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Development of high performance liquid chromatographic method for determination of famotidine and ranitidine.HCl in pharmaceutical preparations

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Abstract

A rapid and sensitive high performance liquid chromatographic method has been developed , validated and applied for the determination of famotidine and ranitidine.HCl in pharmaceutical preparations.The effects of pH, mobile phase composition and concentration of organic modifiers on retention of the investigated drugs were studied. Famotidine and ranitidine.HCl were chromatographically separated from tablets using two columns of different polarity ; Lichrosorb RP-18 (15cm x 4.6mm i.d) 5μm particle size and Lichrosorb RP-8 (25cm x 4.6mm i.d) 5-10 μm particle size with mobile phase composed of 20:80 v/v of acetonitrile in 0.2% diethyl amine in water (pH=3). UV detection was set at 270nm for famotidine and 322nm for ranitidine.HCl . The calibration graph was linear in the concentration range of 20-70μg $.ml^{-1}$ for famotidine and 20-120μg $.ml^{-1}$ for ranitidine. The RSD% was not more than 0.85% and relative error between -0.75-2.08% indicating a good precision and accuracy. The limits of detection were found to be (1.13) and (0.83)μg$.ml^{-1}$ for famotidine and ranitidine.HCl respectively with recoveries ranged from 98 to 99% .

Introduction

The description of selective histamine H_2 - receptor by Black in 1970 was landmark in the history of pharmacology and set the stage for the modern approach to the treatment of acid - peptic disease, which until then had relied almost entirely on acid neutralization in the lumen of the stomach(Goodman & Gilmans,2001).Famotidine and ranitidine -HCl are histamine H_2 receptor antagonists , that potently inhibits gastric acid secretion and commonly used in treatment and prevention of gastric and duodenal ulcers(Langtry et al. ,1989). In the literature,several methods have been proposed for the determination of famotidine and ranitidine including spectrophotometric (Amin et al.,2002,Sadana et al., 1986,Dealmeida et al., 1993 and Chattaral et al., 1989) potentiometric(Tuncel & Atkosar,1989) and coulometric (Nicolic et al., 1995) titrimetric, Electrochemical

(Delgado et al., 1985),capillary electrophoresis(Marine et al.,2002), chemiluminescence (Barnett et al.,1999) ,flow injection analysis FIA(Lopez-Erroz et al.,1996), and high performance liquid chromatographic(HPLC)(Huang et al., 1999,Ficarra et al.,1987 and Biffar et al.,1986)methods. Among these methods HPLC technique was found to be rapid ,simple , versatile , precise and specific.

The success of the HPLC method depends on a good selection of number of factors such as: type , concentration and pH of mobile phase , flow rate , temperature and column dimensions(Otles & Hisil,1993), which were applied in this paper for the simultaneous determination of famotidine and ranitidine in pharmaceutical preparations using a developed HPLC system by optimizing parameters that affecting the separation .

Experimental

1. Reagents and chemicals .

All reagents used were of analytical grade except acetonitrile and methanol , which were HPLC grade . Acetonitrile and methanol were purchased from across organics (Belgium) ,and diethylamine (Merck ,Germany) was used to prepare the mobile phase .o-phosphoric acid (Fluka) was used for adjusting the pH values . Standard medical drugs (as active ingredient) Famotidine was obtained from (S.D.I) and Bilim (Turkey), while ranitidine -HCl was purchased from (S.D.I) and Ranbaxy (India).

2. Preparation of mobile phase and standard solutions .

A mobile phase consisting of 20:80 v/v of acetonitrile in 0.2% diethylamine in water (pH=3).The pH of the mixture was adjusted to 3.0 with (1M) H_3PO_4 . After preparation , it was filtered and degassed in an ultrasonic bath prior to use .

Stock solution (200$\mu g.ml^{-1}$) of ranitidine –HCl was prepared by dissolving 0.05 gm in 250 ml methanol . Stock solution of famotidine (100 $\mu g.ml^{-1}$) was prepared by dissolving 0.025gm in 250ml of mobile phase solution .

3. General procedure for preparation of the sample solutions .

Famosam tablets (Limassol-Cyprus): Each tablet contains 20mg famotidine. A number of famosam tablets were grinded to fine powders ,sieved and 100mg portion of the powder ,equivalent to the average weight of 20 tablets ,was dissoved in about 100ml (mobile phase solution). The the filterate was diluted to 20ml with mobile phase solution .

