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SIMULTANEOUS QUANTITATIVE ESTIMATION OF LISINOPRIL AND HYDROCHLOROTHIAZIDE RESIDUES USING HPLC FOR CLEANING VALIDATION

Imeda Rubashvili [a,b*], Natela Karukhnishvili [b], Khatuna Makharadze [b]

[a]*Petre Melikishvili Institute of Physical and Organic Chemistry, Ivane Javakhishvili Tbilisi State University, 31, Politkovskaya str., Tbilisi 0179, Georgia*
[b]*Quality Control Laboratory, „Aversi-Rational" Ltd, 14, Chirnakhuli str., Tbilisi 0198, Georgia*
[*]*e-mail: rubashvili@yahoo.fr; phone: (+995) 593 56 02 45*

Abstract. The aim of this study was to develop and validate direct - swab and indirect - rinse sampling procedures and a high performance liquid chromatography (HPLC) method for simultaneous quantitative estimation of residues of active pharmaceutical ingredients (API) – lisinopril and hydrochlorothiazide (HCT) in cleaning control samples collected from pharmaceutical manufacturing equipment surfaces after manufacturing of lisinopril/hydrochlorothiazide 20/25 mg uncoated tablets. The swab and rinse sampling procedures were developed and validated in order to obtain a suitable and good recovery (>80%). The acceptance limits of the above-mentioned APIs on the manufacturing equipment surfaces have been established based on pharmacological and toxicological criteria. The new, rapid, specific and selective, developed HPLC method for simultaneous quantitative determination of lisinopril and HCT residues was validated with respect to robustness, system suitability test, specificity, linearity-range, precision, limits of detection and quantitation. The stability of APIs solutions and membrane filter compatibility were studied as well. The method validation was carried out according to ICH Q2 guideline and United States Pharamcopeia requirements. The limit of detection and the limit of quantitation for lisinopril were 0.039 μg/mL and 0.155 μg/mL and for HCT - 0.012 μg/mL and 0.025 μg/mL, respectively.

Keywords: lisinopril, hydrochlorthiazide, swab sampling, rinse sampling, HPLC, validation.

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Introduction

Cleaning validation should be performed in order to confirm the effectiveness of any cleaning procedure for all products contacting pharmaceutical manufacturing equipment. This activity is required by FDA (Food and Drug Administration) and GMP (Good Manufacturing Practice) in pharmaceutical industry and establishes documented evidence with a high degree of assurance that the cleaning procedure effectively removes chemical (the previous product's active pharmaceutical ingredient or cleaning/disinfectant agent) or microbial residues from the manufacturing equipment and facilities below the scientifically predetermined acceptable level. Drug manufacturers must demonstrate that cleaning processes are capable and effective in removing contaminants from the product contact surfaces to the above-mentioned limits. From both regulatory and industry standpoint, cleaning validation is a critical analytical responsibility of the quality assurance system and an important activity which establishes that cross-contamination of the next batch of different pharmaceutical products is under control to ensure the quality of the finished product and patient safety [1-3].

The developed cleaning procedure used in the manufacturing process of a new pharmaceutical product – uncoated tablets of lisinopril/hydrochlorothiazide 20/25 mg must have been inspected and experimentally proven in accordance with the GMP requirements to be suitable and efficient for removal of APIs residues of the above-mentioned product to ensure proper quality and prevent cross-contamination of the subsequent drug product. The need to carry out the cleaning validation was due to the fact that this product is the worst case for the cleaning procedure regarding the solubility of the product's active pharmaceutical ingredients. In order to perform cleaning validation, it was necessary to find a sensitive and specific analytical method combined with appropriate sampling procedures for simultaneous determination of lisinopril and

hydrochlorthiazide (HCT) residues on the manufacturing equipment surfaces.

Lisinopril, (2*S*)-1-[(2*S*)-6-amino-2-{[(1*S*)-1-carboxy-3-phenylpropyl]amino}hexanoyl] pyrrolidine-2-carboxylic acid, is an active pharmaceutical ingredient, which is a potent and competitive inhibitor of angiotensin-converting enzyme (ACE) and is used to treat hypertension and symptomatic congestive heart failure [4]. Hydrochlorthiazide (HCT), 3,4-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxide, is an active pharmaceutical ingredient as well, which is a diuretic medication often used to treat high blood pressure and swelling caused by fluid build-up [5]. Lisinopril can be used alone or in combination dosage form with HCT. The chemical structures of these compounds are shown in Figure 1.

The compendial analytical high performance liquid chromatography (HPLC) procedures for quantitative determination of lisinopril and HCT are described in the monographs of these active substances of the current version of United States Pharamcopeia, respectively. Various HPLC methods for estimation of HCT along with other compounds have been reported in several papers, which described the analysis of HCT, angiotensin-converting enzyme (ACE)-inhibitors and indapamide [6], simultaneous quantification of olmesartan and HCT [7], the analysis of HCT and candesartan cilextil [8], determination of HCT with the major degradation products [9,10]. Analysis of lisinopril along with other components by HPLC has been reported previously [11-13]. Moreover, other methods utilizing HPLC for simultaneous determination of HCT and lisinopril have also been reported [14-16]. A review of the HPLC methods available in the literature revealed that the methods were not appropriate for our analytical purposes. None of the articles discussed the use of HPLC method combined with the sampling procedures in support of cleaning validation. Therefore, a new HPLC method for simultaneous quantitative determination and sampling procedures of the above-mentioned APIs residues on pharmaceutical manufacturing equipment surfaces after manufacturing of dual drug finished product - lisinopril/hydrochlorothiazide 20/25 mg uncoated tablets should be developed and validated.

