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Development and validation of a liquid chromatographic method for determination of lacidipine residues on surfaces in the manufacture of pharmaceuticals☆

María J. Nozal*, José L. Bernal, Juan J. Jiménez, María T. Martín, Francisco J. Diez

Department of Analytical Chemistry, Faculty of Sciences, University of Valladolid, E-47005 Valladolid, Spain

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Abstract

A high-performance liquid chromatographic (HPLC) method for the assay of lacidipine residues in swabs collected from various surfaces involved in drug manufacture is described. The swabbing procedure using two cotton swabs was validated applying a wipe test. An RP-HPLC method, developed to determine low quantities of the drug in the presence of its main impurities, was also validated. To remove drug residues from stainless steel and glass surfaces, the first cotton swab must be soaked preferably in acetonitrile whereas, on vinyl surfaces better results are obtained using methanol. The HPLC method selected involves a C_{12} column, at 40 °C, a mixture of acetonitrile-0.05 M ammonium acetate (88:12, v/v) as a mobile phase and UV detection at 282 nm. Recoveries obtained are strongly dependent on the type of surface tested, being higher on stainless steel. The surface material has also different influence on the drug stability. The method was validated over a range of 0.5–100 μg/400 cm^2 and had a detection limit of 0.1 μg/400 cm^2.

Keywords: Validation; Pharmaceutical analysis; Lacidipine

1. Introduction

Lacipidine, chemically designated as (*E*-4-{2-[3-(1,1-dimethylethoxy)-3-oxo-1-propenyl]phenyl}-1,4-dihydro-2,6-di-methyl-3,5-pyridine-dicarboxylic acid diethyl ester), is a calcium channel blocker developed for oral administration and widely used in therapy since the early 1990s. Antihypertensive effect apart, lacidipine has also shown anti-atherosclerotic and antioxidant effects. It is one of the most vascular selective of the dihydropyridines. It has long duration of action because of its high degree of lipophilicity. The active *trans* form is used in therapy [1–3].

From a physico-chemical point of view, lacidipine is slightly soluble in water, while it is more soluble in some widely used solvents as ethanol, methanol and acetone. It is very sensitive to the action of temperature and light. For this reason, it can be degraded and, after some time, it appears together with some impurities [4], as it can be seen in Fig. 1.

In recent years, the subject of cleaning validation in active ingredient pharmaceutical manufacturing plants has received a large amount of attention from regulators, companies and customers. It is not a new issue since cleaning validation is a requirement mandated by 1963 good manufacturing practice (GMP) regulations (Part 133.4) and 1978 GMP regulations (Section 211.6). The cleaning procedures for the equipment must be validated according to GMP rules and guidelines [5,6].

The main objective of the cleaning validation program is to prevent cross-contamination of products. The effectiveness of the cleaning procedure is checked using a validated analytical method suitable to investigate the traces of residues on surfaces. These residues are usually collected from the surfaces by using, in different ways, cotton swabs [7–10], after that residues are determined by an adequate technique.

Although several analytical methods have been proposed to analyse lacidipine [11–14], its determination as an active ingredient is preferably carried out by high performance

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* Corresponding author. Fax: +34-98-3423013.
E-mail address: mjdnozal@qa.uva.es (M.J. Nozal).

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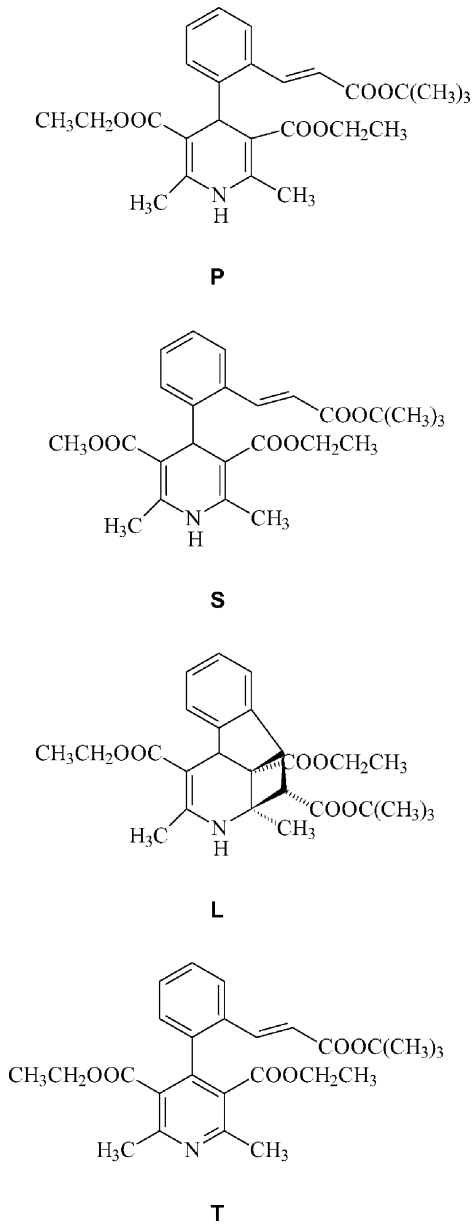

