Original Article

Development and Validation of RP-HPLC-UV Method for Determination of Diclofenac Sodium Residues on Surfaces for Cleaning Validation

Hussein Benachour[a*], Abdelkader Khelifa[b]

[a]Department of Pharmacy, Analytical Chemistry Laboratory, Oran, University 1 Ahmed Ben Bella, [b]Geopharm Pharmaceutical Company, Oran, Algeria

Abstract

In recent years, cleaning validation has achieved a position of increasing in the pharmaceutical industry. It provides assurance to the cleaning procedure that ensures equipment is consistently cleaned from the product, detergent and microbial residues to an acceptable level to avoid cross contamination and adulteration of drug product with other active ingredients. The aim of this study was to demonstrate the applicability of reversed-phase high-performance liquid chromatography coupled with UV detector (RP-HPLC-UV) method for determining the residues of Diclofenac sodium in cleaning control swab samples from equipment surfaces after manufacturing of Diclofenac sodium injection (75mg/3ml) in order to control a cleaning procedure. Diclofenac sodium was evaluated as the worst case. This API is sparingly soluble in water and adherent to surfaces. The acceptable residue limit (*ARL*) of Diclofenac sodium was calculated (0.75 µg/cm²). The analytical method was validated with respect to system suitability test, specificity, linearity-range, accuracy, Repeatability, intermediate precision, limit of detection (LOD) and quantitation (LOQ). These studies were performed in accordance with established guidelines, International Conference on Harmonisation ICH Q2 (R1). The precision of the swabbing procedure and stability of Diclofenac sodium standard solutions were also investigated. The swab sampling method was developed and optimized in order to obtain a suitable recovery (>80%) from stainless steel surfaces 316L. Alpha Texwipe® TX761 polyester Swabs were moistened with diluent (mobile phase) a mixture of Acetonitrile-Water-Orthophosphoric Acid 85 % (600:400:1, v/v/v) (pH 2.5* ± 0.2). The HPLC method was developed in isocratic mode using Hypersil OctaDecylSilyle ODS C18 (250 × 4.6 mm, 5 µm) column at 25°C. At a flow rate of 1.2 ml/min, an injection volume of 20 µl. Detection was carried out at 235 nm. The retention time of Diclofenac sodium was 4.8 min. The calibration curve was linear (the coefficient of determination R^2= 0.9988) over a concentration range 0.05 µg/ml – 12.5 µg/ml. The intra-day, inter-day precision and precision of the swabbing procedure expressed as relative standard deviation were below 5%. The limit of detection and quantitation were 0.014 µg/ml and 0.05 µg/ml respectively. The average recovery of the swabbing method obtained was 87.80 %, when two swabs moistened were used. It is evident that this proposed validated RP-HPLC-UV method with the appropriate swabs Texwipe® TX761 procedure could be applicable for cleaning validation to detect traces levels of Diclofenac sodium residues on pharmaceutical manufacturing equipments.

Corresponding Authors: Hussein Benachour, Department of Pharmacy, Analytical Chemistry Laboratory, Oran, University 1 Ahmed Ben Bella.
Tel: (+213) 665503256
E-Mail: husseindz91@gmail.com

1. Introduction

Cleaning validation is an integral part of current good manufacturing practices in the pharmaceutical industry. Ineffective cleaning procedures may leave residues of the product or cleaning agents in the equipment. Due to these contaminations the purity and potency of the drug may be reduced and patients may show adverse drug reactions. The prevention of cross-contamination was one of the central topics of recent European Union's good manufacturing practices (EU-GMP) [5].

The objective of the cleaning validation is to verify the effectiveness of the cleaning procedure for removal of product residues, degradation products, preservatives, excipients, and cleaning agents as well as the control of potential microbial contaminants. According to the Food & drug administration (FDA) guideline, three different methods of sampling are generally admitted for performing a cleaning control: the direct surface sampling using the swabbing technique, the indirect sampling based on the analysis of solutions used for rinsing the equipment and placebo sampling [1]. A combination of the two firsts methods is generally the most desirable, particularly in circumstances where accessibility of equipment parts can mitigate against direct surface sampling [2].

According to PDA and CGMPs, the determination of cleaning limits and acceptance criteria is a crucial element of a good cleaning validation program [3, 5]. Limits and acceptance criteria should be practical, verifiable, achievable, and scientifically sound [1]. The calculation of an acceptable residue limit *ARL* and the maximum allowable carryover *MACO* of active products in manufacturing equipment should be based on therapeutic doses, toxicological index and general limit 10ppm (part per million) [2]. Chapters 3 and 5 of the EU-GMP guideline have been revised to promote a science and risk-based approach and refer to a "toxicological evaluation" for establishing threshold values for risk identification [13, 14, 15]. The determination of health-based exposure limits for a residual active substance is based on the method for establishing the so-called Permitted Daily Exposure (PDE) as described in Appendix 3 of ICH Q3C (R4) and Appendix 3 of VICH GL 18 [13, 16, 24]. The *ARL* of Diclofenac sodium in the equipment was established in this study.

The analytical methods used to detect residuals or contaminants should be specific, selective and sufficiently sensitive for the substance to be assayed, capable of quantitative estimation or detecting the established acceptable level of the residue or contaminant remaining over the surface after cleaning procedure [2]. RP-HPLC coupled with UV detection is widely used to monitor the efficiency of the cleaning methods due to its high sensitivity, selectivity and automation characteristics. The analytical method was

validated with respect to system suitability test, specificity, linearity-range, accuracy, repeatability, intermediate precision, recovery studies, limit of detection (LOD) and quantitation (LOQ). These studies were performed in accordance with established guidelines ICH Q2 R1 [8]. The stability studies were also performed.

