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Development and validation of a liquid chromatographic method for the determination of amlodipine residues on manufacturing equipment surfaces

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Abstract

In the pharmaceutical industry, an important step consists in the removal of possible drug residues from the involved equipments and areas. The cleaning procedures must be validated and the methods to determine trace amounts of drugs have therefore to be considered with special attention. A high performance liquid chromatographic method for the determination of amlodipine residues in swab samples was developed and validated in order to control a cleaning procedure. The swabbing procedure was optimized in order to obtain a suitable recovery of amlodipine from stainless steel. A mean recovery close to 90% was obtained when two swabs moistened with methanol were used. The residual amlodipine was chromatographed at 25 °C in the isocratic mode on a RP-18 stationary phase using a mobile phase consisting of acetonitrile, methanol and pH 3.0 triethylamine solution (15:35:50 v/v/v). UV detection was performed at 237 nm. The method was shown to be selective and linear into the concentration range varying from 0.39 to 1.56 μg/ml. Accuracy and precision of the method were also studied. The limits of detection and quantitation were evaluated to be 0.02 and 0.08 μg/ml, respectively. The stability of amlodipine at different steps of the sampling procedure and the precision of the swabbing procedure were also investigated.

Keywords: Cleaning validation; Amlodipine; Swabbing recovery; HPLC

1. Introduction

Good manufacturing practice dictates that the equipment necessary to manufacture pharmaceuticals must be maintained in a clean and orderly manner [1]. In many cases, especially in a R&D plant, the same equipment may be used for processing different products and, in order to avoid contamination of the following pharmaceutical product, an adequate cleaning procedure is essential. The cleaning procedure validation describes responsibilities, facilities, cleaning strategies, analytical strategies and residue limit justifications. The cleaning validation consists therefore in two separate activities: the first is the development and validation of the cleaning pro-

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cedure that is used to remove drug from the manufacturing surfaces and the second consists in developing and validating the methods used to quantify residuals from surfaces that are used in the manufacturing environment. To control the effectiveness of cleaning, the analytical method should be selective for the substance considered and has to provide a sufficient sensitivity since the concentration levels of residues are generally low. The objective of the analytical method consists in controlling that the contaminants can be removed from the equipment surface. It is therefore necessary to ensure that contaminants can be recovered from the equipment surface and to determine the level as well as the consistency of recovery.

The sampling is therefore a very important parameter since the conclusions of the cleaning procedure are based on sample results. Indeed, a negative test may be due to a deficient sampling technique. According to the FDA guide [1], two different method of sampling are generally admitted for performing a cleaning control: the direct surface sampling, using the swabbing technique and the indirect sampling based on the analysis of solutions used for rinsing the equipment (rinse method).

The rinse method occurs after the cleaning has been completed and allows the sampling of large surfaces and of inaccessible systems. Moreover, systems that cannot be routinely disassembled can be sampled and evaluated using this technique. However, it must be taken into account that the residue or contaminant may be insoluble or may be physically occluded in the equipment [1].

On the other hand, the swabbing method is a clearly more direct way of sampling. Using this method, areas hardest to clean and which are reasonably accessible can be evaluated, leading to establishing a level of contamination per given surface area. Moreover, residues that are 'dried out' or insoluble can be sampled by physical removal. As the swab sampling does not cover the entire equipment, it is important to define with care the sampling sites. Moreover, due to the nature of this method, it is of great importance to evaluate carefully the material to be used (swabs, solvents...) and to determine the efficiency of the sampling (recovery).

The main objective of this paper is to propose a selective and validated HPLC method for determining residual levels of amlodipine. Amlodipine is a potent dihydropyridine calcium channel blocker used in the treatment of hypertension and angina pectoris [2,3]. A variety of analytical methods dedicated to the analysis of amlodipine have been previously reported. Most of them involve liquid chromatography coupled to UV [4,5], fluorimetric [6], electrochemical [7,8], or mass spectrometry detection [9–12] but some determinations were also performed by thin layer [13,14], micellar electrokinetic [15] and gas chromatography [16,17] or spectrophotometry [18,19]. A LC method for the assay and related substances of amlodipine besilate is also reported in the European Pharmacopoeia [20]. Due to their high sensitivity and selectivity, analytical methods such as liquid [21–25] or capillary gas chromatrography [26] were previously reported to be used for the determination of residues to control cleaning procedures.