***Histac tablet (Ranbaxy labratories limited)*:** Each tablet contains 150mg ranitidine -HCl. A number of tablets were grined into fine powder, sieved and then a portion of the powder (150mg),equivalent to the average weight of 20 tablets,was dissolved in 50ml (methanol).The solution was then vigorously shaked for 30min,then filtered and 1ml of the filterate was diluted to 50ml with methanol .

4. HPLC equipment

The chromatographic system consists of : 1100 series liquid chromatography system (Koria) consisting of a pump LC series CE 1100, high performance detector CE 1200 .Two columns with different polarity were used ;lichrosorb RP-18(15cm x 4.6mm i d) with 5μm partical size and lichrosorb RP-8(25cm x 4.6mm i.d) 5-10μm partical size. A sample injector was 6CE model;with 20ml Injection loop.

Results and discussion

1. Percentage of organic modifier in the mobile phase

The amount of organic modifier present in the mobile phase will influence analytes that are retained by adsorption onto the stationary phase . Acetonitrile was used as a typical mobile phase modifier for this study. It was mixed with 0.2%(v/v) diethylamine in water (pH 3).

The results obtained (Fig.1) indicate that the retention times of the famotidine and ranitidine decreased as the percentage of acetonitrile raised from 10 to 30%.

The best results were obtained when the percentage of acetonitrile in the mobile phase is 20% .

Figure 2 shows the effect of the percentage of diethylamine (DEA) in the mobile phase on the retention times of famotidine and ranitidine. As the DEA increases from 0.1 to 0.2% ,the retention decreases.

2. Effect of pH and buffer concentration .

The effect of pH on the retention time of the analytes and their separation was investigated over the pH range 2.5-5.5 using 0.2% diethylamine solution . The retention time of famotidine and ranitidine decreased with decreasing pH of the mobile phase (Fig. 3) . A good separation of the investigated drugs and short time of analysis were obtained when the pH of mobile phase is 3.0.

3. Effect of flow rate of the mobile phase .

To investigate the effect of the flow rate on the retention time (t_R) of the famotidine and ranitidine , the composition of the mobile phase was held constant with 20% acetonitirile (pH 3.0) at 30c^o . The result (Fig. 4) show that the retention time of the famotidine and ranitidine decreased with increasing the flow rate . The aim of choosing the optimum flow rate is to obtain a short analysis time , which in turn prevents solute band broading ; this finally leads to increase column efficiency(Sawyer et al, 1989). A flow rate of 1ml / minute was selected to obtain maximum resolution in a suitable analysis time.

4. Column temperature .

The effect of column temprature in the range of 30 to 45c^o on the retention time values of drugs was investigated . Generally increasing column temperature in RP-chromatography decreases the (t_R) of the separated bands and increses column efficiency by decreasing mobile phase viscosity , which in turn lowers the column head pressure(Dikran 1996; Knox & Majors, 1975)**.**

5. **Recommended analytical conditions .**

From the optimum experimental condition it can be concluded that the mobile phase containing 20:80 v/v of acetonitrile in 0.2% diethylamine in water (pH=3), and flow rate (1ml / min) would provide good retention time of famotidine and ranitidine as well as an acceptable run time of less than 8 minutes for the separation .

Method validation

Linear detector response for the peak height of famotidine was in the concentration range (20-70) μg.ml^{-1} with a correlation coefficient of 0.9994,showed in fig(5) while the linearity for ranitidine was in the concentration range (20-120) μg.ml^{-1} with a correlation coefficient of 0.9998 showed in fig(6) . The limit of detection of famotidine was 1.13 μg.ml^{-1} and for ranitidine was 0.83 μg.ml^{-1} and the relative standard deviation (RSD) was in the range of (0.15-0.86%) and(0.15-0.52%) for famotidine and ranitidine.HCl respectively .The results (table 1) indicate good accuracy and precision of the proposed method. The indication was based on the calculation of the relative error of the mean observed concentration as compared with the nominal concentration. The relative error at all studied concentrations was less than 2.08% .