The aim of this study was to demonstrate the applicability and to validate a simple and sensitive RP-HPLC-UV method for quantitative determination of Diclofenac sodium residues in cleaning control swab samples from manufacturing surfaces after production of Diclofenac sodium injection 75 mg /3ml and the efficiency of the cleaning procedure.

The novelty of this study compared to the articles published [27, 28] was the specificity of the method, rapidity with a short retention time, the repeatability (precision) on the swabbing technique and the determination of the acceptable residue limit (*ARL*) of Diclofenac sodium in the equipment surfaces. The developed analytical method was performed on the same surface quality of the equipment (stainless steel plates 316L).

Diclofenac sodium was evaluated as the worst case, a non-steroidal anti-inflammatory drug, (NSAID), exhibiting anti-inflammatory, analgesic and antipyretic properties [6]. Diclofenac sodium (Sodium 2-[(2, 6-dichlorophenyl) amino] phenyl] acetate; $C_{14}H_{10}Cl_2NNaO_2$; Mr= 318, 1 ; CAS registry number: [15307-79-6]), white appearance or slightly yellowish, slightly hygroscopic, crystalline powder; Sparingly soluble in water,
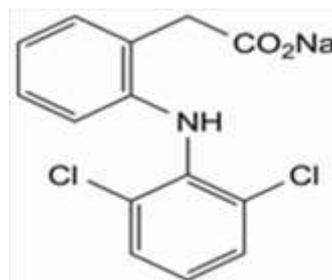

Figure 1. Chemical Structure of Diclofenac sodium.

freely soluble in methanol, soluble in ethanol (96 %), slightly soluble in acetone, having the following structural formula (Figure 1) [7].

2. Material and Methods

2.1. Instruments and Apparatus

The chromatography analysis was performed using Shimadzu Prominence-*i* LC 2030C (Shimadzu Corporation - Japan) equipped with UV detector standard, quaternary pump and auto sampler system. The output signals were monitored and processed using LabSolutions software. The method was developed using Hypersil OctaDecylSilyle ODS C18 (250 × 4.6 mm, 5 μm) column. The analytical balance was from Mettler Toledo, AB204-S (Greifensee, Switzerland) with accuracy ± 0.0001 g. The pH of the mobile phase was measured by a pH meter seven Easy (Mettler-Toledo, Switzerland), Ultra-Sonicator fisher scientific FB 15050, Stainless steel plates 316L & templates (10 cm x 10 cm), were used during development study. Class A Volumetric flasks, pipettes, beakers, measuring cylinders, tubes of Borosil glass were used.

13

2.2. Chemicals and Reagents

Acetonitrile (HPLC grade Merck Lichrosolv®) and Ortho-phosphoric acid 85% (AR grade VWR BDH prolabo), Water (HPLC grade) was obtained from a Milli-QRO water purification system. Reference working standards of Diclofenac sodium batch number 0601004. The extraction–recovery sampling was realized with Knitted Alpha® TX761-Texwipe Swab polyester (An / TW company USA) on a polypropylene handle total head length 16.8mm (0.661"). The mobile phase was filtered through a 0.45 μm Sartorius membrane filter (Gottingen, Germany).The samples were filtered through syringe filters 0.45 μm Millipore Millex-HN Nylon membrane filter (Ireland).

2.3. Method

2.3.1. Chromatographic Conditions

The method was developed using Hypersil OctaDecylSilyle ODS C18 (250 × 4.6 mm, 5 μm) column with isocratic mode. The mobile phase containing a mixture of acetonitrile-Water-orthophosphoric acid 85 % (600:400:1, v/v/v) (pH*2.5 ± 0.2). The flow rate of the mobile phase was set at 1.2 ml/min. The column temperature was maintained at 25°C and the eluted compound was monitored at the wavelength of 235 nm. The injection volume was 20 μl. When the chromatograms are recorded in the prescribed conditions, the retention time was about 4.8 minutes for Diclofenac sodium and the run times were fixed at 7 minutes.

2.3.2. Determination of the Worst Case (API) for Cleaning Validation

According to the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Q7A, if various active pharmaceutical ingredients or intermediates are manufactured in the same equipment and if the equipment is cleaned by the same process, it is acceptable to choose only one substance for cleaning validation [21]. This selection, called worst case selection, should be based on the solubility (listed in pharmacopeias), cleanability and toxicity [5, 21], in addition to the calculation of residue limits based on potency, and stability [1, 21]. The same criterion is considered valid by the World Health Organization WHO [25]. There is no need for the individual validation of cleaning processes and the study of the worst case is acceptable, as long as the selected substance is that presenting the greatest cleaning difficulty [9]. In our study, we selected Diclofenac sodium as a worst case.

2.3.3. Establishing of Acceptable Residue Limit (ARL) for the Cross Contamination Level

FDA does not intend to set acceptance specifications or methods for determining whether a cleaning process is validated. It is impractical for FDA to do so due to the wide variation in equipment and products used throughout the bulk and finished dosage form industries. The firm's rationale for the residue limits established should be logically based on the manufacturer's knowledge of the materials involved and be practical, achievable, and

verifiable [1]. The maximum allowable carryover (*MACO*) is the acceptable transferred amount from the previous (the worst case: Diclofenac sodium 75mg/3ml) to the following product (in our study was Gentamicine sulfate 80mg/2ml). The *MACO* is determined based on the therapeutic dose (NMT 0.1% of the normal therapeutic dose of any product to appear in the maximum daily dose of the following product), toxicity, health based exposure limits and generally 10 ppm criterion (NMT 10 ppm of any product to appear in another product) [2, 5, 13, 25]. Once the maximum allowable residue limit in the subsequent product was determined, the next step was the determination of the *ARL* in terms of the contamination level of active ingredient per the total surface area of equipments [10]. The *MACO* and *ARL* of Diclofenac sodium based on therapeutic dose, 10ppm limit and health based exposure limits were calculated in this study as follows:

2.3.3.1. Determination of MACO and ARL Based on Drug Active Dose or Pharmacological Potency of the Product. Eq. (A.1) & Eq. (A.2)

$$MACO = \frac{TDD\ previous \times MBSnext}{SF \times LDD\ next} \qquad \text{Eq. (A.1)}$$

Where, *MACO* is the maximum allowable carryover (mg), *TDD* previous is the minimal therapeutic dose of previous product (mg), *SF* is a safety factor for injection product (1/1000 [th]), *MBS* next is the minimum batch size of the subsequent product (mg) and *LDD* next is the

largest daily dose of the subsequent product (mg) [3, 4, 12, 22].

$$ARL = \frac{MACO\ [\mu g]}{Total\ contact\ surface\ [cm^2]} \qquad \text{Eq. (A.2)}$$

2.3.3.2. Determination of ARL Based on 10 ppm Criterion Eq. (B.1)

$$ARL = \frac{10 \quad \times \quad MBSnext}{1 \quad \times \quad Total\ contact\ surface\ [cm^2]} \qquad \text{Eq. (B.1)}$$

Where, *MBSnext* is the minimum batch size of the subsequent product (kg) [2, 12].

2.3.3.3. Determination of Health Based Exposure Limits: Calculation of a Permitted Daily Exposure (PDE). Eq. (C.1) & Eq. (C.2)

Determination of health based exposure limits for a residual active substance is based on the method for establishing the so-called Permitted Daily Exposure (PDE) as described in Appendix 3 of ICH Q3C (R4) [16] and Appendix 3 of VICH GL 18 on "residual solvents in new veterinary medicinal products, active substances and excipients (Revision)" [24]. The PDE represents a substance-specific dose that is unlikely to cause an adverse effect if an individual is exposed at or below this dose every day for a lifetime [13]. Determination of a PDE involves (i) hazard identification by reviewing all relevant data, (ii) identification of "critical effects", (iii) determination of the no-observed-adverse-effect level (NOAEL) of the findings that are considered to be critical effects, and (iv) use of several adjustment factors to account for

various uncertainties. Appendices 3 of the ICH Q3C and VICH GL 18 guidelines present the following equation for determination of *MACO* & the PDE:

$$MACO = \frac{PDE \times MBSnext}{LDD \; next} \qquad \text{Eq. (C.1)}$$

$$PDE = \frac{NOAEL \; x \; \text{Weight Adjustment}}{F1 \; x \; F2 \; x \; F3 \; x \; F4 \; x \; F5} \qquad \text{Eq. (C.2)}$$

Where NOAEL is the highest tested dose at which no "critical" effect is observed. Weight adjustment is a standard body weight of 50 kg should be used for human medicinal products. F1: A factor (values between 2 and 12) to account for extrapolation between species, F2: A factor of 10 to account for variability between individuals, F3: A factor 10 to account for repeat-dose toxicity studies of short duration, i.e., less than 4-weeks, F4: A factor (1-10) that may be applied in cases of severe toxicity, e.g. non-genotoxic carcinogenicity, neurotoxicity or teratogenicity, F5: A variable factor that may be applied if the no-effect level was not established. When only an LOEL is available, a factor of up to 10 could be used depending on the severity of the toxicity [13, 16].

2.3.4. Standard Solution Preparation

Working Standard solution of Diclofenac sodium 100 μg/ml was prepared by weighing about 50 mg of substance Diclofenac sodium into 50 ml volumetric flask, added about 20ml of diluent (mobile phase) followed by ultrasonication for 10 minutes in ultrasonic bath to dissolve it, and made up the volume to

50 ml with diluent and mixed. Transferred 5ml of this stock solution into 50 ml of volumetric flask and made up to volume with diluent. Further, dilute 10.0 ml of this solution to100 ml with diluent (Standard solution of Diclofenac sodium 10 μg/ml). Further, dilute 5.0 ml of solution 100 μg/ml to 100 ml with diluent (Standard solution of Diclofenac sodium 5 μg/ml). Further, dilute 0.1 ml of this solution to 100 ml with diluent (Standard solution of Diclofenac sodium 0.1 μg/ml). The standard stock solution was subsequently diluted with diluent to furnish calibration curve (Linearity) in the range of 0.05-12.5μg/ml.

2.3.5. Sample Preparation

There are two general types of sampling that have been found acceptable. The most desirable by FDA is the direct sampling method of equipment surfaces when product contact surfaces are easily accessible. Another method is the use of rinsing water. The swabbing process is a subjective manual process that involves physical interaction between the swab and the stainless steel surfaces and thus may vary from operator to operator. So, a standardized motion protocol is required to establish reproducible and high recoveries. The selected surfaces (10 cm x 10 cm) of stainless steel 316L, previously cleaned using detergent and dried, were sprayed with 1ml of a standard solution, for the positive swab control at three concentrations levels (0.1 ; 5 ; 10 μg/ml), and the plates were allowed in oven VWR to dry (approximate time was 20 min at 30°C). After drying the surfaces were wiped with the first side of the first swab
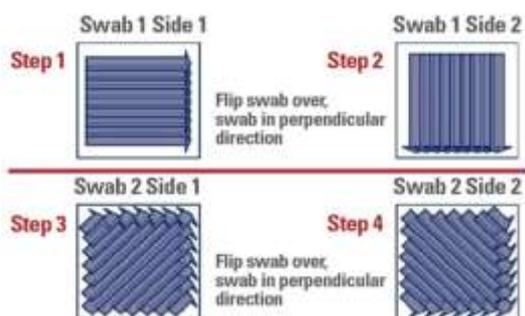
Figure 2. Swabbing patterns.

soaked with diluent (mobile phase), passing it in parallels directions horizontally, to remove the residues from the stainless steel. The other side swab was used to wipe the wet surfaces in parallels directions vertically. We used two swabs to increase the recovery (Figure 2).