The analytical method reported here has been validated considering linearity, accuracy, precision and limits of detection (LOD) and quantitation (LOQ). As the swab sampling was selected, the influence of different parameters on the recovery of amlodipine was evaluated. The nature of the solvents used, the nature of the swabs and the swabbing technique were therefore investigated. The stability of amlodipine was also studied at different steps of the sampling procedure.

2. Experimental

2.1. Equipment

The HPLC system consisted of a L-7100 LaChrom pump, a L-7200 LaChrom autosampler, a L-7360 LaChrom oven and a L-7455 LaChrom diode array detector, from Merck Hitachi (Darmstadt, Germany). A computer equipped with D-7000 HPLC Manager software from Merck was used to control the whole chromatographic system and to collect and treat the data.

2.2. Chemicals and reagents

Amlodipine maleate was supplied by Unichem (Mumbai, India) and qualified as reference substance. Methanol and acetonitrile were of HPLC grade. Phosphoric acid 85% and triethylamine were of analytical grade and purchased from Merck. Ultrapure water was obtained with a Purelab Plus from USF Elga Seral (Ransbach-Baumbach, Germany). Sampling was achieved using CleanFoam TX740 or Absorbond TX762 swabs from Texwipe (Upper Saddle River, NJ). The separation was carried out on a LiChroCART Purospher RP-18e, 5 μm particle size, 125×4.0 mm column from Merck.

2.3. Chromatographic conditions

All chromatographic experiments were performed in the isocratic mode. The mobile phase consisted in a mixture of acetonitrile, methanol and triethylamine solution (15:35:50, v/v/v). The triethylamine solution was prepared by dissolving 7.0 ml of triethylamine in 950 ml of water in a 1-l volumetric flask, then adjusting the pH to 3.0 with phosphoric acid and diluting to volume with water. The mobile phase was degassed with helium prior to use. The flow-rate was set to 1.0 ml/min and the oven temperature to 25 °C. The injection volume was 100 μl and the detection wavelength was set at 237 nm.

2.4. Preparation of calibration solutions

A stock standard solution of amlodipine maleate was prepared in methanol at 3.89 μg free base/ml. This solution was then diluted with a methanol–water mixture (50:50, v/v) in order to obtain the calibration solutions at concentrations of 0.39, 0.58, 0.78, 1.17 and 1.56 μg/ml.

2.5. Sample preparation

The sample preparation for controlling the cleaning step of a manufacturing process is performed as follows:

Rinse the head of two TX762 Absorbond swabs with methanol and let the solvent to evaporate. Soak both swabs with fresh methanol and wipe a 20-cm^2 stainless steel surface area first in a horizontal and secondly in a vertical way, starting from the outside towards the centre. Cut the head of the swabs and introduce them into a 20-ml flask containing 5.0 ml of methanol with which both swabs have been moistened. Add 5.0 ml of purified water and place the solution in an ultrasonic bath for 15 min. Homogenise and inject the test solution into the chromatograph.

2.6. Chromatographic procedure

Hundred microliter aliquots of the samples and the calibration solutions were injected separately into the chromatographic column. The amount of residual amlodipine was determined by comparing the amlodipine peak area obtained for the sample to the linear calibration curve.

3. Results and discussion

3.1. Limit acceptance level

The specific residual cleaning level (SRCL) is defined as a concentration value in mass per unit of surface area and is based on the pharmacological activity of the molecule [21]. The SRCL can be obtained by considering different parameters such as the lowest dosage strength (D) of amlodipine, the smallest batch size manufactured using the equipment train (SBS), the surface area of the entire equipment train used in the manufacturing of the product (S) and a safety factor (SF). The SF has to be adjusted in accordance to the administration route and the toxicity of the product. For an oral formulation, the SF is generally set at 1000 or a higher value [26]

$$\mathrm{SRCL} = \frac{D \cdot \mathrm{SBS}}{\mathrm{SF} \cdot S}. \quad (1)$$

For amlodipine, the SRCL was calculated to be 0.76 μg/cm^2, using 5000 as SF.