Typical HPLC chromatograms of standard solution of famotidine , ranitidine -HCl and the sample test (tablet) spiked with famotidine and ranitidine are shown on (figure 7 and 8 A,B). The retention time of standard solution and the sample test (tablet) were 3.28 and 7.17min for famotidine and ranitidine -HCl respectively .

Applications

The proposed method was applied on two types of drugs using RP-18 column.The results in table (II) show that the recovery is not less than 97.5%.

Table (1): show accuracy data for pharmaceutical preparation

Drug	Nominal concentration μg.ml-1	Mean(n=6) observed concentration / μg.ml-1	Relative Erre %
Famotidine	30.0	29.68	-1.07
	50.0	48.98	-2.08
	70.0	71.44	2.01
Ranitidine -HCl	20.0	19.85	-0.75
	70.0	68.82	-1.71
	100.0	101.5	1.47

Table (2): Assay of determination of pharmaceutical preparations for famotidine and ranitidine.HCl

Drug	Response(mv)	Found(mg)	Recovery %
Famosam-20	25862.088	19.5	97.5
20 mg Famotidine	25901.431	19.5	97.6
tablet	25967.851	19.6	97.9
Histac	58853.432	151	101.3
150 mg Ranitidine	58692.754	151	101.0
tablet	58789.943	151	101.2

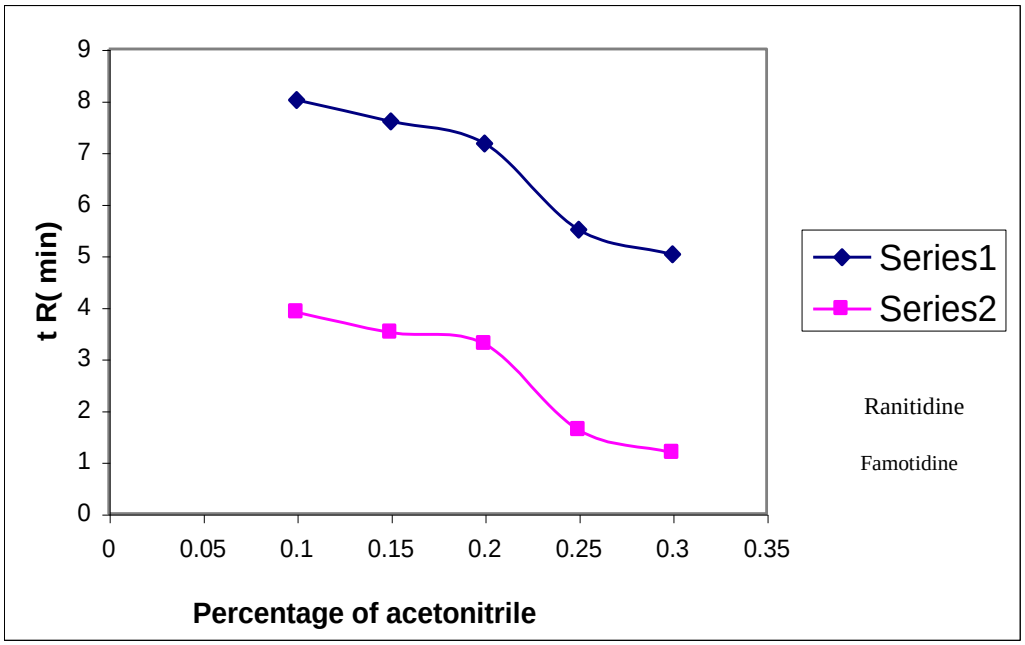


Fig (1) The effect of the organic modifier concentration on the retention of the analytes

