Simultaneous Estimation of Poorly Soluble Drugs by HPLC for CleaningValidation and Cross-Contamination

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Abstract— In order to simultaneously quantify the residues of the active pharmaceutical ingredients (API) lisinopril and hydrochlorothiazide (HCT) in cleaning control samples taken from pharmaceutical manufacturing equipment surfaces after manufacturing of lisinopril/hydrochlorothiazide 20/25 mg uncoated tablets, this study developed and validated direct swab and indirect rinse sampling procedures. To achieve an appropriate and good recovery (>80%), the swab and rinse sample processes were created and validated. Pharmacological and toxicological standards have been used to define the acceptability limits for the aforementioned APIs on the surfaces of industrial equipment. For the simultaneous quantitative analysis of lisinopril and HCT residues, a new, quick, specific, and selective HPLC technique was developed. This approach was verified in terms of robustness, system suitability test, specificity, linearity-range, precision, and limits of detection and quantitation. Additionally, the compatibility of membrane filters and the stability of API solutions were investigated. The method validation was completed in accordance with the US Pharmacopeia's standards and the ICH Q2 guidelines. Lisinopril's limit of detection and quantitation were 0.039 μg/ml and 0.155 g/mL, respectively, while HCT's were 0.012 g/mL and 0.025 g/mL.

Keywords— *Simultaneous, Estimation, Poorly Soluble Drugs, HPLC, Cleaning Validation, Cross-Contamination.*

INTRODUCTION

To verify the efficacy of any cleaning operation for all goods contacting pharmaceutical manufacturing equipment, cleaning validation should be carried out. The FDA (Food and Drug Administration) and GMP (Good Manufacturing Practise) in the pharmaceutical sector require this activity, which establishes documented evidence with a high degree of assurance that the cleaning procedure effectively removes chemical (the active pharmaceutical ingredient or cleaning/disinfectant agent from the previous product) or microbial residues from the manufacturing equipment and facilities below the scientifically predetermined acceptable level. The ability and effectiveness of cleaning procedures to remove pollutants to the levels stated above from product contact surfaces must be proven by drug makers. Cleaning validation is an essential analytical task of the quality assurance system and a significant activity that establishes that cross-contamination of the subsequent batch of various pharmaceutical products is under control to ensure the quality of the finished product and patient safety, both from a regulatory and industry standpoint. In order to ensure proper quality and prevent cross-contamination of the subsequent drug product, 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 GMP requirements to be suitable and efficient for removing APIs residues of the above-mentioned product. The fact that this product is the worst case for the cleaning method in terms of the

solubility of the product's active medicinal components led to the need to carry out the cleaning validation. Finding a sensitive and specific analytical approach along with suitable sample techniques for the simultaneous measurement of lisinopril and hydrochlorthiazide (HCT) residues on the production equipment surfaces was important for cleaning validation. Lisinopril, also known as (2S)-1-[(2S)-6-amino-2-[(1S)- 1-carboxy-3-phenylpropyl]aminohexanoyl] pyrrolidine-2-carboxylic acid, is an active pharmaceutical ingredient used to treat hypertension and symptomatic congestive heart failure. It is a potent and competitive inhibitor of the angiotensin-converting enzyme (ACE). Another active pharmaceutical component is hydrochlorthiazide (HCT), 3,4-dihydro-2H-1,2,4- benzothiadiazine 1,1-dioxide. This diuretic drug is frequently used to treat high blood pressure and swelling brought on by fluid retention. You can take lisinopril by itself or in a dosage that also contains HCT. Figure 1 depicts these compounds' chemical structures.