Fig. 1. Structure of lacidipine, synthesis impurity, light impurity and temperature impurity. P: lacidipine; S: synthesis impurity; L: light impurity; T: temperature impurity.

liquid chromatography, using normal phase on CN columns [4] and, in some instances, RP-HPLC on C_{18} columns [15,16], and UV detection at 240 nm, these RP-HPLC methods do not usually consider the presence of impurities.

Taking the above mentioned consideration into account, the aim of this work has been to develop a method using RP-HPLC that allows the determination at trace level not only of residual lacidipine but also of its frequent impurities caused by temperature and light. We have used the two-swab procedure on stainless steel, glass and vinyl plates, as in other works [17–19] but modifying some steps due to the unstability and different solubility of this compound.

2. Experimental

2.1. Chemicals

The lacidipine certified standard (99.9%, w/w), a mixture test containing three impurities (T, L and S) arisen by effect of temperature, light and synthesis, and the plates of different materials were generously given by Glaxo-SmithKline (Aranda de Duero, Spain). Acetonitrile (ACN) (HPLC-UV) grade was obtained from Lab-Scan (Dublin, Ireland). Ethanol and *n*-hexane (HPLC-UV grade) were obtained from Scharlab (Barcelona, Spain).

Ammonium acetate (99%, w/w), glacial acetic acid, sodium hydroxide, analytical reagents, were purchased to Scharlab (Barcelona, Spain). 0.45 μm Nylon filters were obtained from Millipore (Bedford, MA, USA). Ultrapure water was obtained in a Milli-RO plus system together with a Milli-Q system from Millipore. Absorbent cotton 100% from LauSan (Valladolid, Spain).

2.2. Equipment

The HPLC system used in this study 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. An AE-240 analytical balance from Mettler (Toledo, USA), and a Bransonic 5 ultrasonic bath from Scharlau (Barcelona, Spain).

2.3. Columns

The chromatographic separation was tested on three different columns: a Spherisorb CN column: 5 μm, 250 mm × 4.6 mm, obtained from Supelco (Bellefonte, PA, USA); a Luna C_{18} column: 5 μm, 250 mm × 4.6 mm and a Sinergy C_{12} column: 4 μm, 250 mm × 4.6 mm, both obtained from Phenomenex (Torrance, CA, USA).

2.4. Chromatographic conditions

Using the C_{12} column, the mobile phase used was a mixture of acetonitrile–0.05 M ammonium acetate (88:12, v/v) and was pumped isocratically at a flow rate of 1.0 ml/min and a column temperature of 40 °C. The injection volume was 20 μl and the detection wavelength was set at 282 nm.

Using the Ciano column, the mobile phase used was *n*-hexane–ethanol (94:6, v/v), at a temperature of 25 °C, and a flow rate of 1 ml/min.

Using the C_{18} column, the mobile phase used was acetonitrile–0.05 M ammonium acetate (75:25, v/v), at a flow rate of 1 ml/min and a column temperature of 40 °C.

2.5. Preparation of calibration standards

Stock solution of standard was prepared by accurately weighing lacidipine reference standard (5 mg

approximately) and transferring it into a 50 ml volumetric flask. This standard was dissolved in acetonitrile, dilutions were later prepared with the same solvent to obtain the solutions for calibration. Standards to be used for spiking vinyl surfaces were solved in methanol.

