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Validation of a liquid chromatographic method for the determination of ranitidine hydrochloride residues on surfaces in the manufacture of pharmaceuticals☆

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Abstract

A liquid chromatographic method for determination of the residues of ranitidine hydrochloride on various surfaces employed in drug manufacture is described. Cotton swabs, moistened with a methanol–water (1:1, v/v) mixture were used to remove any residues of drugs from glass, vinyl, and stainless steel surfaces, and gave recoveries of 85%, 78% and 90%, respectively. Residues were determined by high-performance liquid chromatography on a C_{18} column at 25°C with methanol–ammonium acetate (40:60 v/v) pH 6.7 as the mobile phase and detection at 320 nm. The method was validated over a concentration range of 20–10 000 ng/ml and had a detection limit of 2 ng/ml.

Keywords: Validation; Pharmaceutical analysis; Ranitidine hydrochloride

1. Introduction

An important step in the pharmaceutical industry is the postmanufacture removal of possible drug residues from the involved equipment and areas. The procedures used to clean them must be validated according to good manufacturing practice (GMP) rules and guidelines. In the validation scheme, the methods used to wipe the surfaces and to determine trace amounts of drug deserve special attention.

Ranitidine hydrochloride is an H-2 receptor agonist widely used to inhibit gastric acid secretion and has a good efficacy in the treatment of peptic ulcers, so there is a variety of pharmaceuticals and dosage forms where ranitidine hydrochloride is included. Therefore, a great interest in its determination exists, not only in the pharmaceutical formulations, but also in the biological matrices after their dosage; for this reason, several techniques have been proposed to evaluate the drug. Some of them would be supercritical fluid chromatography on cyanopropyl columns [1], polarography at a dropping-mercury electrode [2], differential pulse adsorptive stripping voltammetry [3], ion selective electrodes [4], flow injection analysis [5], immunoassay [6], proton magnetic resonance [7],spectrophotometry [8], or thin-layer chromatography [9–11]. Nowadays the most recommended are capillary electrophoresis [12–14] and,

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above all, high-performance liquid chromatography on C_{18} columns, with different mobile phases, isocratic or gradient, and detection in the UV region, usually between 313 and 330 nm [15–19]. There are also procedures that involve derivatization, pre-column [20] or post-column [21], or use mass spectrometry as detection system [22], but in general, they do not imply a noticeable increase in the sensitivity. This can be explained by the fact that most of the methods are frequently devoted to analyze ranitidine hydrochloride for purity control of tablets, syrups or injections, in which the drug is in relatively high concentration. On the other hand, studies about drug pharmacokinetics or its residues in plasma, urine, etc. generally achieve lower detection limits, about 20–30 ng/ml [12,17,21]. Taking this information into account, we have developed and validated a simple method that allows us to evaluate the possible residues of the drug after removing it from surfaces of pharmaceuticals manufacture areas (glass, vinyl and stainless steel), using our previous experience acquired in the study of the acetylsalicylic acid [23].

2. Experimental

2.1. Chemicals

The ranitidine hydrochloride certified standard and the plates of different materials were generously given by Glaxo Wellcome Factory in Aranda de Duero (Spain). Methanol HPLC UV-grade was obtained from Lab-Scan (Dublin, Ireland). Ammonium acetate, sodium hydroxide and glacial acetic acid, all of analytical grade, were purchased from Scharlau (Barcelona, Spain). Ultrapure water was obtained in a Milli-RO Plus system together with a Milli-Q system from Millipore (Bedford, MA, USA).

2.2. Equipment

The HPLC system consisted of a vacuum degasser, a quaternary pump, an automatic injector with a column oven and a photodiode array detector, all HP Model 1100, from Agilent Technologies (Palo Alto, CA, USA) controlled by an HP Chemstation software. A Bransonic 5 ultrasonic bath was obtained from Scharlau. An AE-240 analytical balance was obtained from Mettler (Toledo, USA).

2.3. Column

The chromatographic separation was carried out on a Luna, 5 μm, 250×4.6 mm, C_{18} column purchased from Phenomenex (Torrance, CA, USA).

2.4. Chromatographic conditions

The mobile phase was a mixture of methanol–0.05 *M* ammonium acetate (40:60, v/v), pH 6.7. The flow-rate was 1 ml/min and the oven temperature 25°C. The injection volume was 25 μl and the detector was set at 320 nm.