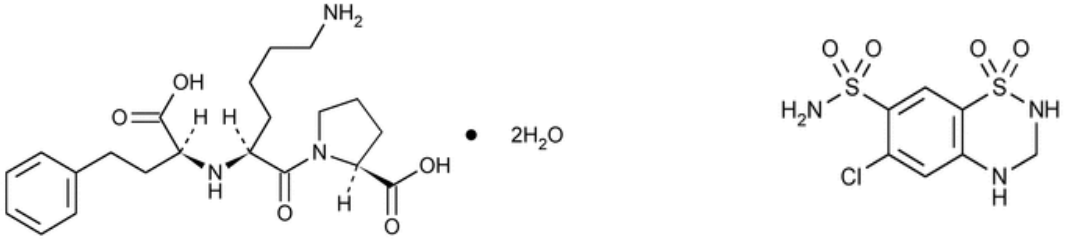
The aim of this study was to develop and validate swab and rinse sampling procedures with respect to very high recovery rate and a new, selective, specific and rapid HPLC method for simultaneous quantitative determination of lisinopril and HCT in cleaning control samples collected from manufacturing equipment surfaces in order to demonstrate the efficiency and removability of the used cleaning procedure. The novelty of the present research is that the HPLC method combined with sampling procedures suitable for cleaning validation has been developed and validated, of which analogue does not exist in the literature and fully responds to the complex analytical tasks for conducting cleaning validation on drug dosage forms such as lisinopril/hydrochlorothiazide uncoated tablets.

Experimental

Materials

The certified analytical standards of lisinopril dihydrate and HCT were supplied by USP (the United States Pharamcopeia) reference standards. The HPLC grade methanol, potassium dihydrogen phosphate, orthophosphoric acid were purchased from Sigma-Aldrich (Germany).

The HPLC grade purified water was prepared using Milli Q Adventage A10 purification system (France). Polyester microswabs (3×2.5×10 mm), teflon template holder, screw cap vials for sampling were purchased from ITW Texwipe (USA). Three stainless steel, anodized aluminium, plastic plates were used as the representative surfaces and durapore polyvinylidene difluoride (PVDF) membrane filters were used in this study. The cleaning procedure was performed using Microbac Forte 1% solution as a disinfectant and cleaning agent, which was purchased from Bode-Chemie (Germany).



(a) ***(b)***

Figure 1. Chemical structures of lisinopril dihydrate *(a)* and HCT *(b)*.

Instrumentation

The HPLC analysis was performed using an Ag 1260 Infinity system and the output signal was monitored and processed using the Chemstation software (USA). SONOREX™ Digital 102P ultrasonic bath DK 102 (Germany), Vortex-Genie™ 2 (USA), shaker 3056 IKA SH 501 DIGITAL Werke (Germany), semi-micro analytical balance CPA 232S Sartorius (Germany), GFL water bath (Germany) were used for sample preparation. All the measuring equipment were appropriately calibrated and qualified. The experiment was carried out in a controlled laboratory area (temperature, t= 22±3°C, relative humidity, RH= 45±15%).

Chromatographic system conditions

The method was developed using the following columns - BDS Hypersil C8(2) 250×4.6 mm, 5 µm (Thermo Scientific) and LiChrospher® RP-8 250×4.6 mm, 5 µm (Merk-Millipore) with an isocratic elution of mobile phase containing a mixture of buffer solution pH 3.0 and methanol (60/40 v/v) filtered through PVDF 0.45 µm membrane filters and degassed; the flow rate of mobile phase was 0.7 mL/min; the UV detection was performed at different wavelengths - 215 nm for lisinopril and 272 nm for HCT; the injected volume was 10 µL; the column temperature was maintained at 40°C.

Validation of analytical HPLC method

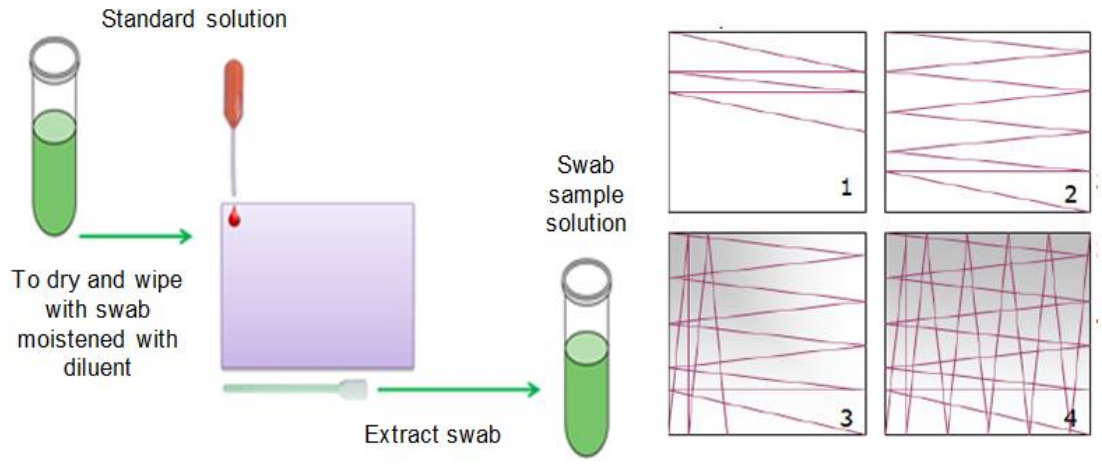
The developed HPLC method was validated with respect to robustness - standard solution stability, membrane filter compatibility test, chromatographic critical factors study using design of experiments (DoE), system suitability test (SST), specificity, linearity-range, precision, limits of detection (LOD) and quantitation (LOQ) according to ICH (International Conference on Harmonisation) guideline and Microsoft Excel 2010 was used for statistical assessment and graphical analysis [17].

