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Stability of ranitidine in injectable solutions

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Abstract

Injectable solutions of ranitidine were prepared by dissolving ranitidine hydrochloride in water for injections. The following buffering system has been used: disodium phosphate (anhydrous), potassium dihydrogen phosphate, and phenol as a preservative. Inert gas (nitrogen) was used to displace oxygen from a solution and reduce the possibility of oxidative changes in the formulation. The solution was poured into 2-ml brown glass ampoules in asceptic condition. Ampoules samples have been stored at three different temperatures. They have been stored at 55 and 40 °C for 6 months, and at 25 °C for 12 months. TLC technique has been used for monitoring related substances, and HPLC technique for monitoring phenol and ranitidine content. It has been shown that only those samples that were stored at 25 °C were actually stable.

Keywords: Stability; Ranitidine; Injectable solution

1. Introduction

The notion of "stability" in pharmaceutics is to be understood as the ability of pharmaceutical product—medicine to maintain its properties within the specified limits during its declared shelf life (Kommanaboyina and Rhodes, 1999). In order to define shelf life for a drug, investigations/tests have to be performed, according to pre-scheduled program, resulting in information about various aspects of stability (chemical, physical, and microbiological) (Matthews, 1999). Essential goals of stability tests performance are as follows:

1. Selection of adequate formulation and primary packaging material.
2. Determination of shelf life and storage conditions for the drug.

Ranitidine (chemical name: 1,1-ethenediamine,*N*-[2-[[[5- [(dimethylamino)methyl] -2- furanyl]- methyl] thio]ethyl]-*N′*-methyl-2-nitro) is a selective H_2 receptor antagonist and powerful inhibitor of gastric acid secretion. It is used for the treatment of various gastrointestinal diseases, primarily in stomach and intestinal ulcer. Ranitidine injection is a clear, colorless to yellow, sterile solution of ranitidine hydrochloride in water for injection. It contains the equivalent of not less than 90.0% and not more than 110.0% of the labelled amount of ranitidine ($C_{13}H_{22}N_4O_3S$). Potency of ranitidine hydrochloride preparations is expressed in terms of the base. Ranitidine injections are available on the European market in form of ampoules in doses of 50 mg/5 ml and 50 mg/2 ml (Fahey, 1987).

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Research made so far on the stability of ranitidine injectable solutions was focused on different drugs in intravenous mixtures (Galante et al., 1990; Inagaki et al., 1998; Nolan et al., 1997), as well as stability testing in so called feeding infusions (Baumgartner et al., 1997; Montoro and Pou, 1991).

Ranitidine hydrochloride is subject to degradation in the presence of humidity, increased temperature, light and atmospheric gases (oxygen). (The Pharmaceutical Codex, 1994). The behavior of ranitidine hydrochloride in aqueous solution is governed by the pH of the solution. The reactive properties of substituted 2-nitro-1,1-vinyldiamino entity allow different hydrolytic behaviour of the ranitidine molecule under different pH ranges. The effect of atmospheric gases (presence of oxygen) on ranitidine in aqueous solution is manifested in hydrolytic degradation. The products resulting from the degradation are S-oxide (*N*-[2-[[[5-[dimethylamino)methyl-2-furanyl]methyl] sulfinyl]ethyl]-*N*′- methyl-2- nitro-1, 1-ethenediamine and N-oxide (*N*-2-[[[dimethylamino)methyl]furan-2-yl]methyl]thio]ethyl]-*N*′-methyl-2-nitroethene-1,1-diamine N-oxide. Two other degradation products under these conditions are N,S-dioxide and sulfone of ranitidine (Haywood et al., 1987). Boiling of ranitidine for 20 min in 1N H_2SO_4 results in 15% loss in potency. Boiling of ranitidine for 20 min in 1N NaOH results in drug degraded by 84.4%. Ranitidine manifests extensive degradation (37.8%) when exposed to 3% H_2O_2 in very short period of time (20 min) at room temperature (Singh and Bakshi, 2000).

The purpose of our research was to determine the stability of aqueous injection solutions of ranitidine (50 mg/2 ml) at different storage conditions (55, 40, and 25 °C) in 6 and 12 months period, respectively.

2. Materials and methods

2.1. Materials

Ranitidine HCl was purchased from UQUIFA (Spain). Phenol was supplied by Merck. Disodium phosphate (anhydrous) and potassium dihydrogen phosphate were supplied by Kemika. All other chemicals used for analysis were of analytical grade. Brown glass ampoules were made of neutral boron silicate glass (tube glass) containing significant amounts of boric oxide, aluminium or alkaline earth oxides. Due to its composition, neutral glass has a high thermal shock resistance and a very high hydrolytic resistance. Coloured glass is obtained by the addition of small amounts of metal oxides, chosen to the desired spectral absorbance. Sterile nitrogen was added to the solution during preparation of the injection, and filling of ampoules with the solution.

