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High-performance thin-layer chromatographic separation of ranitidine hydrochloride and two related compounds

B. Simonovska[a,*], M. Prošek[a], I. Vovk[a], A. Jelen-Žmitek[b]

[a]*National Institute of Chemistry, 1000 Ljubljana, Slovenia*
[b]*LEK d.d., Research and Development, 1000 Ljubljana, Slovenia*

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Abstract

Ranitidine hydrochloride and its two related compounds, used in the USP TLC purity testing of the drug, were separated on a high-performance thin-layer chromatography (HPTLC) RP-18 WF_{254S} precoated plate using methanol–3% NH_4OH (4:1, v/v) as the mobile phase. The main advantage of the proposed HPTLC system over the USP TLC system for testing the purity of ranitidine is a better and more efficient separation of these three compounds in a shorter time and with less consumption of solvents. The system is promising from the point of view of the development of a new method for the TLC purity testing of ranitidine hydrochloride. A video system was used for imaging thin-layer chromatograms. Direct UV densitometric quantitation of the three compounds and a model for the calculation of analytical performance parameters is presented in the second part of the paper.

Keywords: Ranitidine

1. Introduction

Ranitidine is a histamine H_2-receptor blocker that is used in clinical practice for the treatment of duodenal ulcers. The drug is a hydrophilic molecule containing a substituted furano ring (Fig. 1) [1].

According to The United States Pharmacopeia [2], two ranitidine-related compounds, the so-called 'ranitidine related compound A' (5-[[(2-amino-ethyl)thio]methyl] - *N*, *N* - dimethyl - 2 - furanmethan-amine, hemifumarate salt) and 'ranitidine related compound B' (*N*,*N*′-bis[2-[[[5-[(dimethylamino)-methyl] - 2 - furanyl]methyl]thio]ethyl] - 2 - nitro-1,1-ethenediamine) (Fig. 1) are the compounds used, besides ranitidine hydrochloride itself, for testing the purity of the drug. The first compound is not an impurity, but it serves as the suitability check for separation from ranitidine hydrochloride. The second compound is the main impurity to be considered. The European Pharmacopoeia provides a further seven compounds as possible minor impurities in ranitidine hydrochloride. These substances were not available to us, therefore, we cannot report on their chromatographic properties in the high-performance thin-layer chromatography (HPTLC) system used.

2. Experimental

Ranitidine hydrochloride (R), the 'ranitidine related compound A' (A) and 'ranitidine related compound B '(B) were the USP reference standards.

*Corresponding author.

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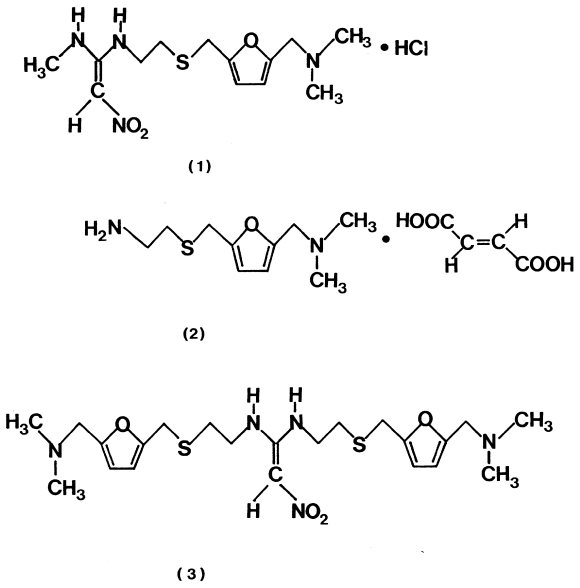

Fig. 1. Structures of ranitidine hydrochloride (1), 'ranitidine related compound A' (2) and 'ranitidine related compound B' (3).