Sample preparation and sampling procedure

Lisinopril and HCT reference standards diluted in a mixture of methanol and water 90/10 v/v were used as a standard solution at the concentration of 10/20 µg/mL and 12.5/25 µg/mL, respectively.

Rinsing and swabbing are two sampling procedures available to demonstrate cleaning validation; the both sampling procedures were used in this study. The swabbing is a subjective manual procedure, which involves physical interaction between the swab and the equipment surface and varies from sampler to sampler. The surface was successively wiped with one swab moistened with extraction solution (diluent – a mixture of methanol and water 90/10 v/v). The scheme of swabbing procedure is shown in Figure 2*(a)*. The swabs were placed in the 5 mL screw-cap test tubes containing 1 mL of the selected diluent. Subsequently, the tubes were placed in an ultrasonic bath for 2 minutes and the solutions were analyzed by HPLC. The rinse samples from uneven surfaces (*i.e.* plastic brush) were collected by rinsing with the fixed volume of the diluent.

Due to the nature of material of the manufacturing equipment surfaces, the three types of material - stainless steel, anodized aluminium and plastic were selected, which were previously cleaned by using disinfectant/detergent and dried before the experiment. The sampling points (hard to clean) were determined based on risk assessment using HACCP (hazard analysis and critical control points).



(a) ***(b)***

Figure 2. The scheme of swabbing procedure *(a)* and sequence (steps 1-4) of swab wiping *(b)*.

The usual standardized swab sampling procedure (procedure I) involved moistening swabs with solvent and swabbing the area to be sampled in an overlapping zigzag pattern – first the surface area was wiped horizontally from one side to the other (back and forth) (1, 2 in Figure 2*(b)*), then, after rotating the swab, vertically (up and down) (3, 4 in Figure 2*(b)*). Fresh surface was exposed and repeatedly wiped to extract the maximum residue. Finally, the swab was stored in a closed and labelled container for estimation. Two other variants - the procedures II and III differ from the procedure I only in the swabbing direction and were only used for the robustness study. According to the procedure II, first the surface area was wiped horizontally from one side to the other and then, after rotating the swab, again horizontally. According to the procedure III, first the surface area was wiped diagonally upwards and downwards, after rotating the swab, again diagonally in the same manner [3,18,19].

Design of experiments

For the robustness test of the developed swab sampling procedure and analytical HPLC method, both quantitative and qualitative factors, selected based on experience were considered. The five factors with their levels for swabbing procedure and HPLC method are summarized in Tables 1 and 2. The percentage recovery rate of each API from the surface and system suitability test parameters – the column efficiency (theoretical plates – *N*), the tailing factor (USP symmetry - A_s), the relative standard deviation *(RSD)* of peak areas *(RSD_A)* and the *RSD* of retention times *(RSD_{RT})* (*n*= 6) and the resolution factor between HCT and lisinopril at 215 nm *(R_s)* obtained from standard solution were used as the response variable for the swab sampling procedure and analytical HPLC method, respectively. The experiment was conducted in 2^{5-2}= 8 runs for five two-level factors.

Validation of sampling procedure

Validation parameters - robustness and accuracy of sampling procedure were studied using the DoE technique. Both developed sampling procedures were checked and the percentage recovery rate (two individual determinations) was determined. The selected surface area of the plates was sprayed with 100 µL (for swabbing) and 5 mL (rinsing) of standard stock solution (lisinopril and HCT at concentration – 100/200 mg/mL and 125/250 µg/mL, respectively) using a micropipette and the solvent was allowed to evaporate. Then swab sampling was performed according to swab wipe standardized procedure as described in sample solution preparation. The swab samples were diluted with the same diluent to 1 mL. For rinse sampling, the surface area was rinsed with approximately 100 mL of diluent and then diluted to a volume with the same diluent to 100 mL, and mixed well. Then it was filtered through a 0.45 µm membrane filter.

The percentage recovery rate was calculated by Eq.(1):

$$Rec(\%) = \frac{A_{rec}}{A_{sp}} \times 100 \quad (1)$$

where, A_{rec} is the peak area of lisinopril/HCT obtained from sample solution (recovered amount);

A_{sp} is the peak area of lisinopril/HCT obtained from spiked solution (amount added) [3].

Table 1

Robustness factors and design of experiments for swab sampling procedure.

No.	*Factor (Xi)*	*Unit*	*Low level (-)*	*Nominal level (0)*	*High level (+)*
1	Surface material *(X1)*	-	Anodized aluminum	Stainless steel	Plastic
2	Swabbing *(X2)*	-	II	I	III
3	Methanol percentage in diluent *(X3)*	%	80%	90%	100%
4	Sampler *(X4)*	-	I Chemist-analyst	-	II Chemist-analyst
5	Amount spiked (lisinopril)	µg	8	10	12
	Amount spiked (HCT) *(X5)*		10	12.5	15

Table 2

Robustness factors and design of experiments for analytical procedure.