2.2. Methods

2.2.1. Tested parameters

(a) Appearance—visually tested clear solution	Slightly yellow to yellow
(b) pH value—potentiometrically determined	6.7–7.3
(c) Related substances	Complies
(d) Phenol content per 2 ml	10.0 mg ± 10%
(e) Ranitidine content per 2 ml	50.0 mg ± 10%

Principle:

- TLC method for related substances (semi-quantitatively).
- HPLC method for phenol and ranitidine contents.

2.2.2. Reagents and chromatographic conditions for TLC method

Related substances

Principle:
TLC

Reagents and conditions of chromatography:

1 Stationary phase
Column: Silica gel GF_{254}, on the plate, dimensions 20 cm × 20 mm; 0.25 mm layer thick

2 Mobile phase
Ethyl acetate/isopropyl alcohol/27% (m/m)–31% (m/m) ammonium hydroxide/purified water (25:15:5:1)

3 Test solution
Injection solution without dilution presents test solution (25.0 mg of ranitidine/1 ml)

4 Standard solution
Dissolve 28.0 mg of ranitine hydrochloride standard into 50 ml volumetric flask in purified water and dilute with purified water to volume (560 μg/ml)

5 Standard solution a
Pipette 5.0 ml standard solution (4) into 10 ml volumetric flask and dilute with purified water to volume (280 μg/ml)

6 Standard solution b
Pipette 2.5 ml standard solution (4) into 10 ml volumetric flask and dilute with purified water to volume (140 μg/ml)

7 Standard solution c
Pipette 1.5 ml standard solution (4) into 10 ml volumetric flask and dilute with purified water to volume (84 μg/ml)

8 Standard solution d
Pipette 0.5 ml standard solution (4) into 10 ml volumetric flask and dilute with purified water to volume (28 μg/ml)

9 Standard solution e
Pipette 1.0 ml standard solution (b) (6) into 10 ml volumetric flask and dilute with purified water to volume (14 μg/ml)

10 Resolution solution
Weigh 12.7 mg of ranitidine related substance A 5-[[(2-aminoethyl)thio]methyl]-*N*,*N*-dimethyl-2-furanmethanamine, hemifumarate salt into 10 ml volumetric flask, dissolve in methanol and dilute with it to the volume

Procedure:

Apply separately 10 μl each of the standard solutions (4–9) and test solution (3) to chromatographic plate (1). In addition, apply separately 10 μl of the test solution (3) to the same plate, and on top of this application, apply 10 μl of the resolution solution (10).

After drying, develop the plate over a path of 15 cm in mobile phase (2).

Allow it to dry thoroughly in air and expose to iodine vapour in a closed chamber until the spots are visible. Examine the plate and compare the intensities of any secondary spots observed in the chromatogram of the test solution (3) with those of the principal spots in the chromatograms of the standard solutions (5–9).

The test is valid when there is complete resolution obtained between the principal spots of the test solution (3) and the resolution solution (10), and if any visible spots are obtained with standard solution (e) (9).

The major secondary spot of test solution (3) is not greater in size or intensity than the principal spot produced by the standard solution (4) (2.0%) and no other secondary spot is greater in size or intensity than the principal spot produced by standard solution (a) (5) (1.0%). The sum of the intensities of all secondary spots obtained from the test solution (3) corresponds to not more than 5.0%.

2.2.3. Reagents and chromatographic conditions for HPLC method

Assay

- 50.00 mg ± 10% (45.00–55.00 mg) of ranitidine/2 ml.
- 10.00 mg ± 10% (9.00–11.00 mg) of phenol/2 ml.