Lisinopril and HCT quantitative determination compendial analytical high performance liquid chromatography (HPLC) methodologies are detailed in the monographs for each of these active ingredients of the most recent United States Pharmacopoeia, respectively. The analysis of HCT, ACE-inhibitors, indapamide, simultaneous quantification of olmesartan and HCT, analysis of HCT and candesartan cilextil, and determination of HCT with the major degradation products are just a few of the HPLC methods for HCT estimation that have been reported in several papers. HPLC analysis of lisinopril and other substances has been documented in the past. The simultaneous measurement of HCT and lisinopril using additional HPLC techniques has also been reported. The HPLC techniques described in the literature were examined, and it became clear that they weren't suitable for our analytical needs. The use of the HPLC method along with the sample techniques in support of cleaning validation was not covered in any of the studies. Therefore, it is necessary to develop and validate a new HPLC method for the simultaneous quantitative determination and sampling of the above-mentioned API residues on pharmaceutical manufacturing equipment surfaces following the production of the dual drug finished product, lisinopril/hydrochlorothiazide 20/25 mg uncoated tablets. The purpose of this study was to create and validate new, selective, specific, and quick HPLC methods for the simultaneous quantitative determination of lisinopril and HCT in cleaning control samples taken from manufacturing equipment surfaces in order to show the effectiveness and detachability of the employed cleaning procedure. The unique aspect of the current study is the development and validation of an HPLC method with sampling techniques that is appropriate for cleaning validation. This method has no analogues in the literature and fully addresses the challenging analytical tasks required to conduct cleaning validation on drug dosage forms like lisinopril/hydrochlorothiazide uncoated tablets.

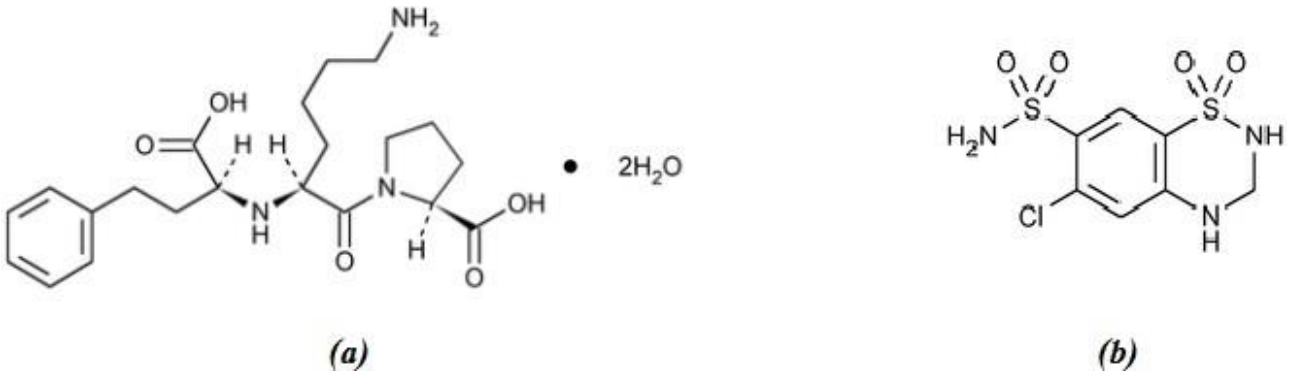

Figure 1- Chemical structures of lisinopril dihydrate (a) and HCT (b)

MATERIALS AND METHODS FOR EXPERIMENTATION

Sigma-Aldrich (Germany) provided the analytical grade phosphoric acid, ammonium phosphate dibasic, sodium hydroxide, and 2-propanol as well as the HPLC grade methanol and 2-propanol. Using the Milli Q

Adventage A10 purification system (Millipore, France), HPLC quality water was created. ITW Texwipe (USA) provided the polyester microswabs (32.510 mm), which were used for sampling. Microbac Forte 1% solution, a disinfectant/detergent obtained from Bode Chemie (Germany), was used during the cleaning process. Ag 1260 Infinity (AG Technologies, USA) was used for the chromatographic analysis. Chemstation software was used to monitor and process the output signal. A pH metre S40 Sevenmulti (Mettler-Toledo, Switzerland) was used to determine the pH of the solutions. For sample preparation, we used the SONOREXTM Digital 102P Ultrasonic bath DK 102 (Germany), Shaker 3056 IKA SH 501 DIGITAL Werke (Germany), Analytical balance CPA 232S Sartorius (Germany), and GFL water bath (Germany). The measuring tools were all certified.