The swabs were placed into a Borosil glass tube containing 5 ml of extraction solution or diluent (mobile phase). The background control sample was prepared from the extraction media. The negative swab control was prepared in the same way as the samples, using swabs, which had been in contact with the stainless steel surfaces without spraying the standard solution of Diclofenac sodium. Subsequently, the tubes were closed and labeled container for estimation and placed in an ultrasonic bath for 15 min. The resulting solutions were filtered through syringe filters 0.45 μm Millipore Millex-HN-Nylon membrane filter and the solutions were analyzed by HPLC–UV, the chromatogram was recorded.

2.3.6. Recovery Rate (REC %) of Swab Sampling From Stainless Steel Surfaces

The recovery studies were performed in order to determine to what entente the residue could be retrieved from the manufacturing equipment surfaces with the selected sampling procedure [4, 17]. The selected surfaces of stainless steel (10 cm x 10 cm) previously cleaned and dried, were sprayed with 1ml of standard stock solution (two concentration levels 10 and 5 μg/ml) and the solvent was allowed to evaporate. Then swab sampling was performed according to swab wipe procedure as described in sample solution preparation. The dilution of samples has been taken into consideration. The calculation formula of recovery % was:

$$REC \% = Au \text{ x } 100 \text{ /As}$$

Where, Au - Peak area of Diclofenac sodium obtained from swab sample solution; As - Peak area of Diclofenac sodium obtained from standard solution [18].

Or

$$REC \% = \text{Recovered Concentration x } 100 \text{ /} \\ \text{Standard Concentration}$$

Where, Recovered Concentration was determined as follows:

$$\text{Recovered Concentration} = Ar \text{ x } Sc \text{ /As}$$

Where, Ar = Average area of sample solution, Sc = Standard concentration in μg /ml, As = Average area of standard solution [4, 17].

17

2.3.7. Method Validation

The method validation was performed in accordance with ICH Q2 R1 guidelines. The following validation characteristics were addressed: system suitability test, specificity, linearity-range, accuracy, Repeatability, intermediate precision, recovery studies, limit of detection (LOD) and quantitation (LOQ) [8]. The stability studies were also performed.

2.3.7.1. System Suitability Testing

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system performance that can be evaluated as such [8]. System suitability was checked by six replicate injections (n=6) of standard solution. Main parameters including: the RSD, % of peak areas (acceptance criteria: < 5.0 %), the RSD, % of retention times (acceptance criteria: <1.0 %), the USP tailing factor (acceptance criteria: 0.8-2.0), the number of theoretical plates N (acceptance criteria: >2000) were measured [11, 18].

2.3.7.2. Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present [8]. The specificity of the method was checked by three replicate injections (n=3) the Diclofenac sodium standard (5 µg/ml), the background control sample (diluent), the negative swab control, the spiked stainless steel (10 cm x 10 cm) plate swabbed as described (positive swab control sample 5 µg/ml).

2.3.7.3. Linearity and Rang

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample within a given range [8]. The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity [8]. From the standard solution of Diclofenac sodium working solutions (100 µg/ml) were prepared at eight different concentration levels ranging from 0.05 µg/ml to 12.5 µg/ml. Three replicate injections (n=3) were performed at each concentration of Diclofenac sodium. The linearity was checked by the correlation coefficient (acceptance criteria: > 0.99) [4], the coefficient of determination (acceptance criteria: >0.98), the relative standard deviation (RSD) of peak areas (acceptance criteria: <5.0 %), the RSD, % of retention times (acceptance criteria: <1.0 %) [11, 18]. A linear regression curve was performed in order to determine the slope, intercept and coefficient of determination.

2.3.7.4. Accuracy

The accuracy or trueness of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an

accepted reference value and the value found [8]. The accuracy of the method was assessed by comparing the analyte amount determined versus the known amount accepted reference value at three different concentration levels (0.1 ; 5 ; 10 μg/ml) with three replicates (n=3). The accuracy is expressed as a percentage of standard value found from accepted reference value with corresponding RSD, %. The main recovery should be within 90.0 – 110.0 % [4] and the RSD, % of percentage recovery should be <5.0 % (acceptance criteria) for each concentration level [11]. The recovery for each concentration level was calculated by the following formula:

$$REC, \% = C_{found} \times 100 / C_{ref}$$

Where, C_{found} − concentration (μg/ml) of Diclofenac sodium obtained value (found); C_{ref} concentration (μg/ml) of Diclofenac sodium as a conventional true value or an accepted reference value [18].

2.3.7.5. Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at two levels: repeatability and intermediate precision [8].

2.3.7.5.1.   Repeatability

Repeatability or intra-assay precision expresses the precision under the same

operating conditions over a short interval of time [8]. The method was estimated by measuring repeatability on three replicate injections (n=3) of Diclofenac sodium standard solutions at three concentrations levels (0.1 ; 5 ; 10 μg/ml) and positive swab samples solutions also at three concentrations levels (0.1 ; 5 ; 10 μg/ml). Swab sample solutions were prepared in the way as described. The intra-assay precision was checked by the RSD, % of peak areas (acceptance criteria NMT 5.0 %) and the RSD % of retention times (acceptance criteria NMT 1.0 %).

2.3.7.5.2. Precision of the Swabbing Procedure

We studied the repeatability of swabbing technique by spraying 1ml of a standard solution 5 μg/ml on stainless steel surface and we continued as described. We released this technique three times, and we injected each swab solution three replicate injections (n=3). The repeatability of swabbing method was checked by the RSD, % of peak areas (acceptance criteria NMT 5.0 %) and the RSD % of retention times (acceptance criteria NMT 1.0 %).

