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Short communication

Stability indicating spectrodensitometric determination of ranitidine hydrochloride using linear and non-linear regression

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1. Introduction

Ranitidine hydrochloride is an H_2 receptor antagonist indicated for the treatment of peptic ulcer [1]. Several methods were reported for the determination of ranitidine. Some of those methods were high performance liquid chromatography (HPLC) [2–16], super critical fluid chromatography [17], thin layer chromatography (TLC) [18], potentiometry [19–21], coulometric [22], polarographic [23,24], Ion-selective electrode [25–27], voltametric [28–30], spectrophotometric [31–34], near infra-red reflectance spectrometric [35] and flow-injection fluorimetric [36] methods. All of those methods used direct linear regression for the construction of calibration curves.

Quantitation by evaluation in situ in the spectrodensitometric TLC technique lies between four kinds of capabilities, i.e. scanning in the transmission mode, the reflectance mode, fluorescence mode, and fluorescence scanning and fluorescence quenching evaluation [37]. A direct relationship between the response and absorption of light by a zone on a layer is more closely approached in the transmission mode than in the reflectance mode. However, linearization of normally non-linear calibration curves can be achieved using several transformation techniques [38].

Ranitidine hydrochloride was determined using thin layer chromatography and then spectrodensitometric quantitation. The relationship between the amount of ranitidine hydrochloride (μg/spot) and the area of the peaks was found to be linear

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within the range 2–12.5, 10–20, and 20–50 μg/spot. No direct linear relationship over the whole range 2–50 μg/spot was found, however, linearization was obtained when using logarithmic function. When non-linear regression was applied, a remarkable improvement in the regression coefficient took place over the range 2–50 μg/spot when using the second and third order functions. Intact ranitidine hydrochloride was separated from the degradation products of ranitidine hydrochloride using the proposed method which enables a stability indicating determination for the cited drug.

2. Experimental

All the chemicals and reagents used were of analytical grade.

2.1. Materials

1 Methanol and acetonitrile (E. Merck Dramstadt, Germany).
2 Ammonia solution 33% (Prolabo, France).
3 Ranitidine hydrochloride (99.3%) supplied by Impex Quimica, Spain.
4 Ranitidine related compound A; (5-[[(2-aminoethyl)thio] methyl]-*N*-*N*-dimethyl-2-furanmethanamine hemifumarate salt), ranitidine related compound B; (*N*,*N*′-bis [2-[[[5-[(dimethylamino) methyl]-2-furanyl]-methyl] thio] ethyl-2-nitro-1,1-ethene-diamine) and ranitidine related compound C; (*N*-[2-[[[5-[(dimethylamino) methyl]-2-furanyl] methyl] sulfinyl] ethyl]-*N*′-methyl-2-nitro-1,1-ethenediamine) U.S.P. reference standards; supplied from the United States Pharmacopeial Convention, Inc.
5 Ranitak tablets, nominally containing 167.4 mg of ranitidine hydrochloride equivalent to 150 mg of ranitidine base per tablet, manufactured and supplied by South Egypt Drugs Industries (Sedico), Egypt.
6 TLC Aluminium sheets 20×20 cm, precoated with silica gel 60 F254 of 0.20 mm thickness, (E. Merck Dramstadt, Germany).

2.2. Apparatus

Shimadzu CS-9000 Dual Wavelength, flying spot scanning densitometer with the following settings:

Reflection mode with zig-zag scanning, optical density 0–8, chart speed 50 mm min^{-1}, monochromator band width 10 nm, minimum area 600, swing width 16 mm, PKF filter 2 and wavelength 312 nm.

2.3. Optimum chromatographic conditions

Different compositions of the mobile phase to obtain an optimum R_f (0.3–0.7) [37] as well as the most uniform and circular spot were tried. The mobile phases tried are mixtures of different proportions of acetonitrile, methanol, ammonia 33% and/or water. Ranitidine hydrochloride (50 mg) was dissolved in 25 ml of methanol, 5 μl aliquots were spotted and eluted with the prepared mobile phases, the plates were dried using a current of air, then the spots were located by exposure to iodine vapours. The spots were compared with one another, concerning the shape and R_f value.