Methanol (HPLC grade) and concentrated ammonia (analytical grade) were obtained from Merck (Darmstadt, Germany). The chromatographic conditions used were as follows:

Chamber: twin trough, Camag (Muttenz, Switzerland). Dimensions: length×width×height=12×4.7×12.5 cm. It was used saturated (lined on the two bigger sides with filter paper that had been soaked thoroughly with the mobile phase and saturated for 0.5 h).

Chromatoplates: 10×10 cm HPTLC plates, precoated with 0.2 mm layer of RP-18 WF_{254S} (Merck, catalog no. 13124), which were used without pretreatment.

Mobile phase: methanol–3% NH_4OH (made with Milli Q water), 4:1, v/v; A 10-ml volume was poured into each part of the chamber.

Separation mode: ascending, only one plate at a time.

Development distance and time: 7 cm and approximately 50 min, respectively.

The compounds were dissolved in methanol. Besides solutions containing each compound separately, a solution containing all three compounds, each at a concentration 0.1 mg/ml, was prepared for calibration. Appropriate amounts of all three compounds (0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 μg) were applied in 5 mm bands, 7 mm apart, 15 mm from the bottom and 10 mm from the left edge of the plate, using the Camag Linomat IV. In the middle of the plate, 100 μg of ranitidine hydrochloride were applied (seven applications on each plate, Fig. 2).

2.1. TLC scanning densitometry

After development of the chromatogram and the evaporation of solvents into the air, the absorbance/reflectance was measured at λ=230 nm (monochromator band width, 10 nm), using the Camag TLC scanner II. The slit length was 2 mm and the slit width was 0.3 mm. The scanner was equipped with a built-in 12 bit ADC, and controlled by an external PC via an R S232 interface. The QTLC-pack software program (KIBK-IFC, 1990) was used. Quantitation was based on peak areas.

2.2. Image processing

The Camag video documentation system, in conjunction with the Reprostar 3, was used for imaging

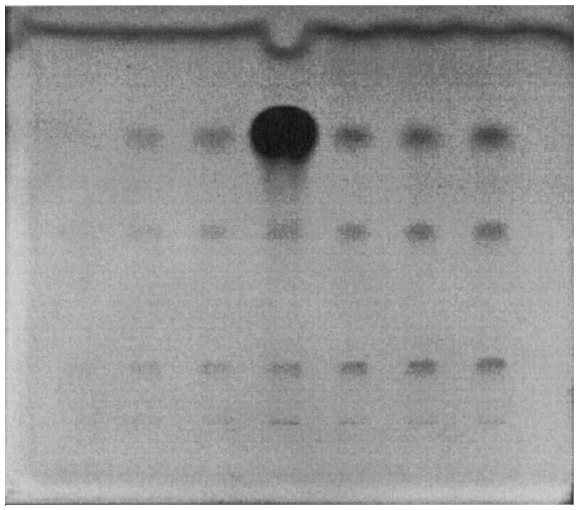

Fig. 2. CCD image of ranitidine hydrochloride and the two related compounds scanned by the Camag video documentation system at λ=254 nm (100 μg of ranitidine hydrochloride spiked with 0.4 μg of related compound A and 0.5 μg of related compound B in the middle of the plate and six calibration points for the three substances: 0.1, 0.2 and 0.4 μg (left) and 0.6, 0.8 and 1.0 μg (right).

and archiving thin-layer chromatograms. The objects were captured by means of a highly sensitive video camera (3×1/2″ CCD camera, model HV-C20 Hitachi, Denshi, Japan). A special digitizing board (frame grabber) assisted rapid processing via a PC system. Image acquisition, processing and archiving were controlled via Video Store 2, a high-performance documentation software program running under Windows 95. All images were obtained by exposing the plate to direct UV light (λ=254 nm), using a UV-blocking filter and attachment lens +2 Dpt for optimum adaptation to the size of the object.

3. Results and discussion

3.1. Separation of the compounds, visual evaluation and storage of chromatograms

All spots were visible under a UV lamp at λ=254 nm. Semi-quantitative visual evaluation was possible. The limit of detection for all three compounds is about 0.1 μg.

