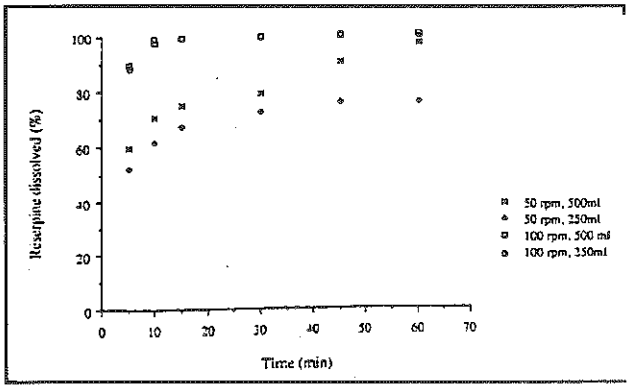

Figure 3. The dissolution profiles of reserpine tablets determined at different conditions (n=3)

The data obtained from dissolution test performed at 50 rpm using 500 mL of dissolution medium, were applied to different kinetics. The values obtained from kinetic evaulation were given in Table 3. When these values were compared with the values obtained from USP XXII dissolution method for reserpine in Table 1, it was observed that r^2 values for HPLC method were higher than that of USP XXII method. It was seen that reserpine dissolution followed Hixson Crowell's kinetic model and the highest r^2 value was obtained.

Furthermore, we compared dissolution data obtained from HPLC method and USP XXII method for tablets containing reserpine, and analysed with two sample analysis statistical test. As a result we observed significant difference between these two methods ($p<0.05$).

Table 3. The kinetic evaluation of data obtained from dissolution test performed at a stirring rate of 50 rpm, a volume of 500 mL for reserpine tablets according to HPLC method

Kinetics	k*	r^{2**}	τ_d^{***}	β^{****}
Zero order	6.64×10^{-3} ($\mu g.min^{-1}$)	0.902	-	-
First order	0.538 (min^{-1})	0.939	-	-
Hixson-Crowell	0.0448(% $\mu g^{1/3}$ min^{-1})	0.963	-	-
RRSBW	-	0.944	6.60	0.751

k* : Dissolution rate constant

r^2 : Determination coefficient

τ_d^{***} : Time at which 63.2 % of reserpine is dissolved

β^{****} : Shape coefficient

CONCLUSION

In this study, we developed a high pressure liquid chromatographic method with fluorescence detection to achieve a sensitive dissolution procedure for tablets containing small quantities of reserpine. This method can be used as an alternative procedure for determining dissolution properties of reserpine in combined with diuretics.

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