The test of impurities was prepared by adding 2 ml of acetonitrile to the vial, which contained 0.025 mg of each compound. All solutions were stored for a maximum of 1 week, in darkness at 4 °C.

2.6. Sample preparation

Two cotton swabs of approximately 0.25 g, previously rinsed exhaustively with water and acetonitrile and then dried under vacuum, were placed into a 50 ml brown screw cap borosilicate test tube and weighed.

The selected surfaces (20 cm × 20 cm) of stainless steel, glass and vinyl, previously cleaned with methanol, then with water, and afterwards, dried, were sprayed with 1 ml of a standard solution of lacidipine, and the solvent was allowed to evaporate. The surfaces were wiped with the first cotton swab, soaked with acetonitrile to remove the residues from glass and stainless steel, and soaked with methanol to remove the residues from vinyl. The other dry cotton swab was used to wipe the wet surfaces. The two swabs were placed in the tube and acetonitrile was added to reach a mass 5.5 g higher than the one obtained before. After that, the tube was placed in the ultrasonic bath, into a mixture of water and ice, for ten minutes and the solution was analysed by HPLC.

3. Results and discussion

3.1. Development of the chromatographic method

To obtain the best chromatographic conditions, the wavelength for detection, the column and the mobile phase composition were adequately selected. The main objective was to develop a reversed-phase liquid chromatographic method that, working in isocratic mode, allowed the determination of lacidipine residues collected by the swab, without interference of its common impurities and in the shortest time.

Lacidipine UV spectrum shows three maxima (240, 282 and 320 nm), the maximum absorbance belongs to the 240 nm band, but examining the spectra of the impurities originated by the action of light or temperature, both present also a strong absorption to the same wavelength, where photodegradation rate is highest. For this reason, we have preferred to select 282 nm, at this wavelength lacidipine shows enough absorption and the calibration has a good linearity and the best signal-to-noise ratio.

Regarding the chromatographic procedure, we assayed first the Ciano column with a mobile phase mixture of *n*-hexane–ethanol, varying the ethanol proportion between 3 and 20% and the temperature between 20 and 60 °C trying to achieve a good separation of all the compounds. The best conditions were *n*-hexane-ethanol (94:6, v/v) as a mobile phase, at a temperature of 25 °C, and a flow rate of 1 ml/min. The retention time for the lacidipine peak (P) was of 8.1 min, whereas for the impurities, the retention times were 5.5 min (temperature, T), 7.6 min (light, L) and 8.9 min (synthesis, S) as can be observed in Fig. 2a. In Table 1, some characteristics of the separation are given.

The same study was carried out using a C_{18} column, the initial mobile phase was a mixture of acetonitrile–0.05 M ammonium acetate (90:10, v/v), then the acetonitrile percentage was varied between 90 and 65% and the temperature between 20 and 60 °C. The best separation was achieved with the mixture acetonitrile–0.05 M ammonium acetate (75:25, v/v), at a temperature of 40 °C, and a flow rate of 1 ml/min. The retention time for lacidipine (P) was 10.0 min, whereas impurities appeared at 9.6 min (T),

Table 1
Chromatographic parameters, obtained with the three columns, for lacidipine (P) and its common impurities (S, L and T)

	Column/mobile phase		
	CN/*n*-hexane–EtOH (94:6, v/v)	C_{18}/ACN–$AcNH_4$ (75:25, v/v)	C_{12}/ACN–$AcNH_4$ (88:12, v/v)
$t_{ret\,P}$ (min) ± R.S.D. (%)	8.1 ± 0.5	10.0 ± 0.5	6.0 ± 0.1
Width 5σ (min)	0.70	0.79	0.40
Symmetry	0.89	0.87	0.98
TF	1.10	1.18	0.96
NTP	1500	6300	6500
k'_P	3.3	3.6	2.0
$R_{P/S}$	1.5	2.5	4.6
$t_{ret\,S}$ (min)	8.9	8.3	5.0
$R_{P/L}$	1.1	1.0	2.5
$t_{ret\,L}$ (min)	7.6	9.3	5.5
$R_{P/T}$	4.8	0.6	1.8
$t_{ret\,T}$ (min)	5.5	9.6	5.7
LOQ_P (μg/ml)	0.06	0.05	0.02

P: lacidipine; S: synthesis impurity; L: light impurity; T: temperature impurity [capacity factor (k'), resolution factor (R), number of theoretical plates (NTP), tailing factor (TF)] ($n = 5$).