No.	*Factor (Xi)*	*Unit*	*Low level (-)*	*Nominal level (0)*	*High level (+)*
1	Flow rate of mobile phase *(X1)*	mL/min	0.6	0.7	0.8
2	Buffer solution of mobile phase *(X2)*	pH	2.8	3.0	3.2
3	Methanol percentage in mobile phase *(X3)*	%	35	40	45
4	Column temperature *(X4)*	°C	35	40	45
5	DAD* wavelength for lisinopril/HCT *(X5)*	nm	213	215	217
			270	272	274

**Diode-array-detection.*

Quantitative estimation of lisinopril/HCT residues

The concentration (μg/mL) of lisinopril/HCT residues in sample solution was calculated by Eq.(2):

$$X = \frac{R_u \times W \times D \times 1000 \times P}{R_s \times 100} \quad (2)$$

where, R_u is the peak area of analyte obtained from the chromatogram of swab sample solution;
R_s is the peak area of analyte obtained from the chromatogram of standard solution;
W is the weighted mass of standard, mg;
D is the dilution factor;
P is the purity of the standard compound, (assay, %).

Methodology to establish acceptance limits

The acceptance limits for the drug residues must ensure the absence of cross-contamination for subsequent batches manufactured in the affected equipment. FDA's guidance for determining residues acceptance limits requires a logical, practical, achievable and verifiable determination practice [2]. The acceptance limits for cleaning validation were based on two pharmacological (the dosage criteria - the patient should not take more than 0.1% of the minimum therapeutic dose of the API of the previous product in the maximal daily dose of the subsequent product) and toxicological.

The maximum allowable carryover (mg) - *MAC* was calculated based on the both above-mentioned criteria [3,18,19].

The *MAC* was calculated based on the pharmacological criteria using Eq.(3):

$$MAC = \frac{TD \times SF \times BS}{LDD} \quad (3)$$

where, *TD* is the minimal therapeutic dose of the studied API of the control product (mg);
SF is a safety factor -1/1000 for solid oral dosage form;
BS is the smallest batch size of the subsequently processed product batch (mg);
LDD is the largest daily dose of the subsequently processed product's API (mg).

The *MAC* was calculated based on the toxicological criteria by Eq.(4):

$$MAC = \frac{NOEL \times SF \times BS}{LDD} = \frac{LD50 \times WA \times SF \times BS}{LDD \times 2000} \quad (4)$$

where, *NOEL* is no-observed effect level (mg/kg);
WA is human average weight calculated on 50 (kg);
2000 is an empirical constant.

The acceptance limits - *AL* for *API* residues in sample solution was calculated using Eq.(5) and Eq.(6) depending on the cleaning procedure. Thus, for sample solution obtained from swabbing:

$$AL = \frac{MAC \times 1000 \times Rec \times A_s \times F}{A_t \times V} \quad (5)$$

And, for sample solution obtained from rinsing:

$$AL = \frac{MAC \times 1000}{V} \quad (6)$$

where, A_s is the sampling area (cm^2);
Rec is the percentage recovery rate of the sampling method;
A_t is the total production line area (cm^2);
V is the volume of sample solution obtained from swabbing/rinsing (mL).

Results and discussion

Establishing acceptance limits

The smallest batch size of the subsequent product was selected for calculating the values of the *MAC*. The lowest obtained value of *MAC* of both APIs – lisinopril and HCT were used to calculate the acceptance limits, given in Table 3.

Table 3

The calculated acceptance limits (μg/mL).

Name of API	*Pharmacological criteria**		*Toxicological criteria***	
	Swabbing	*Rinsing*	*Swabbing*	*Rinsing*
Lisinopril	162.0	120.0	3.45	2.45
HCT	202.0	75.0	1.11	0.83

**Pharmacological criteria were calculated using the values of MAC obtained from Eq.(3);*
***Toxicological criteria were calculated using the values of MAC obtained from Eq.(4).*

The determined concentration of lisinopril and HCT residues in sample solutions should not exceed the established *AL*. According to the current version of good manufacturing practice guidelines (GMP EU Annex 15) the acceptance criteria should be based on a toxicological evaluation [1]. The results of the calculated acceptance limits based on the various approaches show that the strictest limit is the AL based on toxicological criteria. Therefore, this limit should be considered for estimation of the API residues.

Development and validation of sampling procedure

The sampling procedures were developed in order to obtain a suitable and good recovery of APIs residues. The surface (sampling area - 25 cm^2) was successively wiped with one micro polyester swab moistened with diluent. The swabs were spiked with different quantities of lisinopril and HCT. A mixture of methanol and water 90/10 v/v was used as diluent (easy to remove from surfaces by purified water after sampling and easy to check its residues using gas chromatography; the studied APIs residues are soluble in methanol; and the selected diluents ensure the best chromatographic characteristics of the peaks). The sonication time was set to 2-3 minutes.

The robustness of the swab sampling procedure was checked using the recovery rate (Table 4). All the recovery values obtained from the robustness test (8-run design experiment) were more than 86.36%, which approved that the developed swab sampling procedure can be considered robust and none of the examined factors had a significant effect on the swab recovery rate.

The accuracy of the combination of sampling procedure and analytical HPLC procedure was assessed by comparing the analyte amount determined versus the known amount spiked at two different concentration levels (10 and 20 µg/mL for lisinopril and, 12.5 and 25 µg/mL for HCT) with three individual determinations (*n*= 3). The accuracy is expressed as percentage of standard compound recovered from a spiked solution (placebo+standard) with a corresponding *RSD*, %. The average recovery should be within 80.0-120.0% and the *RSD* of percentage recovery rates for three individual determinations should not be more than 4.0% for each concentration level of spiked sample solution (acceptance criteria). The accuracy test results are shown in Table 5. The main recovery rates are more than 82.93% (at two different concentrations *n*= 3), which confirms that the developed sampling procedures have a good recovery.