The visual TLC purity test, according to USP [2], provides an estimation of impurities by visual comparison of their spots with the spots of ranitidine hydrochloride (all spots were visualized using iodine vapors). The test assumes that all compounds have the same response, on a mass basis, to iodine (i.e. the same sensitivities and limits of detection), which has to be considered as an approximation. Our experiments showed that iodine probably can be omitted.

Fig. 2 presents a CCD image of an HPTLC plate that was obtained using the Camag video documentation system. The advantages of this imaging system, which enables acquisition, printing and archiving of images of the thin-layer chromatograms, over densitometry have already been discussed in our previous papers [3–5].

It can be concluded that the positions and shapes of spots and their separation were appropriate.

Small variations in eluent composition affect the position of spots, but not their separation.

Stability during chromatogram development was checked by two-dimensional TLC, using the same eluent in both directions (application of 1.0 μg of each compound). Three spots were situated on the diagonal of the chromatogram, indicating that no decomposition had taken place.

3.2. Calculation of some analytical performance parameters

TLC is regarded as a simple and relatively cheap technique, the disadvantage of which lies in its poor precision. The use of an application device, precoated plates and a modern densitometer improves analytical performance, but the method of calculation of some analytical parameters is also important. Therefore, quantitation of the compounds studied was performed using a model that is applicable in TLC, taken from the literature on statistics in analytical chemistry [6–9]. It can be used in the optimization and also as part of the validation of a method. It is used for the estimation of precision, the limit of detection and the limit of quantitation of the analyte.

Densitograms of pure ranitidine hydrochloride and of a spiked sample are presented in Fig. 3.

Typical densitometric calibration measurements,

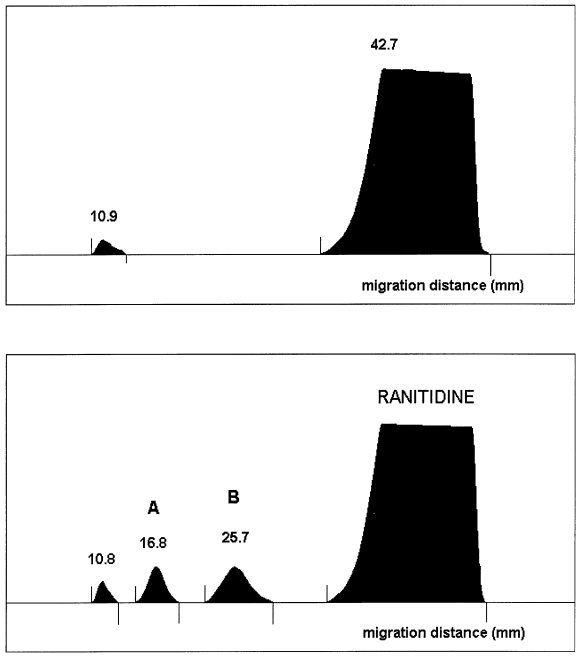

Fig. 3. Spectrodensitometric scan at λ=230 nm of the pure ranitidine hydrochloride (top) and of the same compound spiked with 0.4% of related compound A and 0.5% of related compound B (bottom).

Table 1
Calibration data for the compounds

Amount of standard spotted on plate x_i/μg	Ranitidine ($R_F = 0.67$) y_i/peak area counts	Compound A ($R_F = 0.13$) y_i/peak area counts	Compound B ($R_F = 0.37$) y_i/peak area counts
0.1	3195	3046	1881
0.2	9188	9897	5652
0.4	18 556	19 946	14 779
0.6	31 215	32 537	25 181
0.8	39 722	44 875	33 289
1.0	48 230	53 644	41 209

HPTLC plate, precoated with 0.2 mm layer of RP-18 WF_{254S}.
Mobile phase, methanol–3% NH_4OH (made using Milli Q water), 4:1, v/v.
Scanning densitometry in absorbance/reflectance mode at $\lambda = 230$ nm.

obtained from a plate, are given in Table 1. The ranitidine calibration equation has been calculated for the estimation of possible impurities of an unknown structure, or when results for each separate impurity and their sum have to be expressed on a ranitidine hydrochloride basis.