(i) Development of the Standard Solution- The standard dose of 22 mg, was carefully weighed, transferred to a 25 mL volumetric flask, and then dissolved using a sonicator in 15 mL of methanol and 1 mL of 1 M sodium hydroxide. The mixture was then diluted to volume with the methanol diluent, and then thoroughly mixed. Following that, it was filtered through a Durapore PVDF 0.45 m membrane filter, with the first 5 ml of the filtrate (Stock solution) being thrown away. This solution was diluted to volume in a 10 ml volumetric flask using 1 ml of the solution. The diluent was a 28: 2: 20 mixture of methanol, 1 M sodium hydroxide, and water.

(ii) The Process of Extracting a Sample's Solution- Two sample techniques are offered to show cleaning validation: rinse and swab. FDA prefers to use the swab approach. The physical interaction between the swab and the surface during the swabbing process makes it a subjective manual operation that can differ from operator to operator. So, to establish reproducible recoveries, a standardised motion regimen is needed. A swab was folded diagonally and submerged in the extraction solution. In order to prevent needless drug dilution, excess fluid was squeezed. Wipes were applied horizontally, moving inward from the edges towards the centre. The highest amount of residue was removed by repeatedly wiping a fresh surface. The swab was finally placed for estimation in a jar that was closed and labelled. The swab sampling approach has been employed. The stainless steel surfaces of the equipment $5*5cm^2$ that were chosen (the worst case sampling locations assessed based on risk analysis utilising HACCP) had previously been cleaned using a disinfectant/detergent and dried. One swab that had been soaked with extraction solution (a 28: 2: 20 mixture of methanol, 1 M sodium hydroxide, and water) was used to wipe the surface repeatedly. The test tubes with a 5 mL screw lid and a 1 mL extraction solution were filled with the swabs. The tubes were then submerged in an ultrasonic bath for 5 minutes while the liquids underwent HPLC analysis.

(iii) Conditions for Chromatographic Systems and Equipment- The mobile phase was eluted using an isocratic method and contained a mixture of buffer solution pH 3.0 and methanol (60/40 v/v) that was filtered through PVDF 0.45 m membrane filters and degassed. The UV detection was carried out at various wavelengths. The Ag 1260 Infinity system was used for the HPLC analysis, and the Chemstation software (USA) was used to monitor and handle the output data. To prepare the samples, we used the SONOREXTM Digital 102P ultrasonic bath DK 102 (Germany), the Vortex- GenieTM 2 (USA), the shaker 3056 IKA SH 501 DIGITAL Werke (Germany), the semi-micro analytical balance CPA 232S Sartorius (Germany), and the GFL water bath (Germany). All of the measuring tools were duly qualified and calibrated. The experiment was conducted in a controlled laboratory environment with temperature and relative humidity set at 22°C and 45°F, respectively.

(iv) Analytical HPLC method validation- According to ICH (International Conference on Harmonisation) guidelines, the developed HPLC method was validated for 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), and Microsoft Excel 2010 was used for statistical assessment and graphical representation.

(v) Sample creation and selection methods- A standard solution containing lisinopril and HCT reference standards diluted to concentrations of 10/20 ug/ml and 12.5/25 ug/ml respectively, in a combination of

methanol and water 90/10 v/v was utilised. Two sample techniques, rinsing and swabbing, are available to show cleaning validation; both sampling techniques were used in this investigation. Swabbing is a subjective manual process that differs from sampler to sampler and involves physical contact between the swab and the instrument surface. One swab that had been soaked with extraction solution (the diluent, a 90/10 v/v mixture of methanol and water) was used to clean the surface repeatedly. Figure 2(a) depicts the swabbing procedure's flow chart. The test tubes with 5 mL screw caps and 1 mL of the chosen diluent were filled with the swabs. The solutions were then examined by HPLC after the tubes had spent 2 minutes in an ultrasonic bath. By washing with the predetermined volume of the diluent, rinse samples from uneven surfaces (such as plastic brushes) were obtained. The three materials, stainless steel, anodized aluminium, and plastic, were chosen due to the nature of the materials used in manufacturing equipment surfaces. Prior to the experiment, these materials were washed with a disinfectant or detergent and dried. Utilising HACCP (hazard analysis and critical control points), which assesses risks, the sample spots (hard to clean) were chosen based on the results.