2.3.7.5.3.   Intermediate Precision

Intermediate precision or inter-day precision expresses within-laboratories variations: different days, different analysts and different equipment [8]. The method was estimated by measuring repeatability for three consecutive days, each day, six replicate injections (n=6) of Diclofenac sodium standard solutions and positive swab samples solutions at two concentration levels (5; 10 μg/ml).

Swab sample solutions were prepared in the way as described. The inter-day precision was checked by the RSD, % of peak areas (acceptance criteria NMT 5.0 %).

2.3.7.6. Detection Limit

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. LOD was determined, based on Signal-to-Noise S/N ratio of 3:1 (three times the S/N) according to ICH Q2 R1 guidelines [8]. The LOD was confirmed by injecting six replicate injections (n=6) of dilute solution at detection level and the precision was established. The RSD, % of peak areas should not be more than 5 % (acceptance criteria) and the average of Signal-to-Noise ratio of the responses was also determined.

2.3.7.7. Quantitation Limit

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices. The LOQ was determined, based on Signal-to-Noise S/N ratio of 10:1 (ten times the S/N) according to ICH Q2 R1 guidelines [8]. The LOQ was confirmed by injecting six replicate injections (n=6) of dilute solution at quantitation limit level and the precision was established. The RSD, % of peak areas should not be more than 5 % (acceptance criteria) and the average of Signal-to-Noise ratio of the responses was also determined.

2.3.7.8. Stability of Diclofenac Sodium Standard Solutions

The stability was studied on standard solutions at two concentration levels, 5 and 10 µg/ml. These solutions were stored for 24 hours at room temperature. Each solution was injected six times (n=6). The stability was assessed by determination of percentage assay of standard solutions stored for 24 h at room temperature and comparison with standard solutions freshly prepared.

3. Results and Discussion

3.1. Establishing of Acceptable Residue Limit (ARL) for the Cross Contamination Level

The acceptable residual limit of Diclofenac sodium based on therapeutic dose, 10ppm limit

Table 1. Acceptable residue limit (*ARL*) and the maximum allowable carryover (*MACO*) of Diclofenac sodium obtained through three different calculation methods 10ppm, therapeutic dose and Health based exposure limits.

Criterion based	*MACO* (mg)	*ARL* (µg /cm²)	*ARL* (µg / 100cm²)
10 ppm	40	0.75	75
Pharmacological potency of the product	535.71	10.03	1003
Health based exposure limits	5000	93.65	9365

Table 2. System suitability test results.

Samples	Evaluation				
Blank (mobile phase)	Base line and no peaks detected at the retention time of Diclofenac sodium				
Diclofenac sodium Standard 10 μg/ml (n=6)	RT	Area	Width δ 50%	Number of theoretical plates N	USP Tailing factor TF 5%
1	4.880	304405	0.115	9975.93	1.3
2	4.876	304344	0.115	9959.59	1.3
3	4.875	303783	0.115	9955.50	1.3
4	4.875	304183	0.115	9955.50	1.3
5	4.874	304779	0.115	9951.42	1.3
6	4.872	304607	0.115	9943.25	1.3
Mean	4.875	304350.16	0.115	9956.86	1.3
RSD %	0.0545	0.1140	0	0.1090	0
Evaluation	<1%	<5%	-	N>2000 Efficient column	Between (0.8- 2.0)

Table 3. Specificity results obtained from Diclofenac sodium standard, negative and positive swabs controls.

Samples	Evaluation	
Background control sample (Diluent) & Negative swab control samples	Base line and no peaks detected at the retention time of Diclofenac sodium	
	RT (retention time)	
Sr. No.	Diclofenac sodium standard	Positive Swab Control
1	4.850	4.840
2	4.848	4.840
3	4.848	4.775
Mean	4.848	4.818
% RSD	0.0238	0.7788
Evaluation	<1%	

and health based exposure limits were calculated using total manufacturing line equipments area 53391.2 cm². The calculated *MACO* and *ARL* values were depicted in table 1.

3.1.1. Determination of ARL Based on the Medical or Pharmacological Potency of the Product Eq. (A.1) & Eq. (A.2)

In our study, *TDD* previous (Diclofenac sodium) = 75 mg/day [6], *LDD* next (Gentamicine sulfate 80mg/2ml) = 560 mg/day

[23], *MBS* next (Gentamicine sulfate): 4000 000 mg, SF: Safety factor for injections products $1/1000^{th}$ = 1000 [3]. So, the $MACO_1$ and ARL_1 found in this case were 535.71 mg and 10.03 μg/cm² respectively [3, 4, 12].

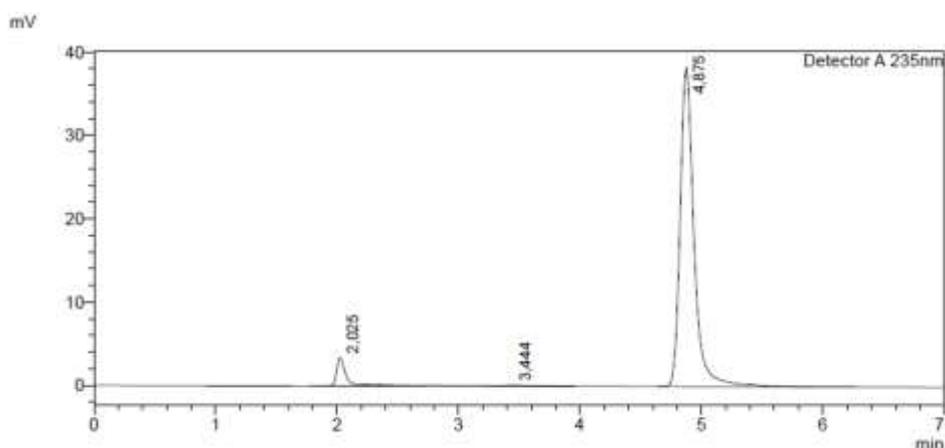### 3.1.2. Determination of ARL Based on 10 ppm Criterion Eq. (B.1), [2, 12]