2.5. Standard solutions

An accurately weighed amount of ranitidine hydrochloride (about 3.0 mg) was dissolved in methanol–water (1:1, v/v) to obtain a solution containing 150 mg/l of ranitidine hydrochloride. This standard was later diluted adequately in the same solvent to prepare the solutions for calibration.

2.6. Sample preparation

The selected surfaces (20×20 cm^2) of glass, vinyl and stainless steel, previously cleaned and dried, were sprayed with 1 ml of a standard solution of ranitidine hydrochloride, and the solvent (methanol–water; 1:1, v/v) was allowed to evaporate. Two cotton swabs of approximately 0.25 g each, previously rinsed with methanol, and then dried under vacuum, were weighed by placing them into a 50 ml screw cap plastic test tube.

The selected surface was wiped with the first swab soaked with methanol–water (1:1, v/v) mixture, passing it in various ways, and the other dry cotton swab was used to wipe the wet surface of the plate. The two swabs were placed into the 50 ml screw cap plastic test tube, and the methanol–water mixture was added to reach a mass 5 g higher than the one obtained before. The tube was placed in the ultrasonic bath for 5 min and the solution was analyzed by HPLC.

3. Results and discussion

3.1. Selection of the chromatographic conditions

The selection of the chromatographic conditions was made trying to get a high peak in a short time, about 6 min, because it is not expected to find other compounds retained on the surfaces. Therefore, attention was focussed on getting the highest sensitivity by choosing the most adequate wavelength, mobile phase and temperature.

When injecting a pure solution of ranitidine hydrochloride, it could be observed that the spectrum had two bands, with maxima at 228 and 320 nm, the latter one higher; for this reason 320 nm was selected to detect the compound.

Regarding the mobile phase, there are several mixtures that have been proposed to analyze the drug in formulations, probably the most commonly used are the mixtures of acetonitrile with salts at different pH values. The mixtures with phosphate buffer at several concentrations and pH values did not appear to be adequate for the determination of trace levels of ranitidine. Another mobile phase very often used in industry, for purity control, is the mixture of methanol–0.1 *M* ammonium acetate (80:20), but when it was employed to analyze the liquid after passing the swabs, a big chromatographic front that coeluted with the peak of the compound appeared. A proportion of 40% of methanol would solve this problem, so the selected mobile phase composition was 60:40. The pH of the mobile phase was also varied between 4.1 and 8.5; in consequence, it was appreciated that the retention time increased gradually with the increase in the pH; because of that, a pH 6.7, given by a solution of 0.05 *M* ammonium acetate, was chosen.

In Table 1 the results obtained for the chromatographic parameters as a function of the injection volume for ranitidine solutions of 0.1 and 0.002 μg/ml are summarized. An injection volume of 25 μl was used, because bigger volumes implied a wider peak without an enhancement of signal-to-noise ratio.

Table 1
Influence of the injection volume on the signal/noise of a 0.002 μg/ml standard

Injection volume (μl)	Signal (mUA)	Noise (mUA)	Signal/noise	Width (5%)
5	–	–	–	–
25	0.0130	0.0028	4.1	0.113
30	0.0135	0.0031	4.3	0.116
40	0.0140	0.0037	3.8	0.118
50	0.0147	0.0040	3.7	0.126

–, not detected.

3.2. Validation of the chromatographic method

Once the chromatographic conditions had been selected the method was validated paying attention to the linearity, precision, accuracy, selectivity, limits of detection and quantitation and stability of standards and samples.

The calibration curve for ranitidine hydrochloride was linear over the concentration range 0.02–10 μg/ml with a correlation coefficient $r^2=0.9999$. The results of the statistical treatment of calibration data were ''*a*'' (slope)=15.3, $s_a=0.25$; ''*b*'' (*y*-intercept)=−0.061, $s_b=0.1$ and $s_{y/x}=0.20$

The precision of the results, reported as relative standard deviation (RSD), were 0.67% and 0.05%, as determined on 10 replicate injections at two different drug concentrations (0.12 μg/ml and 6.2 μg/ml), respectively. An injection precision of less than 5% RSD is considered appropriate for these trace level determinations.