In quantitative TLC, a number of standards are applied to each plate. Each result is calculated on the basis of values obtained for the standards. Linear dependence between signals from the densitometer and the amounts spotted onto the plate can be obtained in a relatively narrow range of amounts spotted. The coefficient of correlation or its square are considered unreliable for confirmation of linearity [7–9]. A visual inspection of points on the calibration curve is always recommended.

The appropriate working range for the linear calibration function was between 0.1 and 1.0 μg. After confirming linearity, a linear regression equation was calculated:

$$y_i = a + bx_i \tag{1}$$

where y_i = signal from the densitometer for standards ('peak area counts'), x_i = amount of standard applied, in μg, and a and b = regression coefficients.

A fundamental assumption is that no errors exist in the x direction (application of standards and test solutions), and that each point on the plot has a normally distributed variation in the y direction, with a standard deviation estimated by $s_{y/x}$ [6,7]:

$$s_{y/x} = \sqrt{\frac{\sum_i (y_i - \hat{y})^2}{n-2}} \tag{2}$$

$\hat{y}$ = value calculated from the regression equation for every y_i obtained from x_i, and n = number of standards.

The value of $s_{y/x}$ enables calculation of the standard deviation of the slope, s_b, and the intercept, s_a [6,7]. It is easy to calculate the result of an analysis as x_d (d = analyte), by inversion of the calibration equation, however, if there is a possibility that the slope and the intercept are subject to error, the following approximate formula for estimating the standard deviation of x_d, s_{xd}, is usually taken [7]:

$$s_{xd} = \frac{s_{y/x}}{b}\sqrt{\frac{1}{m} + \frac{1}{n} + \frac{(\bar{y}_m - \bar{\bar{y}})^2}{b^2 \sum_i (x_i - \bar{x})^2}} \tag{3}$$

where m is the number of replicates, $\bar{y}_m$ is the mean value of y for m replicates, $\bar{\bar{y}}$ is the mean value (spot area counts) of n standards and $\bar{x}$ is the mean value of standards.

The precision of the analytical results of an unknown test solution can be expressed by the confidence interval with a chosen percentage of statistical confidence and $(n-2)$ degrees of freedom for t (Student's t, from tables [6]):

$$\pm t \cdot s_{xd} \tag{4}$$

The results of calculations for the three compounds are presented in Table 2. Precision is expressed in different ways. Taking the calibration data into consideration, an estimation of the standard deviation, s_{xd}, is possible from only one spotting of a test solution on a plate.

There are always a limited number of places on

Table 2
Results of calibration and calculated results of analysis at $y=18\,000$ (arbitrary chosen value) with estimated precision for the three compounds

Parameter	Ranitidine	Compound A	Compound B
a	−1108	−2082	−2727
b	50 567	56 915	44 631
$s_{y/x}$	1275	1104	758
s_a	992	860	590
s_b	1635	1416	971
r	0.9979	0.9988	0.9990
$x/\mu g$	0.378	0.353	0.464
$s_{xd}/\mu g$	0.028	0.025	0.022
$t_{90\%}.s_{xd}/\mu g$	0.059	0.054	0.047
$t_{95\%}.s_{xd}/\mu g$	0.077	0.071	0.061

Number of standards, $n=6$; number of replicates, $m=1$.

the plate for spotting. Table 3 shows the calculated confidence intervals when varying the number of standards and replicates on a plate with seven spotting places. Ranitidine hydrochloride is taken as an example (data from Table 1). With $n=5$, the first standard is omitted, and with $n=4$, the first and last standards are omitted. There are differences in x and s_{yx} values because of differences which arise from new calibration equations.