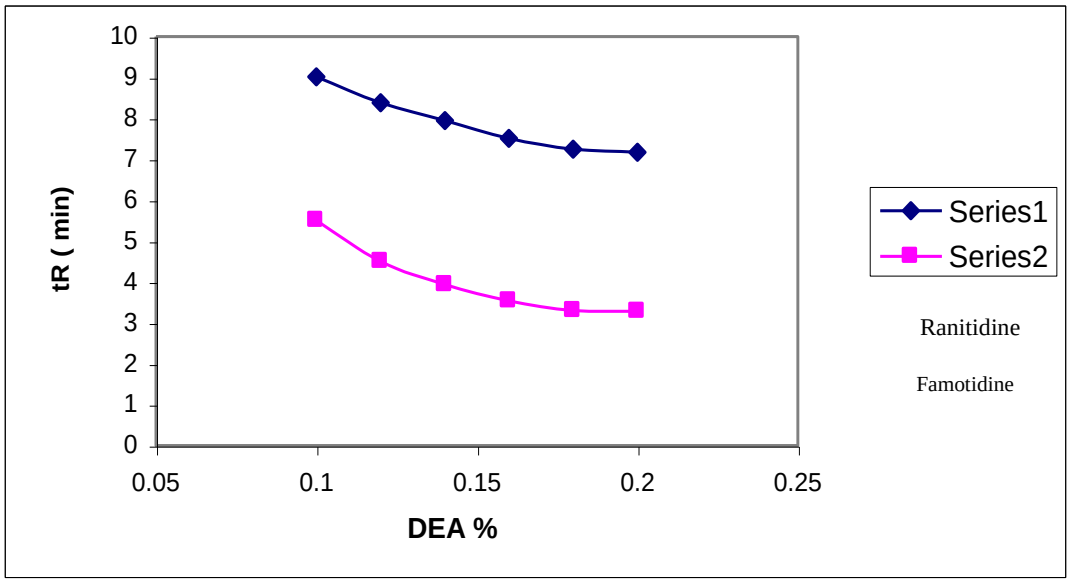
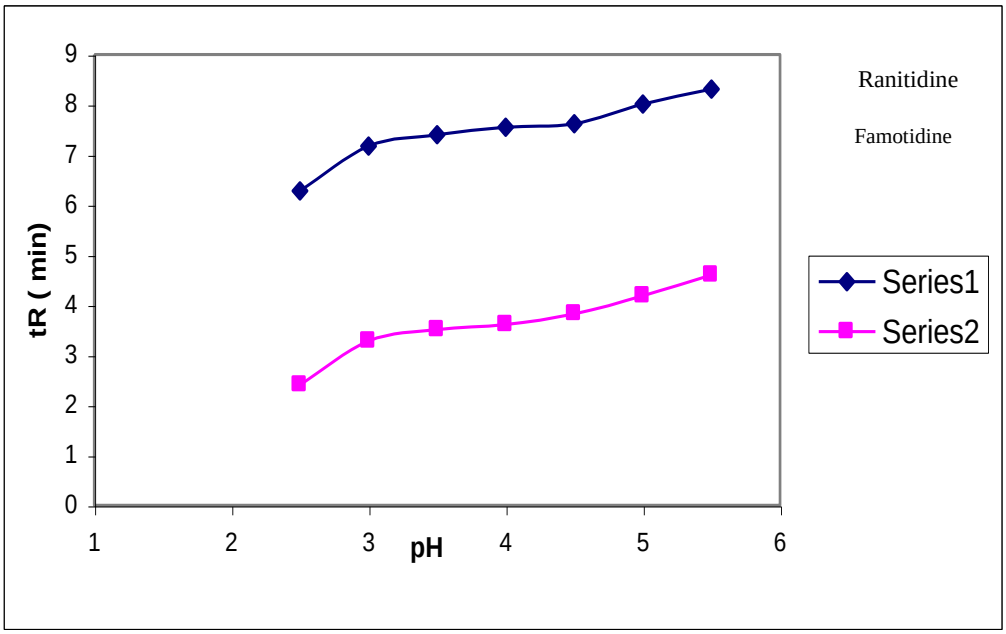


Fig (2): The effect of the Diethylamine concentration on the retention time of the famotidine and ranitidine



Fig(3): The effect or the pH of the mobile phase on the retention time of the famotidine and ranitidine