The accuracy of the method was shown by analyzing different standards five times, and comparing the analytical result to the known added value. The average recovery was calculated at each concentration level. The average recoveries were 99.6% and 99.8% for concentrations of about 0.12 μg/ml and 6.2 μg/ml, respectively. The results obtained were within the acceptable range of 98.0 to 102.0%. No interference was found in the application of the method. To prove this, a cotton swab blank, a surface sample blank and a ranitidine hydrochloride standard were injected. The lack of interference can be observed in Fig. 1.

The limit of detection (LOD) and limit of quantitation (LOQ) were determined by measuring the magnitude of analytical background response by injecting a number of blank samples ($n=5$) and calculating the mean (0.0038 mUA) and RSD=19%.

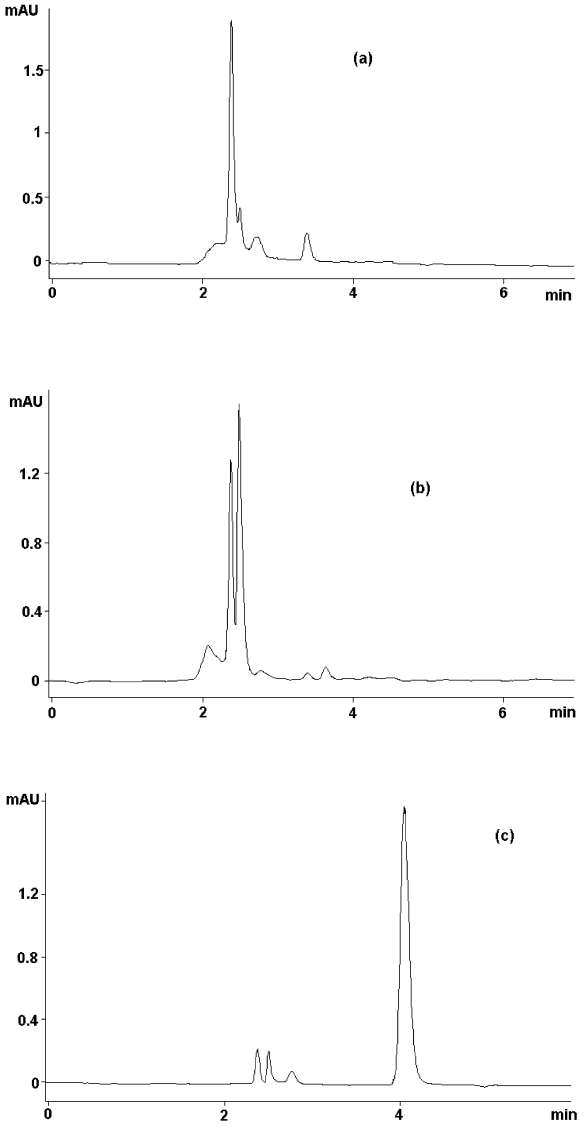

Fig. 1. (a) Chromatogram obtained from a non-spiked cotton swab. (b) Chromatogram obtained from a non-spiked surface. (c) Chromatogram obtained from a 0.88 μg/ml ranitidine hydrochloride standard with the selected conditions: 0.05 *M* ammonium acetate–methanol (60:40), 25°C, 25 μl and 320 nm.

The response standard, plus three times the mean background response provided the LOD. The response standard, plus 10 times the mean background, provided the LOQ. The LOD was 2 ng/ml($S/N=3.8$, RSD=11%) and the LOQ was 20 ng/ml ($S/N=10.4$, RSD=2.5%), see Fig. 2.

The stability of the analytical solutions was determined from the standards and samples at room temperature and at 4°C. These solutions were analyzed after 1, 2, 3, 4, and 7 days. Solutions were analyzed and compared with a freshly prepared standard at each time interval. The standards and samples were found to be stable at room temperature as well as at 4°C, for 7 days. Since the change in concentration was within ±2%, the solutions were considered stable.