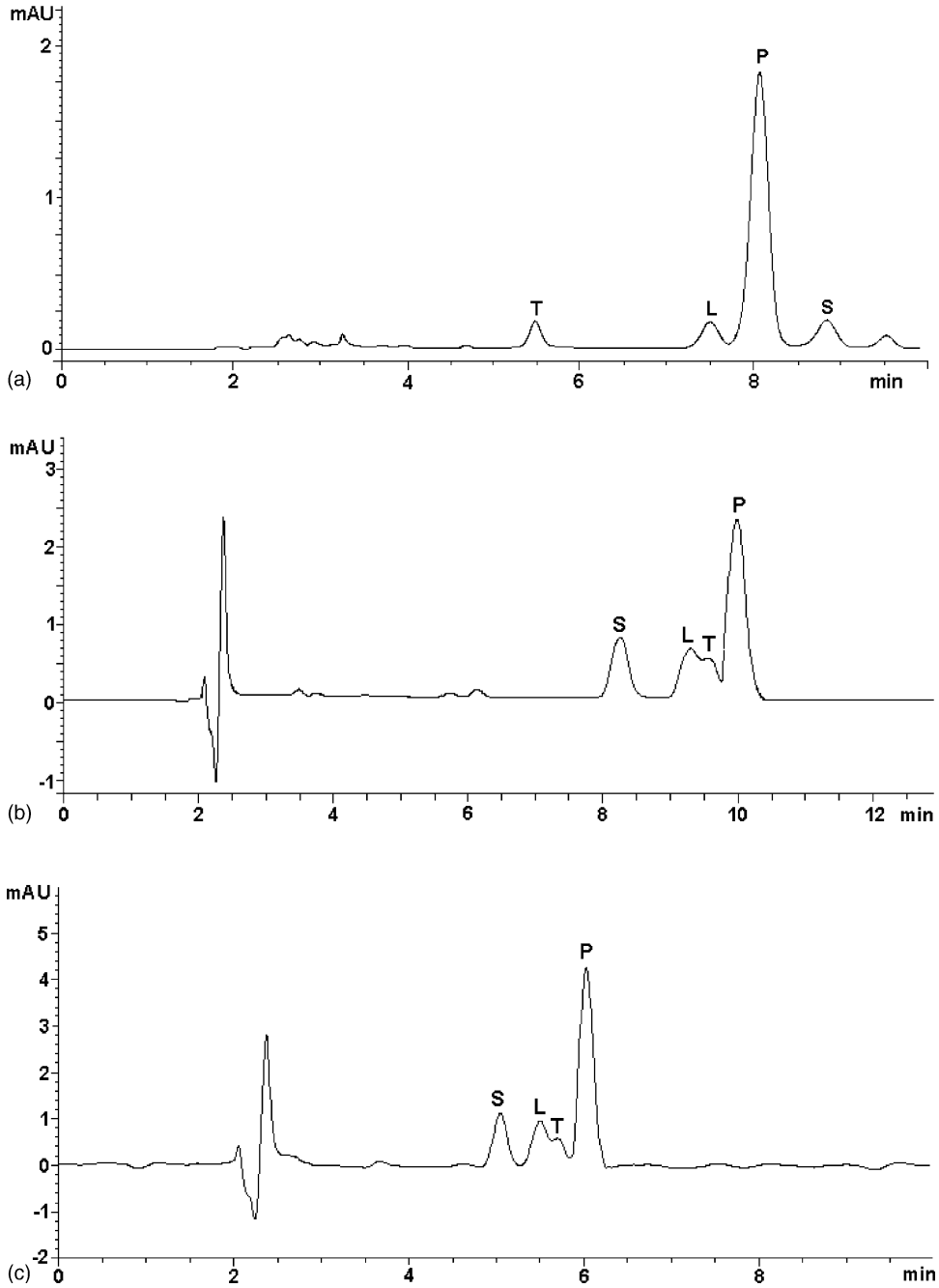

Fig. 2. Chromatograms obtained from standards of lacidipine and their common impurities using different columns with the selected conditions for each one: (a) CN: *n*-hexane–ethanol (94:6, v/v), 25 °C, 20 μl and 282 nm; (b) C_{18}: acetonitrile–0.05 M ammonium acetate (75:25, v/v), 40 °C, 20 μl and 282 nm; (c) C_{12}: acetonitrile–0.05 M ammonium acetate (88:12, v/v), 40 °C, 20 μl and 282 nm.