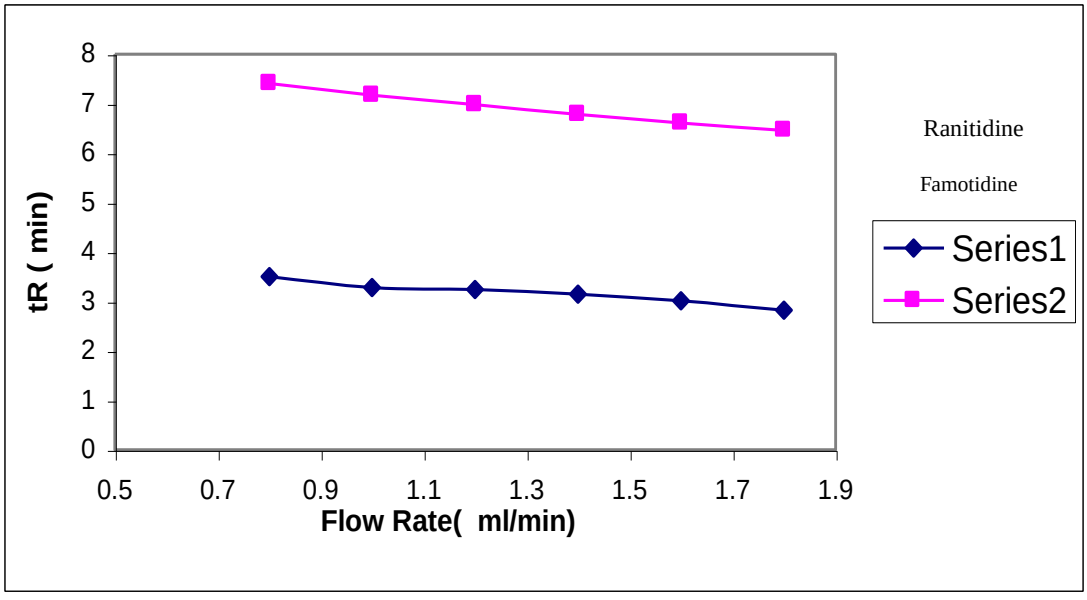
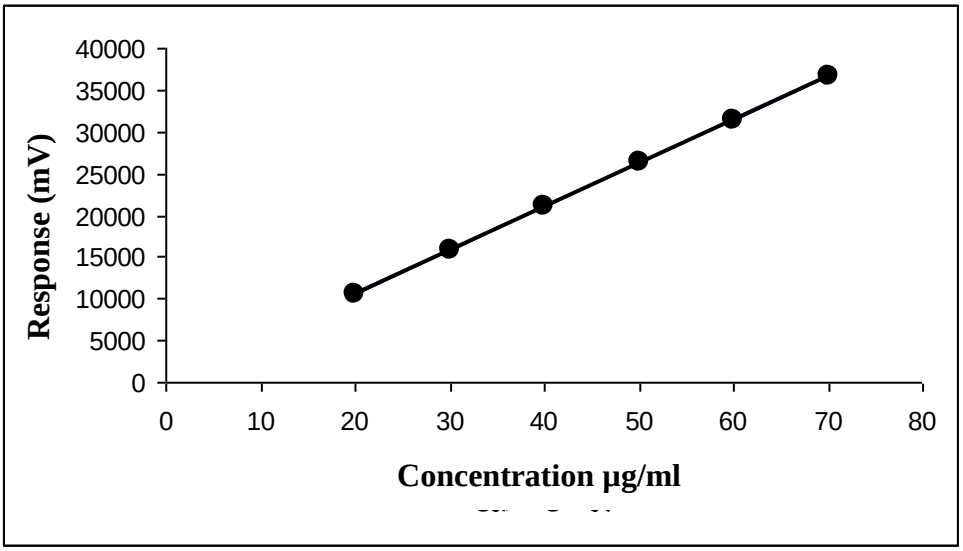
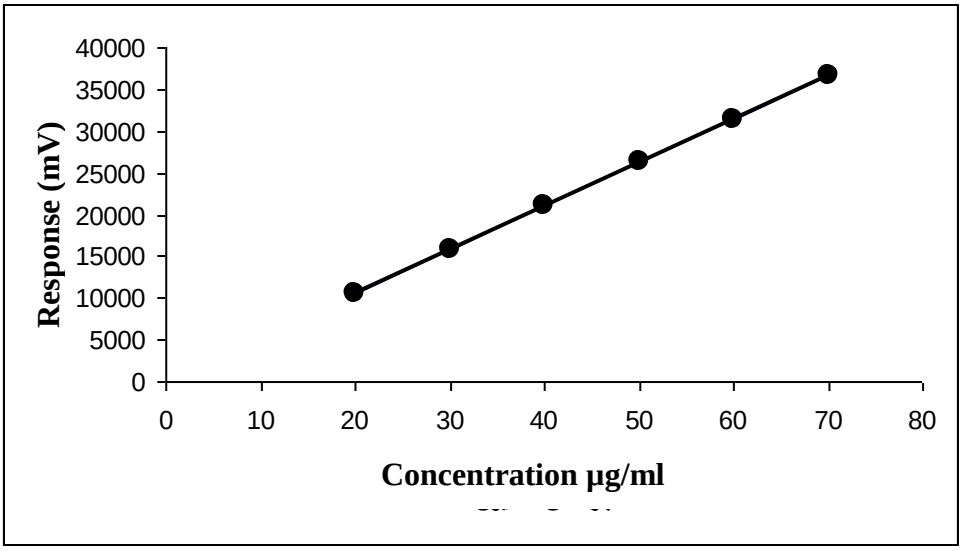