3.3. Sample treatment optimization

Two cotton swabs (0.25 g), cleaned as previously described, were spiked with different quantities of ranitidine hydrochloride ranging from 0.5 to 50 μg, and were placed into a tube. After adding the solvent, the tube was sonicated for 5 min and then, the solution was analyzed by HPLC. In the extraction procedure, three masses (3, 5 and 7 g) of the methanol–water mixture were assayed for each level of ranitidine. The results obtained are shown in Table 2. The analysis of variance (ANOVA) test of two tails ($P=0.95$) was used to prove the homogeneity of the recovery. Significant differences were found among the recoveries for 3, 5 and 7 g but not between the recoveries for 5 and 7 g. Taking into account that it is important not to dilute the sample in excess, in order to obtain the best detection and quantitation limits, 5 g was selected. Using this mass, no significant differences in the recovery were found for the different levels of ranitidine hydrochloride assayed, obtaining a mean value of 97.8% and RSD=2.0%

3.4. Sampling from the surfaces

Three different kinds of cotton swabs: dry, wet and two cotton swabs (the first wet and the second dry), were used to recover each quantity of ranitidine hydrochloride (1.51 μg, 15.1 μg and 30.2 μg) spiked on the different surfaces (20×20 cm^2). As it can be seen in Table 3, the recoveries ranged from 16.5% in the case of the vinyl surface and using one dry cotton swab, to 90.6% in the case of the stainless steel surface and using two cotton swabs. In all cases, the best results were obtained using the two cotton swabs, so this technique was used for the rest

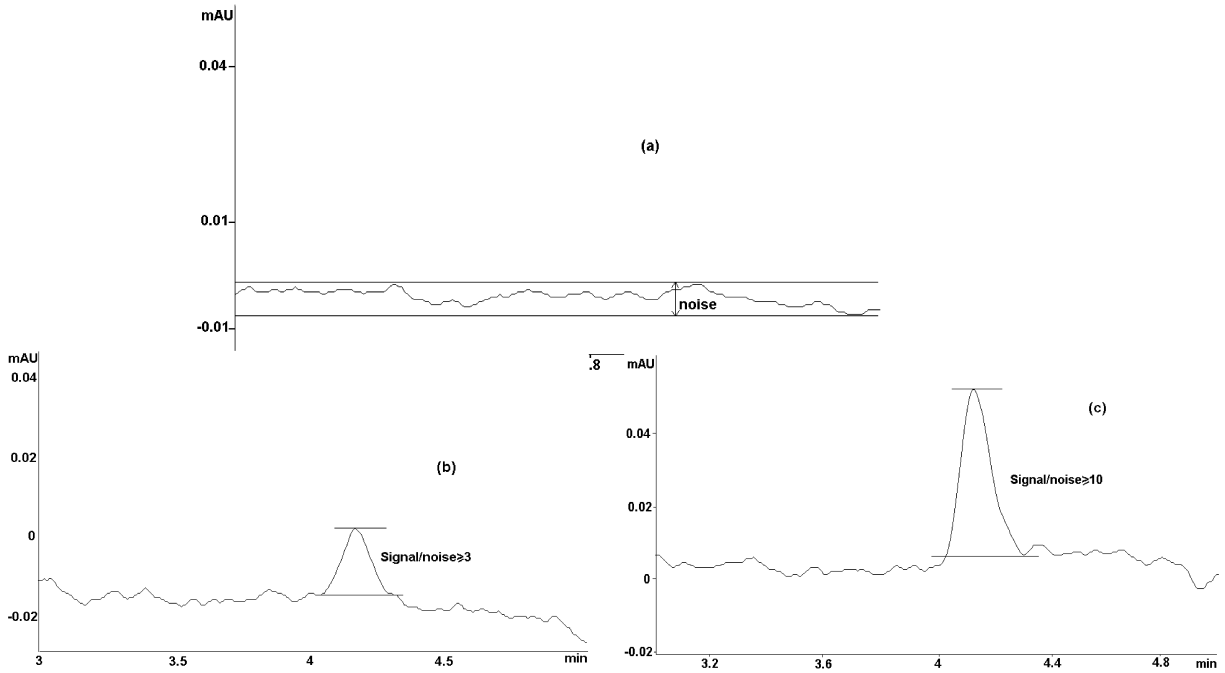

Fig. 2. (a) Chromatogram obtained from blank samples. (b) Limit of detection: chromatogram for a 2 ng/ml ranitidine hydrochloride standard. (c) Limit of quantification: chromatogram for a 20 ng/ml ranitidine hydrochloride standard.