In our study, *MBS* next (Gentamicine sulfate): 4Kg, 10ppm mean: not more than 10 ppm of the previously manufactured product is allowed to appear in the subsequent product

21
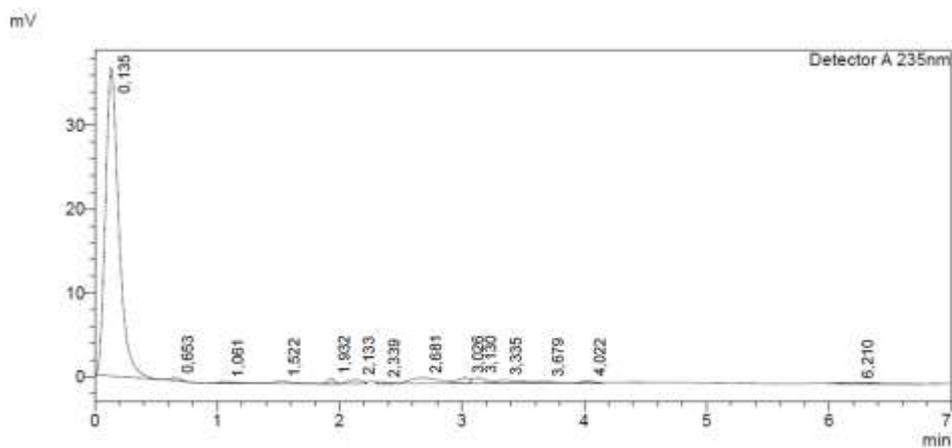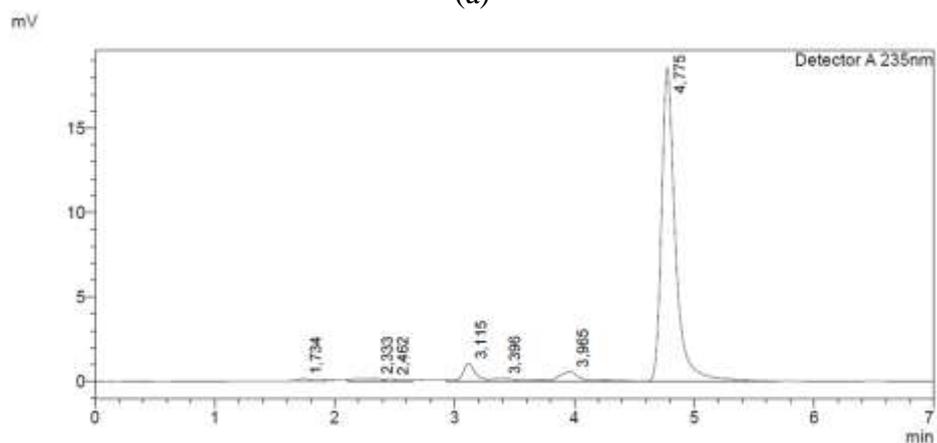
Figure 3. Chromatograms obtained from specificity test: (a) negative swab control, (b) positive swab control, (c) Diclofenac sodium standard solution.

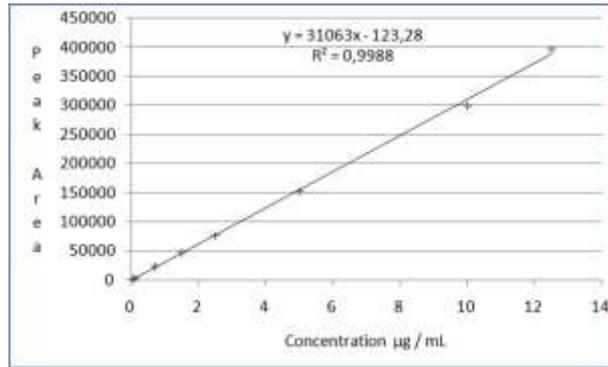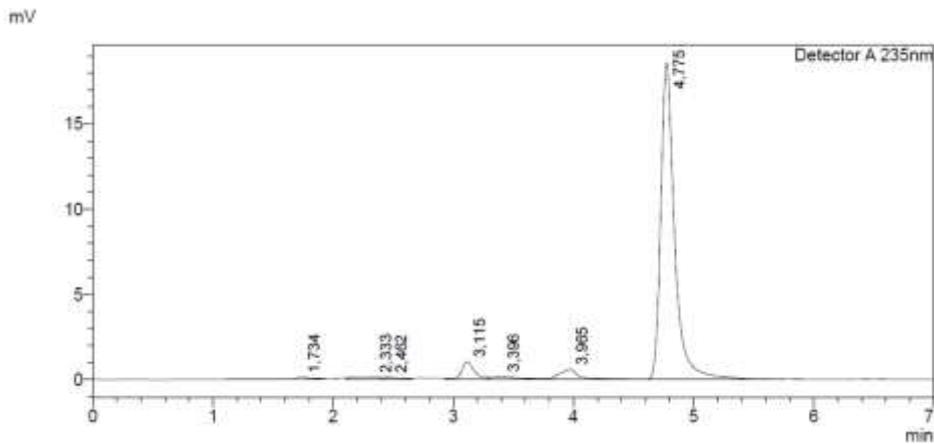(10 mg of previous product in 1 kg of next product). The ARL_2 calculated was 0.75 µg/cm².

Figure 4. Linearity of Diclofenac sodium (the standard deviation values for each concentration were presented as error bars).