Fig (4): The effect of the flow Rate of the mobile phase on the retention time of the famotidine and ranitidine

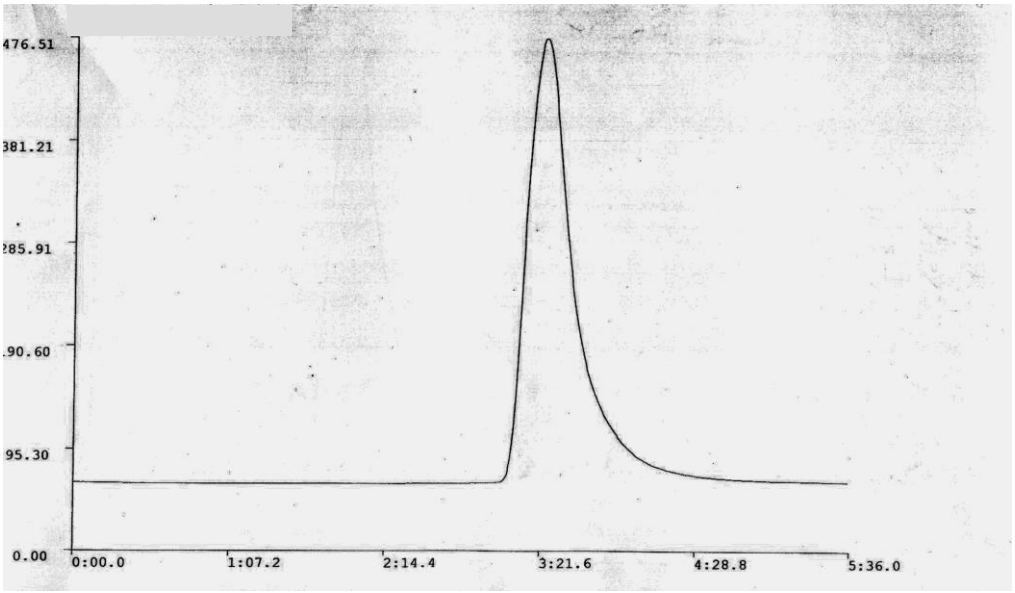


Fig(5):Calibration Curve of the Famotidine



Fig(6):Calibration Curve of the Ranitidine - HCl