Table 2
Recovery of ranitidine hydrochloride from cotton swabs

Ranitidine (μg)	Recovery (%)±RSD (%)		
	Methanol–water (1:1), added		
	3 g	5 g	7 g
0.532	95.9±0.3	97.2±1.4	97.7±2.6
35	95.2±0.5	99.4±0.8	102.8±2.4
50	93.2±2.5	96.0±2.8	95.4±2.7

$n=5$.

of the work. Applying an ANOVA test of two tails ($P=0.95$) to the results obtained using the two cotton swabs, it was found that the recovery was influenced by the type of surface, and not by the level of the analyte spiked, obtaining the lowest recovery for the vinyl surface.

To study the influence of the degree of moisture of the first cotton swab, on the analyte recovery, the three surfaces were spiked with 15.1 μg of ranitidine hydrochloride and different volumes of the metha-

Table 3
Recovery of ranitidine hydrochloride from stainless steel, glass and vinyl surfaces

Drug added (μg)	Dry cotton swab			Wet cotton swab			Double cotton swab		
	Stainless steel Recovery (%) ±RSD (%)	Glass Recovery (%) ±RSD (%)	Vinyl Recovery (%) ±RSD (%)	Stainless steel Recovery (%) ±RSD (%)	Glass Recovery (%) ±RSD (%)	Vinyl Recovery (%) ±RSD (%)	Stainless steel Recovery (%) ±RSD (%)	Glass Recovery (%) ±RSD (%)	Vinyl Recovery (%) ±RSD (%)
1.51 ($n=5$)	23.9±9.9	21.6±23.2	18.1±38.3	87.6±11.2	74.6±28.3	70.4±13.5	92.3±5.0	85.9±7.0	81.2±3.4
15.1 ($n=5$)	34.4±27.8	19.3±48.6	26.3±40.8	74.0±6.5	64.7±9.3	64.4±9.3	94.9±3.8	87.5±5.1	77.4±4.6
302 ($n=5$)	37.2±8.1	24.3±25.5	5.02±48.4	74.6±2.9	61.0±12.9	57.9±5.6	84.5±2.8	81.4±5.2	75.5±5.9
$n=15$							90.6±6.3	85.0±6.3	78.0±5.4

Table 4
Influence of the degree of moisture of the first cotton swab on the analyte recovery from 20×20 cm^2 surfaces

Volume (ml) MeOH–water (1:1)	Stainless steel		Glass		Vinyl	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
0.5 (n=5)	82.8	0.8	77.7	8.1	69.0	12.4
1.0 (n=5)	94.9	3.5	90.5	2.2	77.7	2.5
1.5 (n=5)	91.8	3.7	87.0	1.6	83.9	2.0
2.0 (n=5)	90.0	5.4	83.7	3.5	73.7	4.5
At random (n=5)	93.7	3.4	82.4	4.8	78.3	5.3

nol–water (1:1) mixture were used to wet the cotton swab. For a given surface, the recoveries were similar for all the volumes assayed, except for 0.5 ml, where the recoveries were the lowest (Table 4). This fact could be explained because 0.5 ml are not enough to moist the full surface.

As can be seen in Table 5, using 1 ml of the methanol–water mixture, the mass of the cotton swabs influenced the recovery of the analyte from the stainless steel surface but not from the glass and vinyl ones.

Finally, the effect of the surface size on the recovery was studied. The results obtained for plates of 20×20 cm^2, 25×25 cm^2 and 30×30 cm^2 show that the recovery was not affected by the surface size, obtaining high values in all cases.

4. Conclusions

This work provides a validated procedure for determining ranitidine hydrochloride residues on surfaces in the manufacture of pharmaceuticals.

To extract the ranitidine hydrochloride residues from surfaces a wipe test procedure, using two cotton swabs, is recommended. Recoveries of ranitidine hydrochloride from glass, vinyl and stainless steel surfaces, were higher than 85% in the μg range.

Table 5
Recovery of ranitidine hydrochloride, from 20×20 cm^2 surfaces, in relation with the different masses of cotton swab

Cotton swab (g) (n=5)	Recovery (%)±RSD (%)		
	Stainless steel	Glass	Vinyl
0.10	78.8±4.4	85.8±3.1	76.4±2.4
0.25	94.9±3.5	90.5±2.2	77.7±2.5

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