2.4. Calibration curves

A stock solution of ranitidine hydrochloride in methanol was prepared (10 mg ml^{-1}) from which several dilutions were made. Aliquots (5 μl) from the diluted solutions were spotted on the TLC plates using a 10 μl G.C. Hamilton syringe. Development of the plates was carried out at room temperature without prior saturation for the TLC chamber with the mobile phase. The plates were left to develop $\sim$15 cm away from the point of application. After air drying the plates, the ranitidine spots were visualized under short wavelength UV light ($\lambda = 254$ nm) and then subjected to a densitometric analysis. The peak areas were plotted against concentrations (μg/spot). The procedure was repeated on different days to ascertain the day to day precision. Linear (direct and logarithmic relationships) and non-linear (second and third order functions) were applied and regression equations were computed.

2.5. Application of the method for the determination of ranitidine hydrochloride in bulk powder

A stock solution of ranitidine hydrochloride in methanol was prepared. Several dilutions were made. Different aliquots were spotted on the TLC plates and chromatographed using the conditions mentioned in Section 2.4. The recovered concentrations (μg/spot) were computed using regression equations.

2.6. Application of the method for the determination of ranitidine hydrochloride in Ranitak tablets

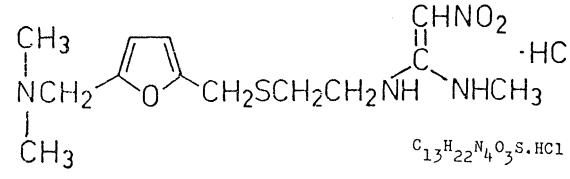
An amount of the powdered tablets equivalent to 200 mg of ranitidine hydrochloride was sonicated with 30 ml of methanol for 10 min, the volume was completed to 50 ml with methanol, the solution was mixed well and filtered, rejecting the first 5 ml of the filtrate. Several dilutions were prepared from the filtrate. Different aliquots were spotted on the plates and chromatographed using the conditions mentioned in Section 2.4. The recovered concentrations (μg/spot) of ranitidine hydrochloride were computed using direct linear regression equations. The accuracy, precision and reliability of the method was ascertained by repeating the procedure and adding known amounts of standard ranitidine hydrochloride and computing the percentage recovery of the added standard.

2.7. Application of the method for the separation and determination of ranitidine from its degradation products

To check the ability of the method to separate intact ranitidine hydrochloride from its degradation products, ranitidine hydrochloride (200 mg) was dissolved in a few drops of water and left in day light in closed bottles for 2 months (this is to achieve degradation of ranitidine hydrochloride). Several dilutions with methanol were subsequently made and aliquots were spotted on TLC plates and chromatographed using the conditions mentioned in Section 2.4. Aliquots of solutions, in methanol, of ranitidine, ranitidine related compound A, ranitidine related compound B and ranitidine related compound C were spotted on the same plate as reference spots. The plate was chromatographed using the proposed method and then subjected to spectrodensitometric scanning.

3. Results and discussion

The mobile phase was composed of acetonitrile:methanol:ammonia (33%):water (125:25:5:10) and gave an almost spherical shaped spot for ranitidine hydrochloride I (Scheme 1) with an R_f value of ~ 0.65 (Fig. 1A). It was found from the experiment that the R_f value increases when the ammonia content of the mobile phase is increased, and that water is necessary for the spherical uniformity of the shape of the spot (Fig. 1B). Different relationships were constructed between the concentration of ranitidine hydrochloride (μg/spot) and the area of the peaks (of the corresponding spots) obtained after densitometric scanning of the plates. Direct linear relationships were obtained over the concentration ranges 2–12.5 μg/spot ($r = 0.9975$), 10–20 μg/spot ($r = 0.9929$) and 20–50 μg/spot ($r = 0.9991$). When a calibration graph was plotted over the concentration range 2–50 μg/spot, direct linear relationship was lost ($r = 0.9703$, Fig. 2A). However, a linear relationship was gained over this range when the logarithmic functions for the concentration and area were used ($r = 0.9971$, Fig. 2B). Two functions of non-linear relationships — second ($r = 0.9972$, Fig. 2C) and third ($r = 0.9995$, Fig. 2D) order functions — were applied to achieve calibration graphs over the concentration range 2–50



Ranitidine Hydrochloride (I)

Scheme 1. Ranitidine hydrochloride.