(a)
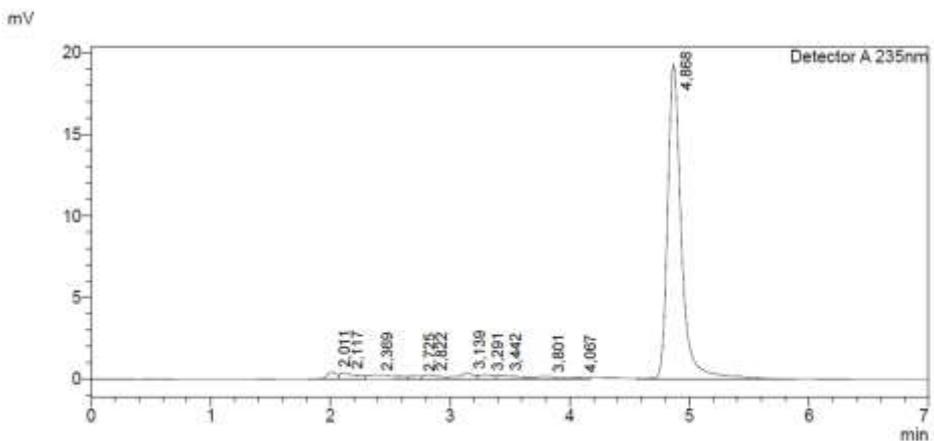
(b)

Figure 5. Chromatograms obtained from Stability studies of Diclofenac sodium standard solutions: (a) freshly prepared 0h, (b) stored solution 24h.

3.1.3. Determination of Health Based Exposure Limits : Calculation of a Permitted Daily Exposure (PDE) Eq. (C.1) & Eq. (C.2)

The NOAEL is the highest tested dose at which no "critical" effect is observed =7mg/Kg/day. Weight adjustment is a standard body weight of 50 kg should be used for human medicinal products. F1=5 for extrapolation from rats to humans F2= 10 to account for variability between individuals,

F3= 10 for study duration of 4 weeks, F4=1 database for Diclofenac sodium no completeness, F5=1 for NOAEL, was used for PDE calculation. The ARL_3 determined was 93.65µg /cm².

As evident from the comparison, the lowest values calculated limit of Diclofenac sodium per surface area in our case was 0.75 µg /cm² or 75 µg / 100cm² [13, 16, 19, 20, 24].

3.2. Validation of Proposed Method

3.2.1. System Suitability Testing

System suitability was checked by six replicate injections (n=6) of standard solution Diclofenac sodium 10 µg/ml. During performing the system suitability test, in all cases, the RSD of the peak areas, the RSD of the retention times, the number of theoretical plates per column or column efficiency and the USP tailing factor comply with acceptance criteria. The results are summarized in table 2.

3.2.2. Specificity

The results are shown in table 3 and figure 3, from which it can be observed that there were no mutual interferences at the retention time of the analyte, which explains the specificity of the method.

3.2.3. Linearity & Rang

The values of the slope, intercept, Calibration curve equation, correlation coefficient, coefficient of determination, and

Table 4. The precision results.

Solution µg/ml (n=3)	Standard solution		Sample solution (Positive Swab Control)	
	RT	Area	RT	Area
10	4.8366	304332	4.84	40093.3333
% RSD	0.1193 < 1%	0.0424 < 5%	0 < 1%	1.7185 < 5%
5	4.84	156702	4.84	24533.6667
% RSD	0 < 1%	0.5060 < 5%	0 <1%	0.1719 < 5%
0.1	4.8333	3171.6666	4.7733	864.3333
% RSD	0.1194 < 1%	2.7634 < 5%	0.1209 < 1%	4.5759 < 5%

Table 5. The repeatability on the swabbing technique.

Sr.No. stainless steel (SS)	Diclofenac sodium Sample solution (Positive Swab Control)			
	Area (n=3)	Mean	Average	RSD %
SS1	25313			
	25475	25262.6667		
	25000			
SS2	24974			
	24529	25152	24982.7778	1.5725 < 5%
	25953			
SS3	24494			
	24578	24533.6667		
	24529			

Concentration range are summarized in table 4. The calibration curve was constructed by plotting response expressed in area units against the corresponding concentration injected of Diclofenac sodium expressed as µg/ml (Figure 4). The high value of the coefficient of determination ($R^2 = 0.9988$) indicated good linearity from 0.05 to 12.5 µg/ml.

3.2.4. Precision

The precision of the method was demonstrated by inter-day and intra-day variation studies.

3.2.4.1. Repeatability

The intra-assay precision was checked by the RSD, % of peak areas and the RSD % of retention times (RT). The RSD, % value of peak areas was less than 5% that illustrate the good precision of the analytical method. The results were summarized in table 5.

3.2.4.2. Precision of the Swabbing Procedure

The RSD, % values found were less than 5%, which illustrate the good repeatability of the swabbing technique. The results were summarized in table 6.

3.2.4.3. Intermediate Precision

The inter-day precision was checked by the

Table 6. The intermediate precision results.

Solution µg/ml Mean (n= 6)	Standard solution			Positive Swab sample		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
10	304408	319169	300220	58330	55958	56959
10	304607	318574	300123	58966	55986	56780
10	304183	318805	300268	58703	56780	57339
10	304405	318504	300059	58312	58116	57359
10	304779	318690	300385	58760	59261	56979
10	304607	318347	300795	56976	56976	57193
Mean	304498.16	318681.5	300308.33	58341.16	57423.8	57101.5
Standard Deviation	212.1328	299.9672	73.8669	319.5814	466.7090	285.4592
Intermediate precision RSD %	3.1280 <5%			1.1162 <5%		
5	154238	158966	148462	24494	24974	26512
5	154111	159883	148449	24578	24529	26492
5	155952	159086	148802	24348	25953	26133
5	156622	159175	148668	23951	25810	26754
5	158111	158697	148600	25000	25411	26315
5	157532	159162	147870	24529	25632	25810
Mean	156094.33	159161.5	148475.16	24483.33	25384.83	26336
Standard Deviation	1028.2028	498.4138	200.1574	116.3844	728.4963	213.2768
Intermediate precision RSD %	3.5596 <5%			3.6472 <5%		

Table 7. The accuracy results.