3.2. Validation of the LC method

The method of determination of amlodipine was adapted from the chromatographic procedure described in the monograph of amlodipine besilate from the European Pharmacopoeia 4th Edition [20]. A Purospher C_{18} end-capped column was preferred to improve peak symmetry. The injection volume was set at 100 μl in order to increase the sensitivity of the method. The validation consisted in studying the linearity of the chromatographic response, the precision, the accuracy, the LOD and LOQ of the LC method as well as the stability of the standard solutions.

3.2.1. Stability of amlodipine standard solutions

The stability was studied on standard solutions at concentrations of 0.39, 0.78 and 1.56 μg/ml. These solutions were stored for at least 24 h at 25 °C, away from direct sunlight and re-analysed by following the proposed method. A t-test ($P = 0.05$; $n = 6$) was performed to demonstrate that no significant degradation of the substance occurred (See Table 1).

3.2.2. Linearity

Linearity was studied in the concentration range 0.39–1.56 μg/ml ($n = 3$; $k = 5$) and the following regression equation was found by plotting the peak area (y) versus the amlodipine concentration (x) expressed in μg/ml:

$$y = 82.9x + 603.7 (r^2 = 0.9997) \quad (2)$$

The determination coefficient (r^2) obtained for the regression line demonstrates the excellent relationship between peak area and the concentration of amlodipine.

3.2.3. Limits of detection and quantitation

The LOD and LOQ of amlodipine were estimated from the intercept ($\bar{a}$) of the regression line and the corresponding residual standard deviation ($S_{y/x}$) [27]. The responses at the LOD and LOQ were estimated by the following expressions, respectively.

$$f(\text{LOD}) = \bar{a} + 3s_{y/x} \quad (3)$$

$$f(\text{LOQ}) = \bar{a} + 10s_{y/x} \quad (4)$$

Table 1
Validation of the LC method for the determination of amlodipine

Validation criterion	Concentration range (μg/ml)	Results
Stability	0.39	$t = 0.69$ ($t_{tab} = 2.57$)
	0.78	$t = 0.22$ ($t_{tab} = 2.57$)
	1.56	$t = 1.24$ ($t_{tab} = 2.57$)
Linearity	0.39–1.56	$y = 82.9x + 603.7$
($n = 3$; $k = 5$)		$r^2 = 0.9997$
LOD		0.025 μg/ml
LOQ		0.084 μg/ml
Precision		
(a) Repeatability		
(R.S.D. (%); $n = 6$)	0.39	2.5
	0.78	2.0
	1.56	0.7
(b) Intermediate precision		
(R.S.D. (%); 3 days; $n = 18$)	0.39	2.5
	0.78	2.0
	1.56	1.0
Accuracy		
(Recovery ± IC (%); $n = 6$)	0.39	98.7 ± 2.2
	0.78	98.3 ± 2.0
	1.56	100.1 ± 0.9

Interval of confidence = IC = $SDt_{tab}/\sqrt{n}$.

Applying this method, LOD and LOQ for amlodipine were found to be 25 and 84 ng/ml, respectively (cf. Table 1).

3.2.4. Precision

The precision of the chromatographic method, reported as relative standard deviation (R.S.D.), was estimated by measuring repeatability and time-dependant intermediate precision on six replicate injections at three different concentrations (0.39, 0.78 and 1.56 μg/ml). The R.S.D. values

presented in Table 1 were less than 3% and illustrated the good precision of the analytical method.

3.2.5. Accuracy

The accuracy of the procedure was assessed by comparing the analyte amount determined versus the known amount spiked at three different concentration levels (0.39, 0.78 and 1.56 μg/ml) with 6 replicates ($n = 6$) for each concentration level investigated. The accuracy defined as mean% associated with an interval of confidence (IC; $P = 0.05$) shows that the LC method developed for the determination of amlodipine can be considered as accurate within the concentration range investigated (cf. Table 1).

3.3. Optimisation of the extraction procedure

Recovery studies were performed on stainless steel plates with a predefined 20 cm^2 surface area. Different solutions containing 77.9, 155.8 and 311.5 μg/ml of free base amlodipine were prepared by diluting a stock solution (778.8 μg/ml) with methanol. 50 μl of these solutions were applied onto stainless steel plates with predefined 20 cm^2 surface areas and were allowed to evaporate in order to obtain amlodipine residues of 0.19, 0.39 and 0.78 μg/cm^2. The head of the Absorbond swabs were rinsed with methanol and dried prior to use. The total surface of the plates were successively wiped first in a horizontal and secondly in a vertical way, starting from the outside towards the centre, with one or two swabs moistened with the appropriate solvent (Table 2). The head of the swab(s) was (were) cut and placed into a 20-ml volumetric flask containing 5.0 ml of the solvent with which the swab has been soaked. Five microliter of water were then added to each volumetric flask. These were capped and sonicated for 15 min. The extract was finally transferred into an autosampler vial.