To estimate the compatibility of the used swab material - polyester (ITW Texwipe swab, USA), the standard solution and extracted swab solution added standard of the same concentration were prepared and injected (Figure 3). This test confirms the existence of desorption of lisinopril/HCT residues from the swab material. The compatibility of swab material was evaluated quantitatively by the calculated percentage difference between peak areas obtained from standard solution and extracted swab solution added standard which should not be more than 3.0% (acceptance criteria).

Table 4

Robustness test results for the swab sampling procedure.

Experiment	*Factors*					*Mean recovery, %*	
no.	*X1*	*X2*	*X3*	*X4*	*X5*	*Lisinopril*	*HCT*
1	+	+	+	+	+	93.25	98.55
2	+	+	-	+	+	92.50	91.91
3	+	-	+	-	+	91.55	87.11
4	+	-	-	-	-	96.65	86.36
5	-	+	+	-	-	93.77	95.00
6	-	+	-	-	+	89.25	94.21
7	-	-	+	+	-	87.74	88.33
8	-	-	-	+	+	92.66	87.92

Table 5

The accuracy results.

API residue	*Spiked sample solution, µg/mL*	*Recovery rate, %*			*RSD (n= 3)*	*The main recovery rate, % (n= 3)*
		I	*II*	*III*		
			Swabbing			
Lisinopril	10	95.96	97.27	93.25	1.75	96.40
	20	96.77	98.01	97.15	0.53	
HCT	12.5	95.77	97.07	93.16	1.71	95.17
	25	94.25	93.78	96.96	1.48	
			Rinsing			
Lisinopril	10	87.58	81.53	85.23	2.94	85.70
	20	89.25	83.14	87.44	2.96	
HCT	12.5	82.88	80.59	84.99	2.17	82.93
	25	83.15	82.45	83.50	0.53	

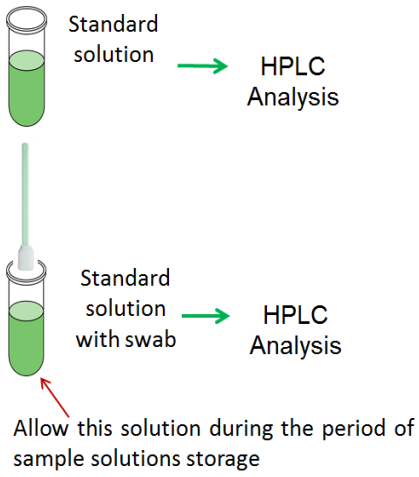


Figure 3. The scheme of compatibility testing of swab.

The calculated percentage difference is 0.43% for lisinopril and 0.25% for HCT. Hence, the lisinopril and HCT residues desorb from the swab and the swab material does not effect on the determination of the above-mentioned APIs residues.

Optimization of chromatographic system conditions and robustness study

The final chromatographic conditions were determined by optimizing the system operational parameters: wavelength for detection, composition of the mobile phase, flow rate, nature of stationary phase and checking the system suitability parameters: theoretical plates, tailing factor, peak purity, resolution, *etc*.

The calibration curve showed good linearity for the trace level quantitative estimation at 215 and 272 nm for lisinopril and HCT, respectively. Five critical factors (*X1* - flow rate of mobile phase; *X2* - buffer solution of mobile phase; *X3* - methanol percentage in mobile phase; *X4* - column temperature; *X5* - DAD wavelength for lisinopril/HCT) were selected and small variations (low and high levels) were induced in the nominal values of the method. An 8-run design experiment was performed to assess the effect of each factor in the system suitability test results. Table 6 shows the design experiments results of the robustness test for the developed HPLC method. The variability of resolution factor is 12.32% but the minimal value of the resolution factor is not bellow the acceptance criteria (>7.0).

Validation of analytical HPLC method

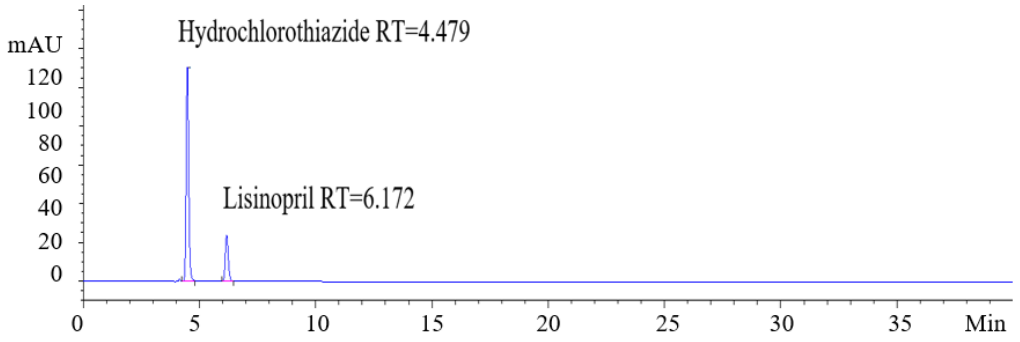
The specificity test was checked using the standard solution, the spiked swab and rinse sample solutions, and the blank solution. This solution was prepared in the same manner as the spike sample solution but no standard was used. The specificity test results have shown that there is no interference from the extracted blank and the diluent at the retention time (RT) of analyte peak. The lisinopril and HCT peaks were pure and the purity factor (999.988 for lisinopril and 999.995 for HCT) was more than the purity threshold (990.0). Figures 4 and 5 show the chromatograms obtained from the standard solution and the blank solution, respectively.