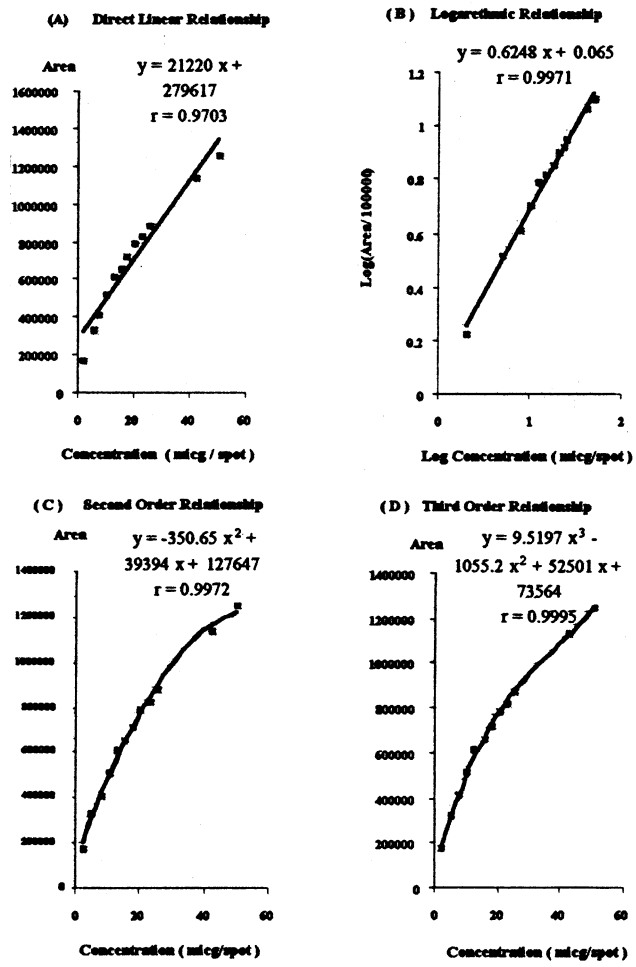


Fig. 1. Different regression functions for ranitidine hydrochloride: (A) linear; (B) logarithmic; (C) second order; and (D) third order.

μg/spot. Microsoft Excel 97, Software Computer Program was used to calculate the regression coefficient values (r) for the second and third order functions while the Software of a Shimadzu UV-1601PC Spectrophotometer (the Quantitative mode) was used to calculate the recovered amounts of ranitidine hydrochloride. A calculator was used to calculate the regression coefficient (r) and the recovered amounts of ranitidine hydrochloride in case of the direct and logarithmic linear relationships.

Table 1 shows the percentage recovery of ranitidine hydrochloride obtained using a direct linear relationship over the ranges 2–12.5, 10–20 and 20–50 μg/spot, respectively. The mean percentage recovery was found to be 99.48, 102.13 and 101.08% over the ranges 2–12.5, 10–20 and 20–50 μg/spot, respectively.

Concerning the application of the suggested method for the determination of ranitidine hydrochloride in tablet form, the results obtained are presented in Table 2. The mean percentage recovery of the added ranitidine hydrochloride standard using the direct linear function over the concentration range 2–12.5 μg/spot was found to be 99.64%.

For the spectrodensitometric technique, the shape of calibration curves for absorption mea-

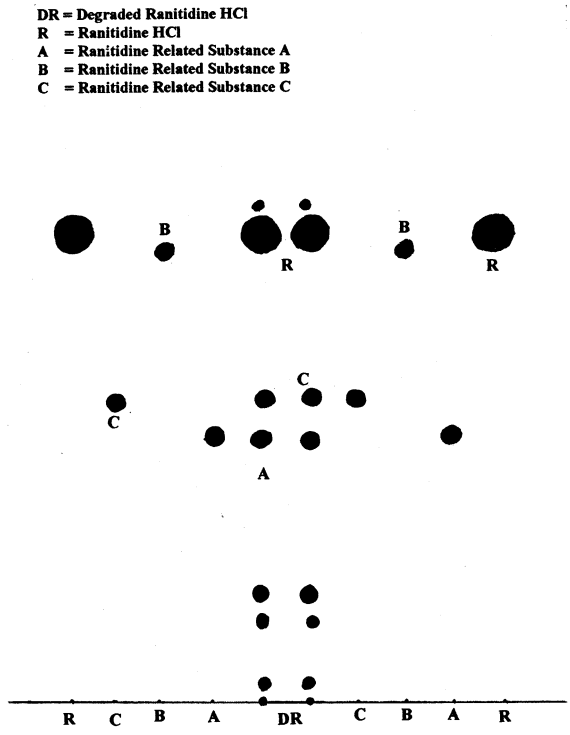


Fig. 2. Chromatogram of degraded ranitidine hydrochloride showing spots of intact ranitidine and separated degradation compounds.

surements in the reflectance mode is generally non-linear. They are generally comprised of a psuedolinear region at a low sample concentration, curving towards the concentration axis at higher concentration [38]. Table 3 shows a comparison between the percentage recovery of ranitidine hydrochloride using different methods of calibration over the range 2–50 μg/spot. It is obvious from the results that there is an improvement in the RSD when using logarithmic linearization or non-linear calibration functions. As a result, one can suggest that there is no reason to omit some obtained data, when linearity is not obtained. With the availability of computer programs which make it easy to compute such relationships, one should try different relationships programs.