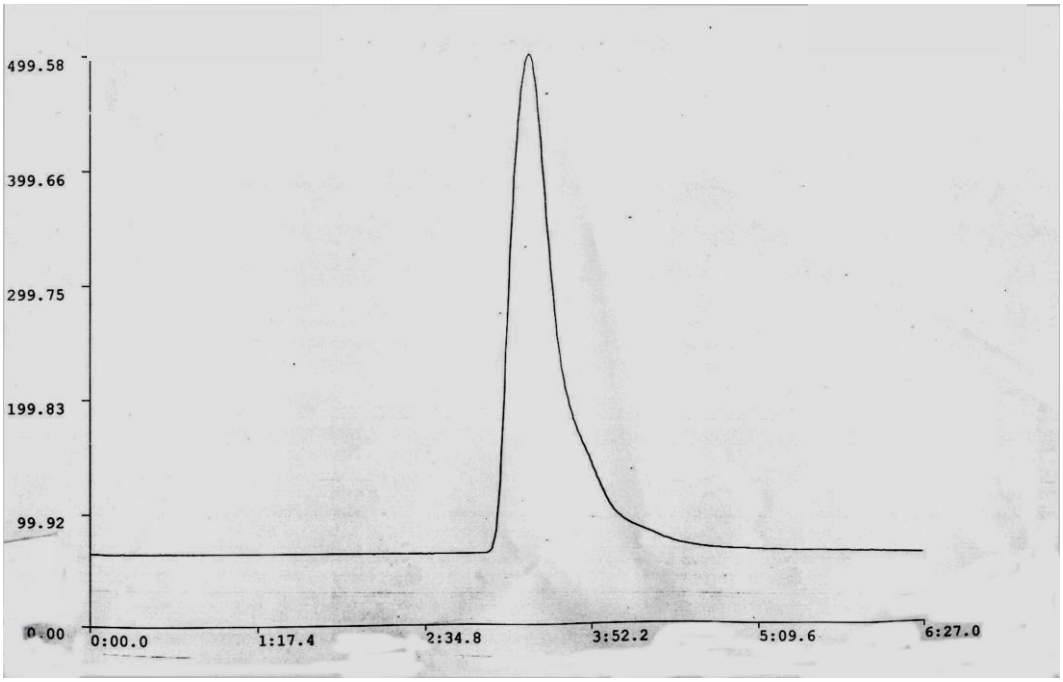
Response(mV)



t_R(min)

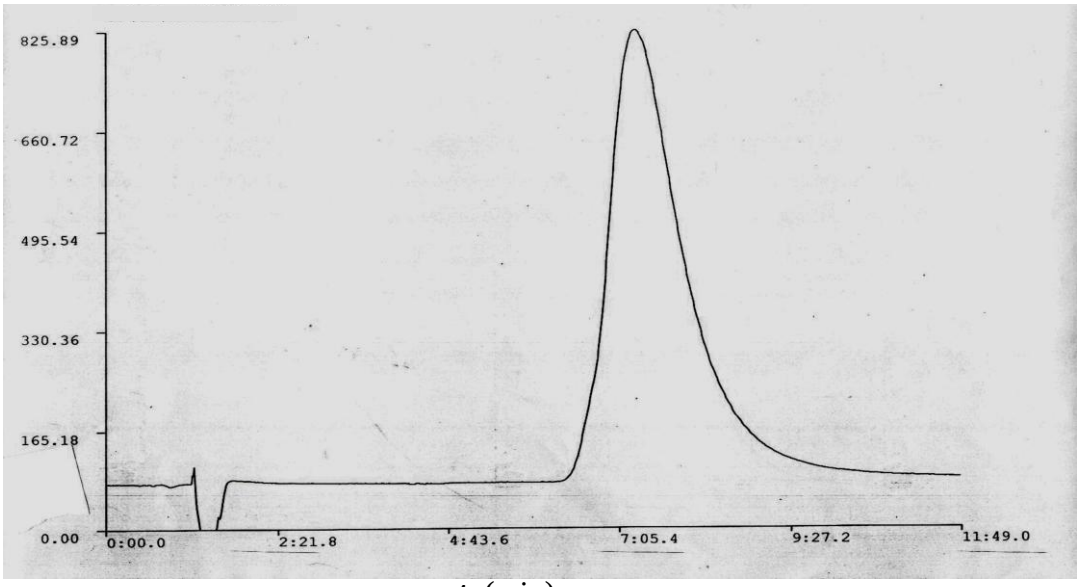
Fig (7. A) Chromatograms of Standard Solutions of Famotidine on column Rp – 18

Response(mV)



t_R(min)