In order to study the linearity-range, the working solutions were prepared at eight different concentration levels (the range was 0.155-20.0 µg/mL for lisinopril and 0.025-25.0 µg/mL for HCT) and injected by six replicates (n= 6) for each concentration level. The linearity was checked by the square of correlation coefficient (acceptance criteria: >0.998), the *RSD* of peak areas (acceptance criteria: <5.0%) at all concentration levels excluding the last concentration level which should not be more than 10%, the *RSD* of retention times (acceptance criteria: <1.0%). The calibration curves were constructed by plotting the peak area against the corresponding concentration of the injected working standard solutions that indicate a perfect linearity for each compound. Figure 6*(a)* and *(b)* shows the linearity plots for lisinopril and HTC, respectively.

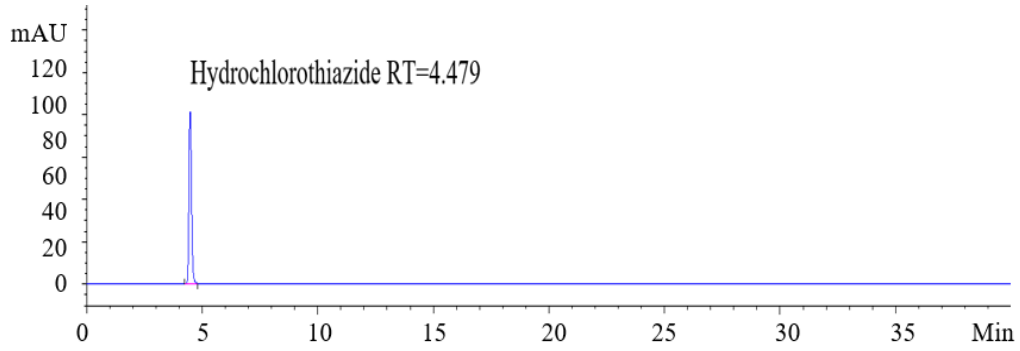
Table 6

Robustness results of recovery study for analytical procedure.

Experiment no.	*Factors*					*Resolution factor (Rs)*
	X1	*X2*	*X3*	*X4*	*X5*	
1	+	+	+	+	+	7.61
2	+	+	-	+	+	7.55
3	+	-	+	-	+	7.85
4	+	-	-	-	-	7.92
5	-	+	+	-	-	8.11
6	-	+	-	-	+	7.86
7	-	-	+	+	-	7.22
8	-	-	-	+	+	7.23



(a)



(b)

Figure 4. Chromatograms of the standard solution recorded at 215 nm *(a)* and 272 nm *(b)*.

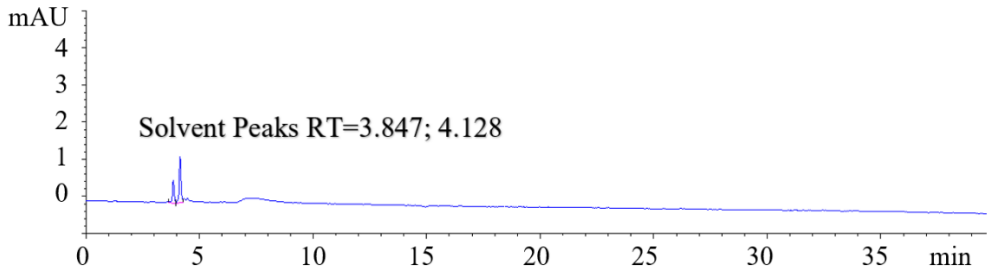
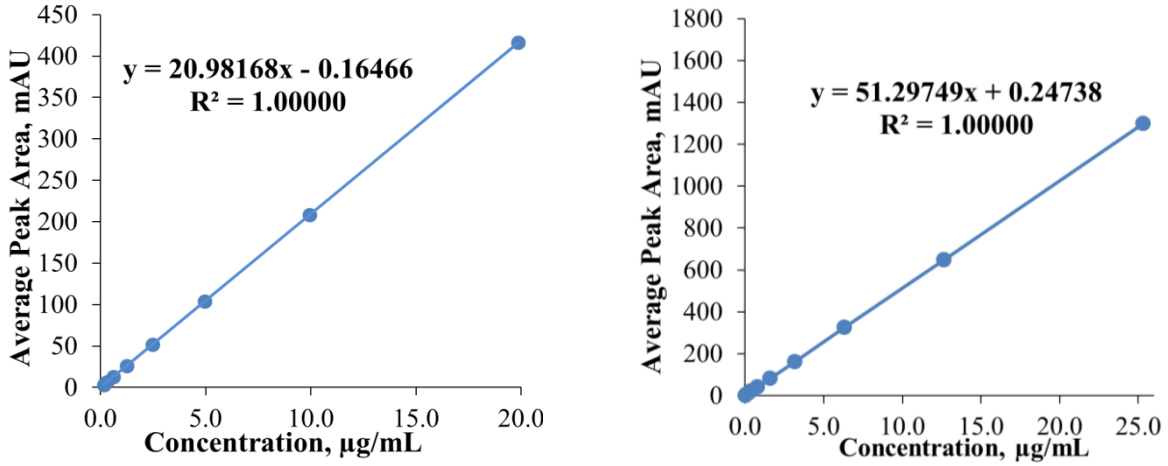


Figure 5. Chromatogram of the blank solution.



(a) ***(b)***

Figure 6. The linearity (calibration) curve for lisinopril at 215 nm *(a)* and HCT at 272 nm *(b)*.