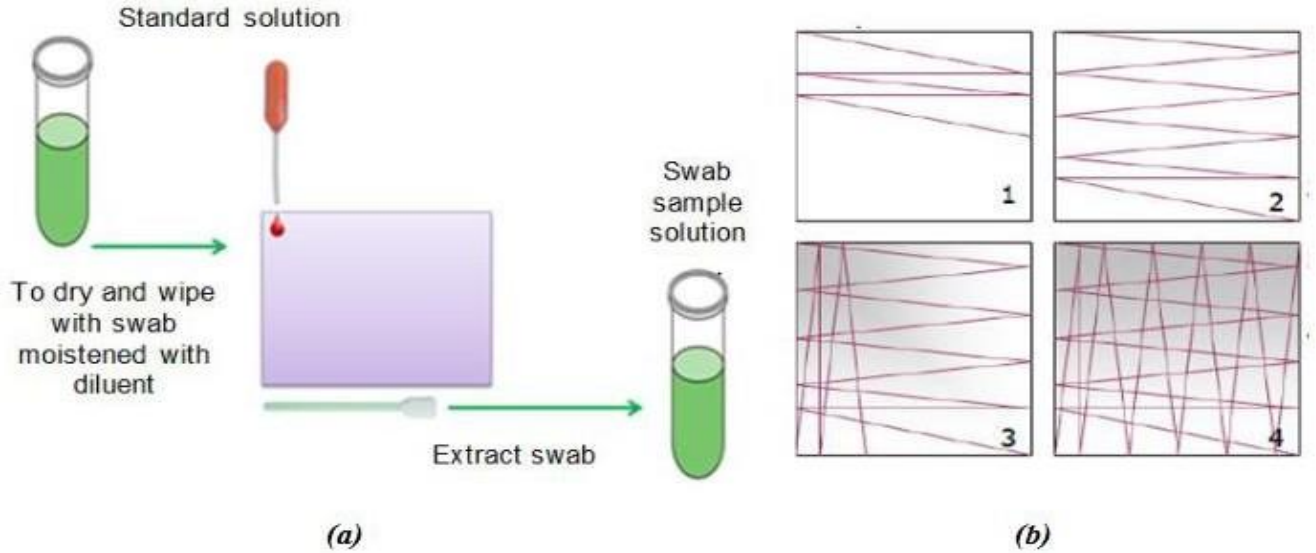

Figure 2- The scheme of swabbing procedure (a) and sequence (steps 1-4) of swab wiping (b)

The typical standardised 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 (back and forth) in Figure 2(b), then, after rotating the swab, vertically (up and down) in Figure 2(b). The highest amount of residue was removed by repeatedly wiping a fresh surface. The swab was then placed in a sealed container with a label for estimating. Two further versions, procedures II and III, were solely used for the robustness investigation and differ from process I only in the orientation of the swab. The surface area was initially wiped horizontally from one side to the other in accordance with process II, and then again after rotating the swab. In accordance with step III of the technique, the surface was first wiped diagonally up and down, and then again in the same manner after rotating the swab.

(vi) Creating experiments- Both quantitative and qualitative parameters were taken into account for the robustness test of the designed swab sampling procedure and analytical HPLC method. These factors were chosen based on experience.

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 1- Robustness factors and design of experiments for swab sampling procedure

Tables 1 and 2 provide a summary of the five components and their respective values for the swabbing procedure and HPLC method. The response variable for the swab sampling procedure and analytical HCT was the percentage recovery rate of each API from the surface and system suitability test parameters, including the column efficiency (theoretical plates - N), the tailing factor (USP symmetry - As), the relative standard deviation (RSD) of peak areas (RSDA), the RSD of retention times (RSDRT) (n=6), and the resolution factor between HCT and lisinopril at 215 nm.