Spotting only three standards is unacceptable, even at $m=4$ (i.e. spotting the same test solution four times) because of the large t values. Taking all influences of the variables into consideration, the experimental conditions for the desired precision can be calculated from a small number of preliminary experiments. It is obvious that small plates are economic with regard to determining the best separation conditions, but not with regard to routine quantitative work with many samples.

The limit of detection and the limit of quantitation are also important analytical parameters. They can be statistically defined and calculated from the calibration equation [10,11].

The limit of detection can be defined as value x at y_{LOD}, which is significantly different from the blank signal. This y_{LOD} signal lies at the upper confidence limit for the blank ($x=0$) [10,11]:

$$y_{\mathrm{LOD}} = a + s_{y/x} \cdot t \cdot \sqrt{1 + \frac{1}{n} + \frac{\bar{x}^2}{\sum_i (x_i - \bar{x})^2}} \qquad (5)$$

All symbols were explained previously. From the regression equation, x_{LOD} is calculated (Table 4).

The limit of quantitation (x_{LOQ}) is about twice the limit of detection. Signals between y_{LOD} and y_{LOQ} (from the regression equation for the x_{LOQ}) mean approximate quantitation. Better precision means a lower limit of detection. The number and distribution of standards also has some influence on the limit of detection.

The mathematical expressions for different ana-

Table 3
Calculated changes in precision under different spotting conditions for ranitidine hydrochloride

Variant	n	m	$x/\mu g$	$s_{y/x}$	$s_{xd}/\mu g$	$t_{90\%}.s_{xd}/\mu g$	$t_{95\%}.s_{xd}/\mu g$
1	6	1	0.378	1275	0.028	0.060	0.078
2	5	2	0.371	1350	0.025	0.059	0.080
3	5	1[a]	−	1350	0.031	0.073	0.099
4	4	3	0.372	1215	0.019	0.055	0.082
5	4	1	−	1215	0.027	0.079	0.116
6	3[a]	1	0.370	1345	0.028	0.177	0.356
7	3[a]	4	−	1345	0.019	0.116	0.241

n = number of standards, m = number of sample test solution replicates with an average area count of $y=18\,000$.
[a] Calibration data obtained following the application of 0.2, 0.4 and 0.6 μg of ranitidine hydrochloride.

Table 4
Statistically estimated limits of detection and quantitation for the three compounds

Compound	x_{LOD}/μg	y_{LOQ} to y_{LOD}/ peak area counts	x_{LOQ}/μg
Ranitidine hydrochloride	0.09	3376 to 7994	0.18
Related compound A	0.07	1801 to 5886	0.14
Related compound B	0.06	0 to 2629	0.12

lytical parameters in the model under consideration seem rather complicated, however, if appropriate software is used, the calculations should not be difficult.

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References

[1] C. López-Erroz, P. Vinas, N. Campillo, M. Hernandez-Córdoba, Analyst 121 (1996) 1043.
[2] The United States Pharmacopeia, 23 (1995) 1362.
[3] I. Vovk, M. Prošek, J. Chromatogr. A 768 (1997) 329.
[4] I. Vovk, A. Golc-Wondra, M. Prošek, J. Planar Chromatogr. 10 (1997) 416.
[5] I. Vovk, M. Prošek, J. Chromatogr. A 779 (1997) 329.
[6] E.L. Bauer, A Statistical Manual for Chemists, Academic Press, New York, London, 1971.
[7] J.C. Miller, J.N. Miller, Statistics for Analytical Chemistry, Ellis Horwood, Prentice Hall, New York, 1993.
[8] W. Wegscheider, Fresenius' J. Anal. Chem. 349 (1994) 784.
[9] Ferenczi-Fodor, A. Nagy-Turak, Z. Végh, J. Planar Chromatogr. 8 (1995) 349.
[10] A. Hubaux, G. Vos, Anal. Chem. 42 (1970) 849.
[11] J.E. Bailey, E.A. Cox, J. Assoc. Off. Anal. Chem. 61 (1978) 1404.