The limit of quantitation (*LOQ*) was estimated to be ten times the s/N ratio; the limit of detection (*LOD*) was estimated to be three times of s/N ratio (acceptance criteria). The quantitation limit was achieved by injecting a series of stepwise diluted solutions and the precision was established at the specific determined level. The *RSD* of peak area should not be more than 10% (acceptance criteria). The determined limits of quantitation and detection of lisinopril and HCT by HPLC are presented in Table 7.

Table 7

The LOQ and LOD of HPLC method.

Parameter	*Value*	
	Lisinopril	*HCT*
LOQ, μg /mL	0.155	0.025
LOD, μg /mL	0.039	0.012
RSD of peak areas for LOQ (*n*= 6)	2.001	3.343
RSD of retention times for LOQ (*n*= 6)	0.050	0.073
s/N for LOQ	18.23	14.25
s/N for LOD	4.03	7.98

In order to check the chromatographic system performance, the system suitability test was performed by using six replicate injections (*n*= 6) of the standard solution at the concentrations – 10 μg/mL and 12.5 μg/mL, respectively. The following parameters - the *RSD* of peak areas, the *RSD* of the retention times, the peak tailing factor (the USP coefficient of the peak symmetry), the column efficiency - the number of theoretical plates and resolution factor between HCT and lisinopril were measured. The results are summarized in Table 8.

The precision of the analytical method was estimated by measuring repeatability (intra-day precision) and time-dependent intermediate precision (inter-day) on six replicate injections of standard solution and on six individual determinations of lisinopril and HCT in sample solution at the same concentrations (10 and 12.5 μg/mL for lisinopril and HCT, respectively).

This validation parameter was studied during the accuracy study of sampling procedures. Sample solutions were prepared according to the description in the experimental section. The intermediate precision (inter-day) was carried out on a different day. The intra-day precision was checked by the *RSD* of the determined concentrations (μg/mL) for three individual determinations of lisinopril and HCT which should not be more than 4.0%; The intermediate precision was checked by the *RSD* of six individual determinations (totally inter-day and intra-day determinations) of lisinopril and HCT which should not be more than 4.0%, the percentage difference, which should be more than 5.0% and *F*-test which should not be more than 19. The precision study results given in Tables 9, 10 and 11 are within the acceptance criteria indicating that this method has a good precision.

The standard solution stability was checked three times: initially, and after 24 h and 48 h of storage at room temperature against a freshly prepared standard solution. The stability was checked using two standard solutions and by the percentage difference between the peak areas of the standard solution stored at room temperature and the freshly prepared one which should not exceed 3.0% (acceptance criteria). The bias in terms of peak area between two standard solutions should be within 0.98-1.02 (acceptance criteria). The percentage difference between the peak areas obtained with two standard solutions, one stored at room temperature for 24 h and another prepared freshly, is 1.3% and 0.51% for lisinopril and HCT, respectively. This gives the confidence that APIs residues are stable within 48 h and the residues concentration does not change in sample solutions during cleaning validation process.

Table 8

The system suitability test parameters results.

Parameter	*Lisinopril*	*HCT*	*Acceptance criteria*
Column efficiency	>11766	>8178	>2000
RSD of peak areas (*n*= 6)	0.113%	0.127%	<2.0%
RSD of retention times (*n*= 6)	0.018%	0.024%	<1.0%
Tailing factor (USP symmetry*)	0.84	0.85	0.8÷1.2
Resolution factor between HCT and lisinopril	7.95		>7

**USP symmetry is the coefficient of the peak symmetry S= $W_{0.05}$/2f where,*
W= peak width at 5% of peak height,
f= time from width start point at 5% of peak height to RT.

Table 9

The precision repeatability results for standard solution.

Injection no.	*Lisinopril*		*HCT*	
	Peak area, mAU*	*RT**, min*	*Peak area*, mAU*	*RT**, min*
1	208.40	6.034	650.71	4.484
2	208.50	6.032	650.07	4.482
3	208.04	6.042	651.14	4.489
4	209.06	6.033	650.10	4.482
5	208.54	6.036	651.24	4.487
6	208.61	6.034	651.74	4.487
Average	208.53	6.035	650.83	4.485
RSD	0.330	0.004	0.666	0.003

** The instrument error for peak area ± 0.1 mAU;*

*** The instrument error for RT ± 0.01 min.*

Table 10

The precision results for sample obtained with swab sampling solution, µg/mL.

Sample solution no.	*Precision repeatability (intra-day)*		*Intermediate precision (inter-day)*	
	Lisinopril	*HCT*	*Lisinopril*	*HCT*
1	17.52	23.52	17.24	22.27
2	17.74	23.31	16.93	22.69
3	17.58	24.24	17.38	23.03
Average	17.61	23.69	17.18	22.66
RSD (*n*= 3)	0.115	0.488	0.230	0.381
RSD (*n*= 6)			0.286	0.685
Percentage difference			2.47	4.44
F-test			6.04	1.64

The PVDF membrane filter compatibility was evaluated using a standard solution and by calculating the percentage difference between peak areas of filtered and non-filtered standard solutions which should not be more than 0.5% (acceptance criteria). The percentage difference between peak areas of filtered and non-filtered standard solutions is 0.24% and 0.12% for lisinopril and HCT, respectively, which gives the confidence that the adsorption of each analyte does not occur on the used filter.