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.*

Table 2- Robustness factors and design of experiments for analytical procedure

LISINOPRIL AND HCT RESIDUAL ESTIMATION IN SAMPLES AFTER SWABBING AND RINSING

For the purpose of sampling API residues from the surfaces of manufacturing equipment, both swabbing and rinsing techniques were used. The API residue concentrations were given in g/mL. Following the production of three batches in a row of the completed drug product, uncoated tablets of lisinopril/hydrochlorothiazide 20/25 mg, samples for equipment cleaning were taken from various sampling sites. Following sampling, the equipment surfaces were repeatedly washed with pure water to eliminate any remaining methanol from the surfaces. Gas chromatography was used to evaluate the final cleaned sections for methanol residues. The approved HPLC method was used to quantify the presence of lisinopril and HCT residues in swab and rinse samples. Table 3 presents the outcomes. The typical chromatograms obtained from the sample solution are shown in Figure 3. The diluent and one unidentified substance (RT=21 min) that was recovered from the swab material are responsible for the secondary peaks that were visible on the chromatograms. The measured levels of lisinopril and HCT residues are below the approved cross-contamination acceptability limits. The established standard operational cleaning technique for cleaning production equipment surfaces is effective enough to remove the aforementioned APIs from the cleansed surfaces and eliminates the possibility of following completed products becoming contaminated.

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

Table 3-The results of lisinopril and HCT residues analysis

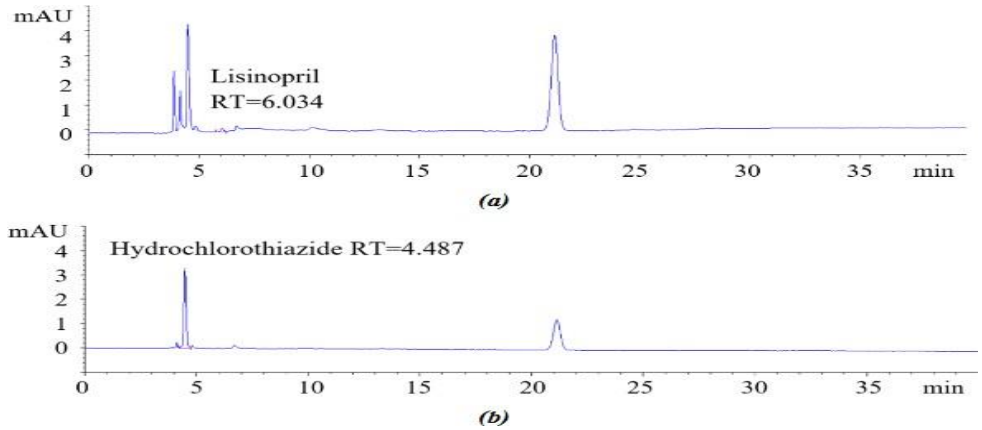

Figure 3- Chromatograms of the sample solution recorded at 215 nm (a) and 272 nm (b)

CONCLUSION

For the simultaneous quantitative assessment of lisinopril and hydrochlorthiazide (HCT) residues on surfaces of pharmaceutical equipment used in the manufacturing process, an analytical HPLC approach combining swab and rinse sampling techniques was devised. To show cleaning validity, lisinopril/hydrochlorthiazide 20/25 mg uncoated pills were employed. Regarding precision, accuracy, robustness, specificity, system adaptability test, and linearity-range, the analytical method was validated over concentration ranges of 0.025 ug/ml to 25 g/mL for HCT and 0.155 g/mL to 20.0 g/mL for lisinopril. Both the swab and rinse sampling techniques that had been devised were shown to be reliable and accurate with high recovery rates (>80%). Swab/blank solution interferences were not seen. The concentrations of cleaning control sample solutions did not change for a while between sampling and injecting into the HPLC system since standard solutions of both substances were stable within 48 hours. As a result, the results show that both API residues can be removed from equipment surfaces using the conventional cleaning process. Lisinopril and HCT concentrations in sample solutions are substantially lower than the predicted acceptable limit of cross-contamination of the subsequent completed product (3.45 g/mL by swabbing and 2.45 g/mL by washing, respectively). Other pharmaceutical quality control laboratories may successfully employ the tested sampling protocol and HPLC technology to maintain the cleaning validation process for lisinopril and HCT residues following the production of uncoated tablets.

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