9.3 min (L) and 8.3 min (S). It can be observed, Fig. 2b, that the elution order has changed: all impurities elute before lacidipine. In Table 1, some characteristics of the separation are also given.

Taking the low polarity of lacidipine into account we thought of testing other RP columns, specially a C_{12} one, with a similar mobile phase. With this column, we obtained a good separation using a mixture of acetonitrile–0.05 M ammonium acetate (88:12, v/v), a temperature of 40 °C, and a flow rate of 1 ml/min. The peakwitdh had been notably reduced in comparison with that one obtained with the C_{18} column, and the retention times had also been shortened, so lacidipine peak (P) appeared at 6.0 min, and those corresponding to impurities at 5.8 min (T), 5.6 min (L) and 5.2 min (S,) respectively. In Fig. 2c and Table 1, the results obtained are shown.

Taking into account the results obtained with the three columns assayed, we finally chose the C_{12} one because the

quantitation limits obtained were the lowest, without interferences and in the shortest runtime.

3.2. Validation of the chromatographic method

The validation has been carried out following the ICH guidelines [20] and IUPAC technical report of 2002 [21], determining selectivity, limit of quantitation and detection, linearity, precision and trueness.

3.2.1. Selectivity

Selectivity has been checked by injecting a standard of lacidipine and its impurities. In Fig. 2c, it can be observed that there are not mutual interferences.

3.2.2. The detection limit (LOD) and quantitation limit (LOQ)

LOD and LOQ were determined by measuring the magnitude of the analytical background response, by injecting a number of solvent blank ($n = 6$). We deduced the LOD and LOQ values from the response standard, plus 3 and 10 times the mean background response, respectively. The values obtained were LOD: 0.008 μg/ml (S/N = 4.8; R.S.D. = 4.8%) and for LOQ: 0.02 μg/ml (S/N = 10.6; R.S.D. = 2.8%).

3.2.3. Calibration and linearity

Plotting the area values in function of the concentration for ten standards, the graph obtained was a straight line of intercept not significantly different from zero, which confirmed the linearity through the range studied and the lack of bias.

The statistical parameters of the calibration were: a (slope) $= 121.4 \pm 0.34$; b (y-intercept) $= 1.04 \pm 1.37$ ($b \rightarrow 0$; no bias); $S_{y/x} = 3.44$; $r^2 = 0.9999$; $t_{exp} > t_{tab(n-2,0.95)}$.

3.2.4. Precision

Precision was evaluated by the same analyst making six determinations on the same sample and for three different concentrations. Results obtained show that R.S.D.s varied from 0.06 to 2.1% (for quantitation limit).

3.2.5. Spiking/recovery and trueness

Spiking/recovery and trueness were determined by injecting six standards from three different concentrations (one of them the quantitation limit). The average percentage recoveries obtained were comprised within 98.5 and 100.2% with R.S.D.s of 0.8–2.3% (for quantitation limit). A t-test revealed the absence of significant differences between them.

3.3. Extraction procedure

Two cotton swabs were spiked with different quantities of lacidipine ranging from 0.2 to 40 μg, and were placed in the brown tube. In the extraction procedure, three volumes (5, 7 and 10 ml) of the acetonitrile were assayed for each quantity of lacidipine. We select 5.5 g of acetonitrile(equivalent to 7 ml added) because lower limits of quantitation and lower R.S.D.s were obtained.

The extraction procedure was validated in terms of selectivity, quantitation limit, precision and trueness.

3.3.1. Selectivity

A blank from two cotton swabs placed in the tube, wetted with acetonitrile and sonicated for 10 min was analysed. It can be observed in Fig. 3 that the blank contribution to the background was minimal.

3.3.2. Limit of quantitation

It was carried out by spiking a pair of cotton swabs with low quantities of lacidipine and comparing the signal with the blank of cotton swabs. The background signal of the cotton swab sample, plus ten times the mean background response provided the LOQ. The quantitation limit was set up at 0.2 μg (S/N = 10.3; $R.S.D._{(n=6)}$ = 3.0%).