Estimation of lisinopril and HCT residues in samples from swabbing and rinsing

Both swabbing and rinsing procedures were performed for APIs residues sampling from manufacturing equipment surfaces. The APIs residues were expressed in µg/mL. After manufacturing of three consecutive batches of finished drug product - uncoated tablets of lisinopril/hydrochlorothiazide 20/25 mg, equipment cleaning samples were collected from different sampling points. After sampling, the equipment surfaces were rinsed with purified water for several times to remove residual methanol on surfaces. The last rinsed portions were checked using gas chromatography to detect methanol residues. Swab and rinse samples were tested immediately to estimate lisinopril and HCT residues using the validated HPLC method. The results are shown in Table 12. Figure 7 shows typical chromatograms obtained from the sample solution. The secondary peaks that appeared on the chromatograms belong to the diluent and one unknown compound (RT= 21 min) extracted from the swab material.

The determined concentrations of lisinopril and HCT residues are below the established acceptance limits for cross-contamination. The standard operating cleaning procedure established for cleaning of manufacturing equipment surfaces provides enough efficacy in order to remove the above-mentioned APIs from the cleaned surfaces and excludes the risk of cross-contamination of the subsequent finished product.

Table 11

The precision results for sample obtained with rinse sampling solution, µg/mL.

Sample solution no.	*Precision repeatability (intra-day)*		*Intermediate precision (inter-day)*	
	Lisinopril	*HCT*	*Lisinopril*	*HCT*
1	16.15	20.79	15.77	21.28
2	15.05	20.61	16.03	20.89
3	15.83	20.88	16.23	20.88
Average	15.68	20.76	16.01	21.02
RSD (*n*= 3)	0.566	0.138	0.231	0.228
RSD (*n*= 6)			0.206	0.219
Percentage difference			2.08	1.25
F-test			6.02	0.36

Table 12

The results of lisinopril and HCT residues analysis.

Sampling procedure	*Number of sampling points*	*The determined concentration range of residues, µg/mL*		*Acceptance limit, µg/mL*	
		Lisinopril	*HCT*	*Lisinopril*	*HCT*
Swabbing	10	0.19÷0.67	0.06÷0.46	3.45	1.11
Rinsing	3	0.28÷0.62	0.24÷0.69	2.45	0.83

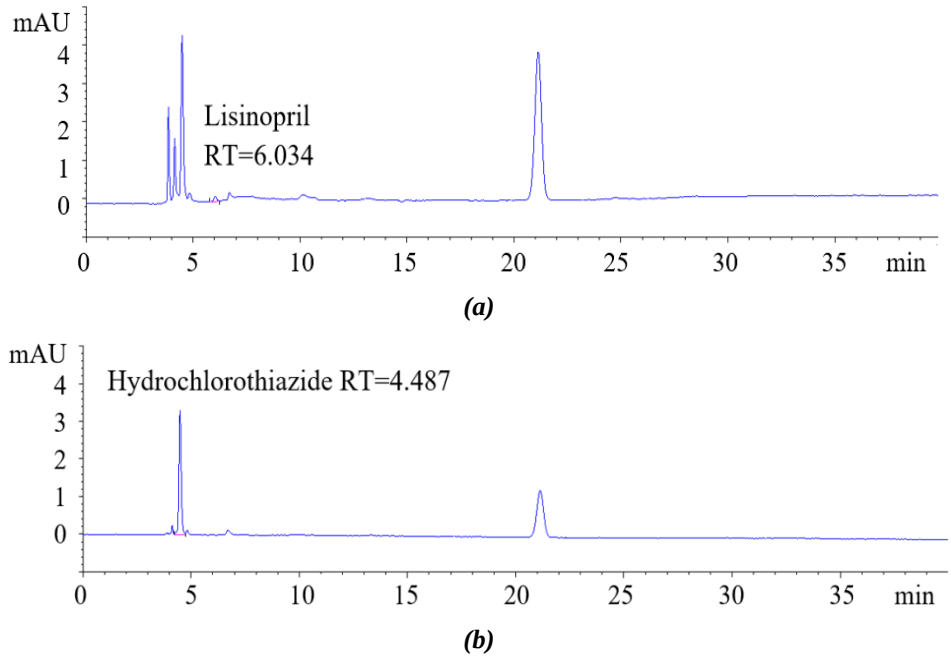


Figure 7. Chromatograms of the sample solution recorded at 215 nm *(a)* and 272 nm *(b)*.

Conclusions

An analytical HPLC method combined with swab and rinse sampling procedures was developed for simultaneous quantitative determination of lisinopril and hydrochlorthiazide (HCT) residues on surfaces of pharmaceutical equipment used in the manufacturing process. The lisinopril/hydrochlorthiazide 20/25 mg uncoated tablets were used to demonstrate cleaning validation. The analytical method was validated with respect to precision, accuracy, robustness, specificity, system suitability test and linearity-range over the concentration range from 0.155 µg/mL to 20.0 µg/mL for lisinopril and from 0.025 µg/mL to 25 µg/mL for HCT.

Both developed swab and rinse sampling procedures were found to be robust and accurate with high recovery rate (>80%). No interferences from swab/blank solutions were observed. Standard solutions of both compounds were stable

within 48 hours; therefore, the concentrations of cleaning control sample solutions did not change for a time from sampling to injecting into HPLC system. Hence, the obtained results confirm that the standard cleaning procedure is adequate and effective for removing both APIs residues from equipment surfaces. The determined concentrations of lisinopril (<3.45 µg/mL by swabbing and <2.45 µg/mL by rinsing) and HCT (<1.11 µg/mL by swabbing and <0.83 µg/mL by rinsing) in sample solutions are much lower than calculated acceptance limit of cross-contamination of the next finished product.

The validated protocol of sampling and HPLC method may be successfully used by other pharmaceutical quality control laboratories to sustain cleaning validation process for lisinopril and HCT residues after manufacturing of uncoated tablets.

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