3.3.1. Influence of the swabbing technique on recovery

The influence of different solvents and the number of Absorbond swabs used to recover different amounts of amlodipine applied on the 20 cm^2 stainless steel surface areas was investigated. Three concentration levels (0.19, 0.39 and 0.78 μg/cm^2, $k = 3$) with 6 replicates per concentration ($n = 6$) were investigated. Water, methanol and a mixture of water and methanol (50:50, v/v) were tested. The residues were collected by using one or two Absorbond swabs, the second one being dry or moistened with the selected solvent. For each condition tested, an analysis of variance (ANOVA, $P = 0.05$) was carried out to satisfy that the recovery was not dependent of the concentration level investigated and that the mean recovery could therefore be calculated ($n = 18$).

As it can be seen in Table 2, the recovery obtained when water was used as solvent is clearly lower than those observed when methanol or methanol–water mixture were used. Moreover, the amount of amlodipine recovered was found similar when the sampling was performed with only one swab moistened with either methanol or methanol–water mixture. However, when a second dry swab was used, a slightly higher recovery was observed using methanol as solvent. An increase of the recovery was further achieved when two Absorbond TX762 swabs moistened with methanol were successively used for collecting amlodipine. A mean recovery of about 90% was then obtained.

Table 2
Recovery of amlodipine using the swabbing technique

Solvent	Number and type of swab used	Mean recovery (%) ± IC (%); $n = 18$
Water	1 Abs. TX762	62.7 ± 2.7
Methanol–water (1:1, v/v)	1 Abs. TX762	71.0 ± 1.9
Methanol	1 Abs. TX762	71.7 ± 4.7
Methanol–water (1:1, v/v)	2 Abs. TX762, first moistened, second dry	75.2 ± 2.4
Methanol	2 Abs. TX762 first moistened, second dry	79.2 ± 3.6
Methanol	2 Abs. TX762, both moistened	89.9 ± 3.0
Methanol	2 CF TX740, both moistened	71.6 ± 1.1

Abs. = Absorbond TX762; CF = CleanFoam TX740.

A different type of swab, CleanFoam TX740, was also tested in the same conditions but the efficiency of the recovery of amlodipine was found to be lower than the one observed when Absorbond TX762 swabs were used.

3.3.2. Selectivity

During the sample preparation, some potential contaminant substances extracted from the Absorbond swabs could interfere with the quantitation of amlodipine. The selectivity was studied by comparing a blank solution and an amlodipine test solution. The test solution was prepared by wiping a plate spiked with 7.78 μg of amlodipine, according to the optimized swabbing technique. The blank solution was prepared in the same way without sampling. No sources of interference were observed at the retention time of the analyte. The chromatograms of both solutions are presented in Fig. 1.

3.3.3. Stability of amlodipine

The stability of amlodipine was considered at different steps of the sampling procedure: on the equipment surface (stainless steel), in cleaning swabs (undiluted) and in the extraction solutions. 50 μl of the amlodipine solution (155.8 μg/ml) were applied onto six sets of six stainless steel predefined 20 cm^2 surface areas. Two series were allowed to remain undisturbed for up to 24 and 72 h, respectively. The four other sets were wiped immediately using two swabs soaked with methanol as mentioned in the sample preparation section. Two series of six swabs were placed into the 10-ml volumetric flasks and allowed to remain undisturbed. The last two series of swabs were placed into the volumetric flasks containing 5.0 ml of methanol with which both swabs have been soaked. Five microliter of water were added and the volumetric flasks were carefully stoppered. The samples were stored at ambient conditions away from direct sunlight. The extracted residues were analysed using the proposed LC method. The results of the stability studies after 24 and 72 h are presented in Table 3.

The results indicate that amlodipine is stable in cleaning swabs for at least 72 h since the recoveries observed are higher than 85%. Amlodipine is less stable in the extraction solutions, although their analysis can be performed in the 24 h. The recovered quantities of amlodipine collected from stainless steel are lower than 60% after 24 h, implying that the equipment has to be swabbed immediately after the completion of the cleaning process if a good information on the cleaning of the equipment is needed.