3.3.3. Precision

Precision was determined by spiking six pairs of cotton swabs with three different quantities of lacidipine, obtaining R.S.D.s from 0.09 to 2.07% (the last one belonging to the lowest concentration).

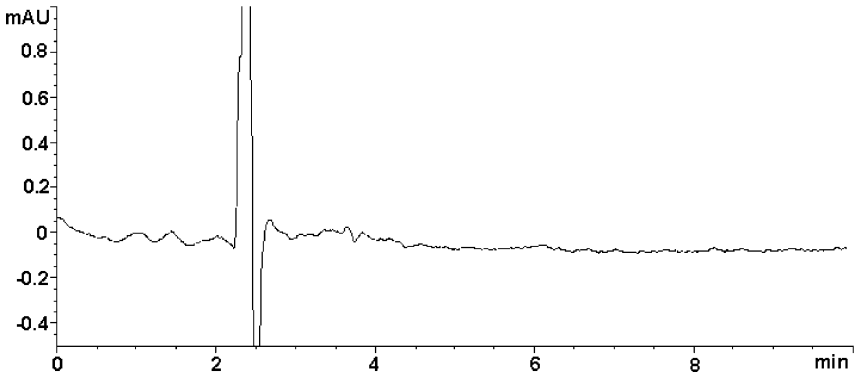

Fig. 3. Chromatogram obtained from a non-spiked cotton swab under the selected conditions: C_{12} column, acetonitrile–0.05 M ammonium acetate (88:12, v/v), 40 °C, 20 μl and 282 nm.

3.3.4. Spiking/recovery and trueness

These were determined by spiking six pairs of cotton swabs with three solutions at different concentration, obtaining average recoveries ranging from 101.3 to 99.8% and R.S.D.s from 0.1 to 3.7%. A *t*-test revealed the absence of significant differences between different levels of spiking.

3.4. Application of the developed and validated method to the quantitative determination of lacidipine from stainless steel, glass and vinyl surfaces

Each plate, previously cleaned with methanol, water and then dried, was spiked with 1.0 ml of different standard solutions, in order to apply approximately 1, 5, 8 and 25 μg of lacidipine on surfaces. The plates were allowed to dry, and the drug residues were removed by wiping the surface with the cotton swabs in a way that assured that the entire plate was thoroughly cleaned.

The first cotton swab was initially wetted with methanol, the excess of the solvent was removed pressing it with the stainless steel tweezers. The second dry cotton swab was used to remove the remainder solvent on the surface. The recoveries obtained were somewhat low, an 85% from stainless steel, a 73.7% from glass and a 70.4% from vinyl. Trying to increase these recoveries we assayed acetonitrile for wetting the first cotton swab, and in fact, the recoveries on stainless steel and glass plates increased (see Table 2). However, in the case of vinyl plates, an obstacle arose: it seems that acetonitrile removes some components of the plate material leading to the appearance of peaks in the chromatogram that avoided the correct determination of the target compounds (Fig. 4). Other solvents than methanol (ethanol, isopropanol, water) were added to the first cotton swab, trying to increase the recovery on vinyl surfaces. All attempts were unsuccessful. For this reason, we recommend the use of acetonitrile for wetting the first cotton when stainless steel and glass surfaces are considered whereas methanol is more advisable to work with vinyl plates. The suggested procedure consists of the following steps: (1) Each 20 cm × 20 cm pre-cleaned plate is spiked with 1.00 ml of lacidipine solutions, (2) the first cotton swab (0.25 g) is wetted with acetonitrile (for vinyl surfaces the swab is wetted with methanol), (3) the plates are left to dry, (4) the drug residues are removed by wiping the surfaces with the first cotton swab in a way that assures that the entire plate is thoroughly cleaned (horizontally, vertically and diagonally), (5) the second dry cotton swab (0.25 g) is used to remove the remainder of the solvent from the surface, (6) finally, both cotton swabs are placed in the tube and 5.5 g of acetonitrile are added, they are sonicated for ten minutes and a portion of the solution is analysed by the HPLC proposed method.