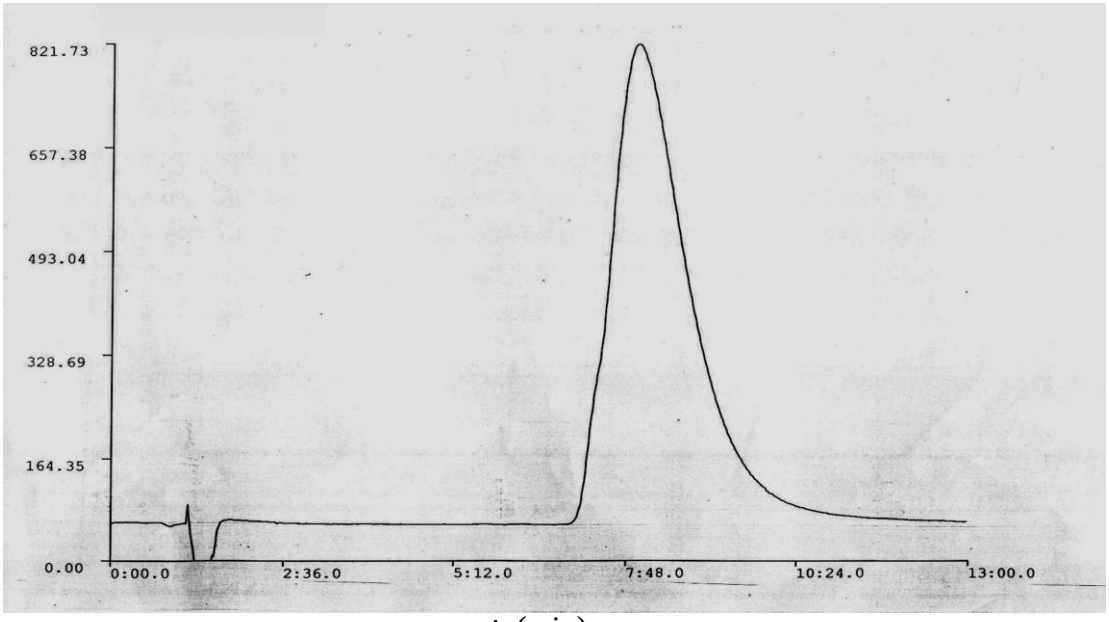
Fig (7. B) Chromatograms of Sample (Famosam 20) containing 20 mg of famotidine on column Rp- 18



Response(mV)

t_R(min)

Fig (8. A) Chromatograms of Standard Solutions of Ranitidine – HCl on column Rp- 18



Response(mV)

t_R(min)

Fig (8.B) Chromatograms of Sample (Histac) containing(150mg) of Ranitidine – HCl on column Rp- 18

Conclusions

The proposed HPLC method employing liquid phase extraction for pharmaceutical preparation samples , is simple and convenient for the determination of famotidine and ranitidine in tablet samples . The typical assay time is about 8 minutes , the method has been developed and validated for the quantitative determination of famotidine and ranitidine in pharmaceutical preparations .The validation data demonstrate good precision , recovery and accuracy indicating the reliability of the proposed method .

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تطوير تقنية كروماتوغرافيا السائل ذات الاداء العالي لتقدير الفاموتيدين والرانتدين في المستحضرات الصيدلانية

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الخلاصة

باستخدام كروماتوغرافيا السائل ذي الاداء العالي- الطور العكوس (RP-HPLC)تم استحداث طريقة سريعة وحساسة لتقدير عقاري الفاموتيدين والرانتدين - هايدروكلورايد في المستحضرات الصيدلانية. تم فصل هذين العقارين وقدرا في الحبوب بهذه الطريقة الكروماتوغرافية باستخدام عمودين مختلفي القطبية وهما Lichrosorb RP-18(١٥سم x ٤,٦ ملم قطر داخلي)و Lichrosorb RP-8(٢٥سم x ٤,٦ ملم قطر داخلي) مع طور متحرك مكون من ٨٠:٢٠حجم /حجم من الاسيتونايترايل في ٠,٢% ثنائي اثيل امين في الماء(عند دالة حامضية =٣) تم دراسة تاثير الـ pH و مكونات الطور المتحرك وتركيز المحور العضوي على زمن الاحتجاز و القياس عند طول موجي 270 نانوميتر و 322 نانوميتر للكشف عن الفاموتيدين والرانتدين هايدوركلورايد على التوالي و كانت خطية منحنى المعايرة لمدى من التراكيز(20-70) و (٢٠-١٢٠) مايكروغرام/مل للفاموتيدين والرانيتيدين على التوالي. كما أظهرت نتائج الدراسة ضبط ودقة جيدين حيث أن الانحراف القياسي النسبي RSD% لم يتجاوز0.85% والخطأ النسبي تراوح بين ٠,٧٥ و٢,٠٨% وكان حد الكشف 1.13 مايكروغرام / مل للفاموتيدين و 0.83 مايكروغرام / مل للرانتدين مع أسترجاعية تراوحت بين ٩٨ و ٩٩% .