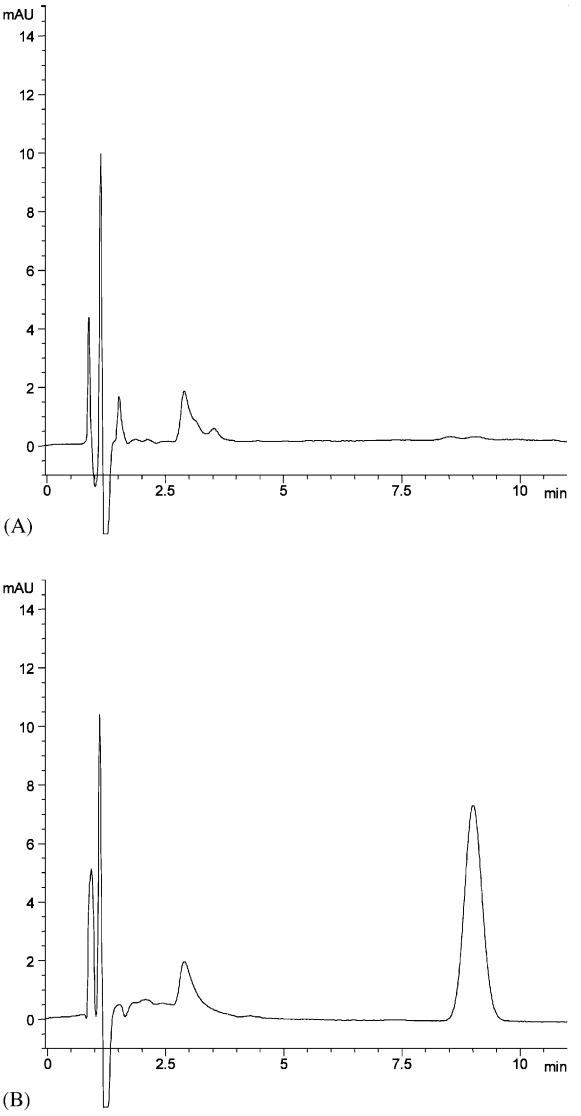

Fig. 1. Chromatograms of (A) a blank solution and (B) an amlodipine test solution (0.78 μg/ml).

Table 3
Stability of amlodipine at different steps of the sampling procedure

Procedure step	Mean recovery (%) ± IC (%); $n = 6$	
	24 h	72 h
Stainless steel	56.1 ± 10.9	36.9 ± 4.0
Cleaning swabs	87.5 ± 3.3	86.1 ± 1.6
Extraction solutions	84.7 ± 2.1	81.6 ± 2.1

Table 4
Precision of the swabbing procedure

Amount of amlodipine (μg/cm^2)	R.S.D. (%); $n = 6$; 3 days	
	Repeatability	Intermediate precision
0.19	4.2	9.0
0.39	5.0	5.8
0.78	6.8	6.8

3.3.4. Precision of the swabbing procedure

The precision of the swabbing procedure was evaluated by considering the repeatability and the intermediate precision at three different concentration levels of amlodipine (0.19, 0.39 and 0.78 μg/cm^2). Repeatability was achieved by wiping consecutively six stainless steel 20 cm^2 surface areas spiked with the appropriate amount of amlodipine. The procedure was performed over 3 days. The results, expressed as R.S.D., are included in Table 4. The obtained values were less than 7 and 9% for repeatabilty and time-dependent intermediate precision, respectively, and revealed the good precision of the swabbing procedure.

4. Conclusion

A SRCL was proposed for amlodipine and set at 0.76 μg/cm^2 of the equipment train used in the product manufacture. An analytical method was developed and validated in order to determine such residual amounts of amlodipine after the cleaning procedure. The chromatographic technique was demonstrated to be sensitive, linear, accurate and precise in the concentration range studied. The swabbing protocol was optimized to obtain effective and reliable recoveries. The sampling procedure selected consists in using two Absorbond TX762 swabs previously moistened with methanol. It must be noted that the swab sample must be performed immediately after the completion of the cleaning process to avoid underestimation of the residues of drug remaining on the equipment surface.

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