Principle
HPLC

Reagents and conditions of chromatography:

1 Stationary phase
Column: LiChrospher® 100 RP-18; 125 mm × 5.0 mm; 5 μm

2 Buffer solution
Solution A
Dissolve 0.115 g of disodium hydrogen phosphate ($Na_2HPO_4 \cdot 2H_2O$), Mr 177.99) in 100 ml of ultrapure water

Solution B
Dissolve 0.045 g of potassium dihydrogen phosphate (KH_2PO_4, Mr 136.09) in 100 ml of ultrapure water
Mix 100 ml of solution A with 700 ml of ultrapure water, adjust pH to 7.0 with solution B, add 8.35 g of sodium hexanesulphonate and dilute with it to 1000 ml

3 Mobile phase
Mixture methanol/buffer solution (2) (40:60)

4 Solvent
Mixture methanol/ultrapure water (40:60)

5 Primary solution of ranitidine standard
Weigh 28 mg of ranitidine hydrochloride standard into 25 ml volumetric flask, dissolve in solvent (4) and dilute with it to the volume

6 Primary solution of phenol standard
Weigh 10 mg of phenol standard into 50 ml volumetric flask, dissolve in solvent (4) and dilute with it to the volume

7 Standard solution
Pipette 1 ml of primary ranitidine standard (5) and 1 ml of primary phenol standard (6) into 25 ml volumetric flask and dilute with mobile phase (3)

8 Test solution
Pipette 1 ml of ampoule content into 25 ml volumetric flask, dilute with solvent (4) to volume. Dilute 1 ml of this solution with mobile phase (3) to 25 ml

9 Flow rate
1.3 ml/min

10 Column temperature
65 °C

11 Injection volume
20 μl

12 Detection UV
280 nm

Procedure:

Separately inject 20 μl of standard solution (7) and test solution (8) into the chromatograph, record the chromatograms and measure the responses for major peaks. Chromatogram of standard solution (7) is given in Fig. 1, and chromatogram of test solution (8) in Fig. 2. Relative retention time of phenol peak is about 0.6.

Calculate ranitidine and phenol content according to responses of principal peaks in the chromatogram of test solution and standard solution:

$$\frac{P_p \cdot m_{st} \cdot 0.896 \cdot 25 \cdot 25 \cdot 2}{P_{st} \cdot 25 \cdot 25} = \frac{P_p \cdot m_{st} \cdot 0.896 \cdot 2}{P_{st}} = \text{mg of ranitidine}/2\,\text{ml} \quad \text{(a)}$$

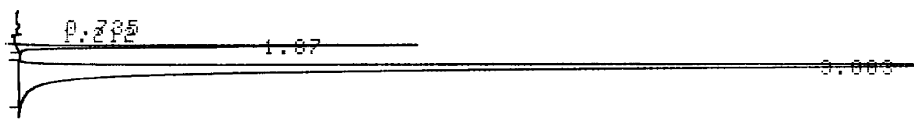
where, P_P is the peak response of ranitidine obtained from test solution; P_{st}, the peak response of ranitidine obtained from standard solution; m_{st}, the weight of ranitidine hydrochloride standard, in mg; 0.896, the conversion factor of ranitidine hydrocholride to ranitidine (314.41/350.87 = 0.896).

$$\frac{P_p \cdot m_{st} \cdot 25 \cdot 25 \cdot 2}{P_{st} \cdot 50 \cdot 25} = \frac{P_p \cdot m_{st}}{P_{st}} = \text{mg of phenol}/2\,\text{ml} \quad \text{(b)}$$

where P_p is the peak response of phenol obtained from test solution; P_{st}, the peak response of phenol obtained from standard solution; m_{st}, the weight of phenol standard, in mg.

3. Results and discussion

Tests have been done in buffered, aqueous solutions of ranitidine HCl (50 mg/2 ml) filled in brown glass ampoules (V = 2 ml). During the 6-month period some samples were exposed to increased temperatures: 55 °C (Table 1) and 40 °C (Table 2), while



CHROMATOGRAM 1 MEMORIZED

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	1.87	21334			10.2678	
2	3.003	186442			89.7322	

Fig. 1. Chromatogram of standard solution (7).

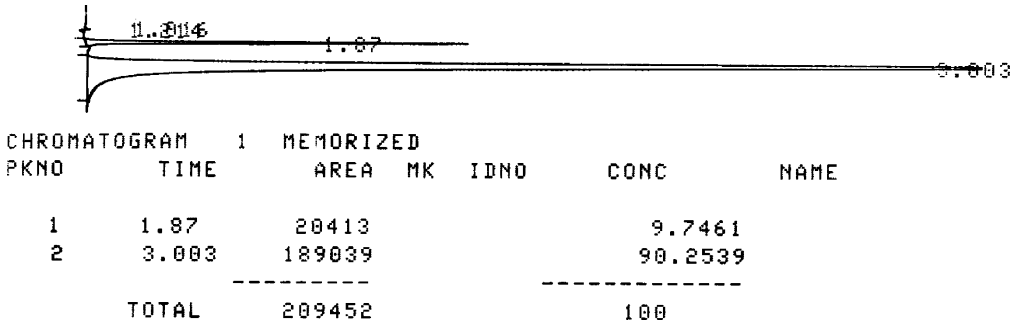


Fig. 2. Chromatogram of test solution (8).