Table 2
Recovery of lacidipine from spiked surfaces (400 cm^2) applying the two-cotton swab procedure

Lacidipine added (μg) ($n = 5$)	$R \pm$ R.S.D. (%)		
	Stainless steel	Glass	Vinyl[a]
1	93.5 ± 5.6	80.5 ± 3.5	–
5	92.8 ± 6.1	83.7 ± 5.3	69.6 ± 7.7
8	94.0 ± 6.6	83.4 ± 6.2	70.2 ± 6.0
25	90.3 ± 7.2	81.9 ± 2.0	71.3 ± 9.6
Mean ($n = 20$)	92.6 ± 5.8	82.3 ± 4.3	70.4 ± 7.4

R: recovery; R.S.D.: relative standard deviation; (–) < limit of quantification.
[a] Swab wetted with MeOH.

3.4.1. Validation

Applying an analysis of variance (ANOVA) test of two tails ($P = 0.95$) to the results, it was appreciated that recovery was influenced by the type of surface, and not influenced by the amount of drug on the surfaces, obtaining the lowest recoveries from the vinyl surface, and the highest recoveries were obtained from stainless steel plates. Results obtained on different surfaces are shown in Table 2.

Using this procedure, it is possible to determine quantities of spiked drug lower than 1 μg on stainless steel and glass, and 5 μg on vinyl, on surfaces of 400 cm^2.

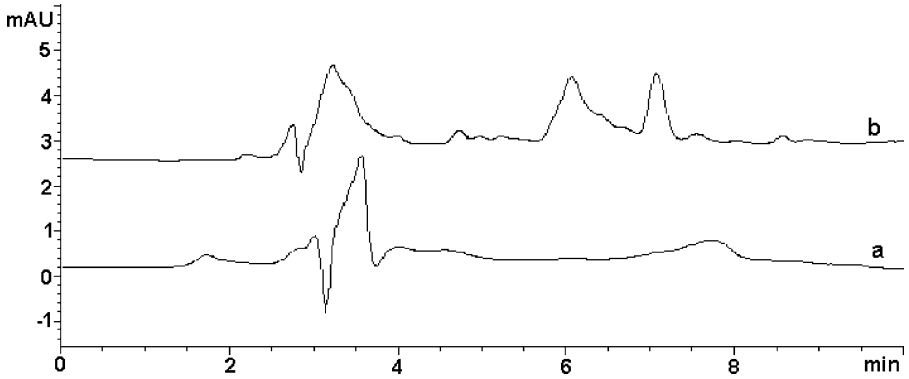

Fig. 4. Chromatograms obtained from cotton swabs moistened with methanol (a) or acetonitrile (b) passed through a 20 cm × 20 cm vinyl surface, and analysed under the selected conditions.

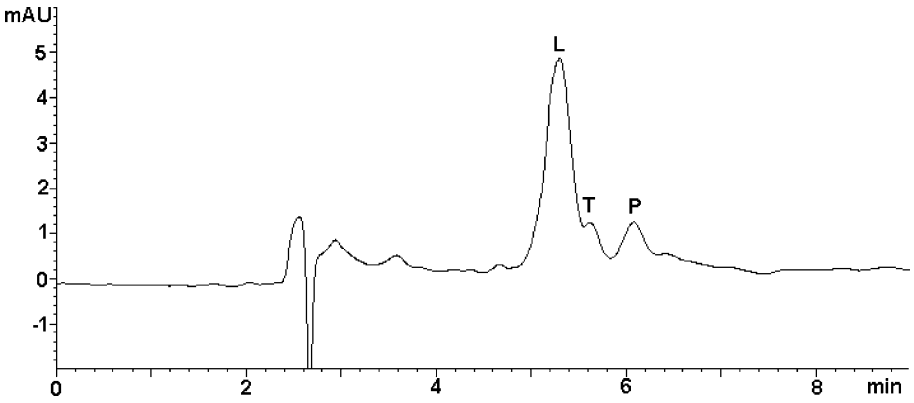

Fig. 5. Chromatogram of residues obtained by the double swab method, from a 20 cm × 20 cm stainless steel surface spiked with 50 μg of lacidipine. Extract stored for 3 days at 25 °C and light and then analysed.