Ranitidine hydrochloride is easily degradable particularly in the presence of moisture and light [39]. This method was tested for its ability to differentiate between intact ranitidine hydrochloride and its degradation and related compounds. Fig. 3 shows the location of the spots of intact ranitidine hydrochloride and its degradation and

Table 2
Determination of ranitidine hydrochloride in Ranitak tablets

Labeled ranitidine (μg/spot)	Added ranitidine standard (μg/spot)	%Recovery
2.40	2.76	101.60
2.40	3.68	98.82
3.20	4.60	98.51
	Mean	99.64 ± 4.22%
	RSD	1.71%

Table 1
Determination of ranitidine hydrochloride in bulk powder using spectrodensitometric technique.

Concentration range (2–12.5 μg/spot)		Concentration range (10–20 μg/spot)		Concentration range (20–50 μg/spot)	
R.HCl taken (μg/spot)	% Recovery	R.HCl taken (μg/spot)	% Recovery	R.HCl taken (μg/spot)	%Recovery
3.0	97.10	12.0	103.10	25.0	97.9
4.0	99.60	14.0	100.86	33.0	104.25
6.0	100.86	16.0	103.40	–	–
8.0	101.43	18.0	101.17	–	–
12.0	98.52	–	–	–	–
Mean	99.50 ± 2.18%		102.13 ± 2.07%		101.08 ± 38.10%
RSD	1.79%		1.28%		4.44%

Table 3
Comparison between several methods of regression for computing of recovered amounts of ranitidine hydrochloride

Concentration of ranitidine HCl taken (μg/spot)	%Recovery			
	Direct linear regression	Logarithmic linear regression	Second order non-linear regression	Third order non-linear regression
3.0	−99.23*	92.37	76.71	96.15
4.0	−22.68*	92.52	86.92	96.12
6.0	51.31	100.65	96.95	96.87
8.0	88.17	101.22	103.31	99.46
12.0	118.36	108.38	108.46	103.46
14.0	117.11	104.82	104.16	99.91
16.0	121.51	106.94	105.38	102.36
18.0	119.64	104.78	102.82	101.03
25.0	108.56	95.37	93.27	95.59
33.0	104.20	93.60	93.50	102.22
RSD	105.68%**	6.17%	10.10%	2.97%

* Negative results of recovery due to bad linearity.
** Abnormal high RSD value due to the negative results.

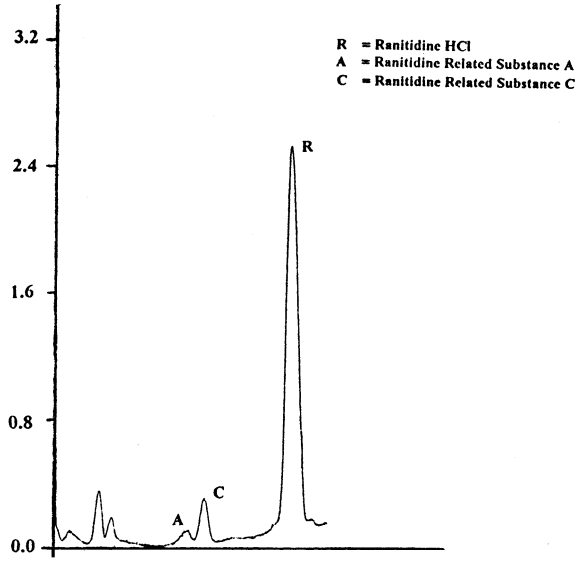


Fig. 3. Chromatogram of degraded ranitidine hydrochloride after spectrodensitometric scanning at 312 nm.

related compounds. Six degradation and related compounds were efficiently separated from the main spot of ranitidine hydrochloride. Ranitidine related compound A and ranitidine related compound C were separated and identified in comparison to reference spots, while ranitidine related compound B was not separated in the chromatogram. The other four degradation products which were separated need further investigations to be identified. The efficient separation of intact ranitidine hydrochloride from the degradation and related compounds facilitates the determination of the intact ranitidine hydrochloride without interference from the degradation products which makes the method suitable for the stability study of ranitidine hydrochloride (Fig. 3).

No statistical significant difference concerning precision and accuracy was found when the method was compared with the official method for the determination of ranitidine hydrochloride.

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