Table 1
Samples stored at 55 °C

Time (month)	Appearance	pH	Ranitidine content	Related substance	Phenol content
Initial	+	6.90	48.26	Complies	9.62
2	+	6.98	50.85	Failed[b]	10.12
3	Failed[a]	7.04	48.70	Failed[b]	9.99
6	Failed[a]	7.16	39.15	Failed[b]	10.21

[a] Dark yellow, clear solution.
[b] The major secondary spot of test solution was greater in size than the principal spot produced by the standard solution (2%) and other secondary spots were greater in size than the principal spot produced by standard solution (1%). The sum of intensities of all secondary spots obtained from the test solution was more than 5%.

Table 2
Samples stored at 40 °C

Time (month)	Appearance	pH	Ranitidine content	Related substance	Phenol content
Initial	+	6.90	48.26	Complies	9.62
2	+	6.98	49.94	Complies	10.73
4	+	7.00	48.65	Complies	10.18
6	Failed[a]	7.01	47.09	Failed[b]	10.19

[a] Dark yellow, clear solution.
[b] The major secondary spot of test solution was greater in size than the principal spot produced by the standard solution (2%) and other secondary spots were greater in size than the principal spot produced by standard solution (1%). The sum of intensities of all secondary spots obtained from the test solution was more than 5%.

others were stored at temperatures not exceeding 25 °C during a 12-month period (Table 3).

It has been noticed in samples kept at 55 °C that there was no significant change of pH value and phenol content in the period of 6 months. The appearance has changed in 2 months. Solution's colour changed from light yellow to brown. Ranitidine content decreased below the declared value (50.0 mg ± 10%) in

Table 3
Samples stored at 25 °C

Time (month)	Appearance	pH	Ranitidine content	Related substance	Phenol content
Initial	+	6.90	48.26	Complies	9.62
3	+	7.02	51.25	Complies	9.75
12	+	6.90	50.85	Complies	10.16

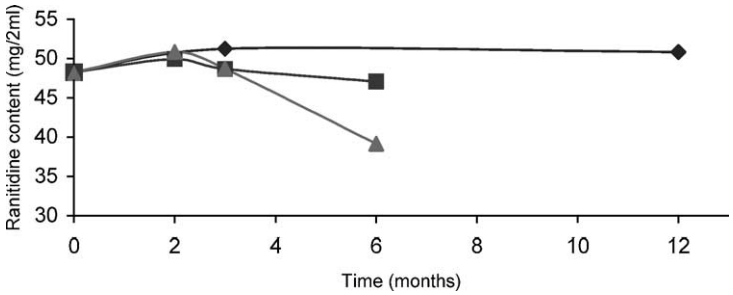


Fig. 3. Ranitidine content as a function of time at (▲) 25 °C, (■) 40 °C and (◆) 55 °C.

Table 4
Ranitidine content as a function of time at three temperatures

Time (month)	Ranitidine content		
	25 °C	40 °C	55 °C
Initial	48.26	48.26	48.26
2	–	49.94	50.85
3	51.25	48.65	48.70
6	–	47.09	39.15
12	50.85	–	–

6 months. The amount of related substances has exceeded allowed limits even 1 month after the test.

No change has been detected in pH values of samples and phenol content in samples stored at 40 °C in the period of 6 months. Ranitidine content was decreased, but within allowed limits. The appearance and quantity of related substances have changed after 6 months of the testing.

No changes in test parameters have been noticed at samples stored at 25 °C in the period of 12 months.

Summary results of ranitidine content as a function of time at three temperatures are presented in Table 4. These results are also presented in diagram (Fig. 3).

4. Conclusion

The test results demonstrate that aqueous injection solutions of ranitidine hydrochloride are unstable at increased temperatures (55 and 40 °C). The changes of solution appearance and content of active ingredient and related substances were determined. Solution stored at temperatures not exceeding 25 °C during 12 months period was stable.

During the production of ranitidine injection, nitrogen should be used to displace oxygen from a solution and ampoules, as well as reduce the possibility of oxidative changes in the formulation.

Buffering system should be added to maintain a required pH (6.7–7.3) because a change in pH may cause significant alterations in the rate of degradative reactions. At neutral pH, as it was found during stability tests with solutions injection buffered to pH 7.0, hydrolysis of ranitidine is particulary slow.

Ranitidine injection should be stored below 25 °C and protected from light (brown glass ampoules).

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