3.5. Stability of solutions

Knowing that lacidipine degrades with temperature and light, a study of the stability of the compound through the whole procedure was done for a week and under different conditions. The stability of the standard solutions was determined preparing a set of them, that was stored in three different conditions (darkness: 25 °C, light: 25 °C, darkness: 4 °C). After 1, 2, 3, 4 and 6 days, these aged solutions were reanalyzed against freshly prepared standard solutions. The common acceptance criterion for stability was that the variations of recoveries were less than ±2%.

The stability of the swab samples was determined by spiking similar surfaces directly with solutions of the drug and measuring them immediately after applying the cleaning procedure and repeating it along 7 days, always comparing those data with the ones obtained on freshly prepared standard solutions. In this case, the acceptance criterion for stability in swabbing was that the variations of recoveries were less than ±5%.

After 2 days, the average concentration of swab samples and standards, stored at light and 25 °C, reduced to 40% of its initial concentration. In Fig. 5, the chromatographic peaks of the degradation can be observed, being those caused by the radiation bigger. Therefore, it is recommended to keep the sample solutions in absence of light at 4 °C. The purity peak has been evaluated in each case obtaining purity factors 990–1000 on standards stored in absence of light at 4 °C.

The stability was studied on the three different surfaces obtaining similar results in stainless steel and glass, but the stability of lacidipine extracted from vinyl surfaces was slightly lower, this could be due to the fact that some compounds of the material could be extracted by methanol, which could make the degradation faster, even if the sample was stored at 4 °C. Results of stability of samples and standards are shown in Table 3. Therefore, it is advisable to carry out the extraction and further HPLC analysis as soon

Table 3
Variation with time of the recoveries and R.S.D.s obtained for lacidipine standards and samples taken from different surfaces

	Days after preparation or collection				
	1	2	3	4	6
Standard					
Light: 25 °C	64.7 ± 1.0	39.1 ± 1.3	14.2 ± 1.8	–	–
Dark: 25 °C	98.1 ± 0.8	95.6 ± 1.8	90.1 ± 2.8	88.1 ± 4.3	81.1 ± 5.4
Dark: 4 °C	99.2 ± 1.0	99.2 ± 1.3	99.5 ± 1.3	99.5 ± 1.2	99.2 ± 1.3
Stainless steel					
Light: 25 °C	52.1 ± 1.2	37.2 ± 1.8	17.5 ± 2.0	–	–
Dark: 25 °C	98.6 ± 0.8	87.6 ± 1.3	82.0 ± 1.7	78.1 ± 1.5	75.1 ± 1.5
Dark: 4 °C	100.0 ± 0.5	99.4 ± 0.8	98.8 ± 1.1	98.5 ± 1.3	98.1 ± 1.8
Glass					
Light: 25 °C	56.2 ± 2.2	41.3 ± 2.1	25.1 ± 3.0	–	–
Dark: 25 °C	95.2 ± 1.1	88.7 ± 1.1	86.5 ± 0.3	81.6 ± 2.8	77.5 ± 0.8
Dark: 4 °C	99.5 ± 1.2	99.3 ± 1.1	99.2 ± 1.3	99.5 ± 1.3	99.1 ± 1.3
Vinyl					
Light: 25 °C	54.0 ±1.8	34.0 ± 1.8	10.5 ± 2.3	–	–
Dark: 25 °C	97.1 ± 2.4	84.0 ± 1.8	78.0 ± 1.5	75.1 ± 2.2	67.2 ± 2.0
Dark: 4 °C	98.8 ± 1.8	98.7 ± 2.2	98.4 ± 2.1	98.3 ± 1.8	94.0 ± 3.0

(–) < limit of quantification.

as possible, after applying the swabs, in order to prevent the transformation of the lacidipine caused mainly by light.

4. Conclusions

A rapid and sensitive reversed-phase high performance liquid chromatographic method using a C_{12} column to determine lacidipine residuals in manufacturing pharmaceutical production surfaces has been developed and found to be accurate and precise. The HPLC method is suitable for the analysis of residues on surface samples in the range of 0.5–100 μg. The unstability of samples of lacidipine collected from vinyl is higher than that obtained from stainless steel or glass surfaces, so it is recommended to carry out the extraction and analysis as soon as possible.

The highest recoveries are obtained on stainless steel plates and the lowest on vinyl. Those recoveries are strongly dependent on the surface material (stainless steel, glass or vinyl) and also on the solvent used to wet the first cotton swab.

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