Reference value µg/ml	Peak area found (n=3)	Value found µg/ml	The percentage recovery, %	The mean recovery, %	RSD of percentage recovery, %
10	304408	9.803	98.03	98.05	0.0412
	304405	9.803	98.03		
	304607	9.810	98.10		
5	156622	5.046	100.92	100.96	0.5067
	155952	5.024	100.48		
	157532	5.075	101.50		
0.1	3110	0.104	104	103.66	0.5569
	3080	0.103	103		
	3133	0.104	104		

Table 8. LOQ and LOD of the method.

Parameter	Value
LOQ[a], µg /ml	0.050
LOD[b], µg /ml	0.014
RSD of peak areas, % for LOQ (n=6)	1.993
RSD of peak areas, % for LOD (n=6)	1.909
S/N[c] for LOQ	11.75
S/N for LOD	7.938

[a] Limit of Quantitation;
[b] Limit of Detection;
[c] Signal-to-noise ratio.

Table 9. Recovery of Diclofenac sodium from spiked stainless steel plates.

Standard solution concentration µg /ml	Mean area (n=3)	Amount spiked on stainless steel plate (µg)	Area (n=3)	Recovered concentration µg /ml	REC %	Average REC %	RSD %
10	307829.3	10	58341.1	9.47	94.70	93.53	1.11
			57423.8	9.32	93.20		
			57101.5	9.27	92.70		
5	154576.9	5	24483.3	3.95	79.00	82.06	3.77
			25384.8	4.10	82.00		
			26336.0	4.26	85.20		

RSD, % of peak areas. The data obtained revealed that the method exhibited a good intermediate precision with less than 5% RSD for the Diclofenac sodium standard Solutions and positive swab samples at two concentration levels when analyzed on three consecutive days. The results are summarized in table 7.

Table 10. Stability studies of Diclofenac sodium standard solutions.

Standard solution µg/ml (n=6)	% Assay	
	0 h	24h
10	98.6	100.26
5	100.6	102.4

3.2.5. Accuracy

The percentage of recovery obtained and the RSD % of percentage recovery calculated were well within limits of acceptance criteria which indicate the accuracy of the method. The accuracy results are shown in table 8.

3.2.6. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD and LOQ were determined based on Signal-to-Noise S/N ratio of 3:1 and 10:1, respectively according to ICH Q2 R1 guidelines. The LOD and LOQ for Diclofenac sodium were found to be 0.014 and 0.050 µg/ml respectively. At LOQ level, RSD of Diclofenac sodium area from six replicate injections (n=6) of standard solution (0.050 µg/ml) was found to be 1.993 % with average of Signal-to-Noise 11.75. At LOD level, RSD of Diclofenac sodium area from six replicate injections (n=6) of standard solution (0.014 µg/ml) was found to be 1.909 % with average of Signal-to-Noise 7.938 [18]. Lower values of LOD and LOQ demonstrated that the method is enough sensitive to quantify trace level amount of Diclofenac sodium. The results are summarized in table 9.

3.2.7. Recovery Studies

Recovery of Diclofenac sodium from spiked stainless steel plate (10cm x 10cm) was done by swab sampling. The results of the proposed methods are depicted in table 10. High values of recovery *REC %* demonstrated that the swabbing method was enough reliable to recover trace level amount of Diclofenac sodium from stainless steel surfaces. RSD % values less than 5% (n=3) shows a good precision of the swabbing procedure.

3.2.8. Stability of Diclofenac Sodium Standard Solutions

The results show that, the retention time and the percentage assay of Diclofenac sodium standard solutions stored remained almost unchanged. No changes on the chromatograms of the stored solutions were found and no additional peaks were registered when compared with the chromatograms of the freshly prepared solutions, thus indicated that no significant degradation within the indicated period, so the standard solutions were stable for at least 24 hours.

4. Conclusion

Summing up the results, fast and efficient RP-HPLC-UV method was developed and

validated to quantify residues of the active pharmaceutical ingredient Diclofenac sodium on stainless steel surfaces using swab sampling, in support cleaning validation of pharmaceutical manufacturing equipment. Validation studies showed that the RP-HPLC-UV method was simple, rapid, specific, linear, precise, accurate and sensitive to quantify the *ARL* determined 0.75 µg/cm². To extract the Diclofenac sodium residues from the surfaces, a wipe test procedure using two polyester swabs Alpha Texwipe® TX761 is recommended. The recovery obtained from the stainless steel 316L surfaces was more than 87 % and there was no interference from the polyester swab. Stability studies show that Diclofenac sodium standard solutions were, at least, stable over the investigated 24 hours at room temperature. The overall procedure can be used to determine trace levels of Diclofenac sodium residues in production equipment surfaces to confirm the efficiency of the cleaning validation program.

Acknowledgements

References

[1] *US Food and Drug Administration: Guide to inspections validation of cleaning processes. https://www.fda.gov/ICECI/Inspections/InspectionGuides/ucm074922.htm* (Accessed May 26, 2018).

[2] Health Products and Food Branch Inspectorate. Guidance Document Cleaning Validation Guidelines, Guide-0028, Canada, (2008) 1-9.

[3] PDA Parenteral Drug Association. Guidance for Industry, Technical Report No. 29, Points to consider for cleaning validation, Washington, 52 (1998) 1-29.

[4] APIC Active Pharmaceutical ingredients Committee. Guidance on aspects of cleaning validation in active pharmaceutical ingredient plants, (2016) 1-56.

[5] EudraLex European Commission. EU Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use, Qualification and Validation, Brussels, (2015) 15 (4): 11-12.

[6] *Eureka santé Vidal*: *Diclofenac sodium SANDOZ injectable 75 mg/3 ml, France. https://eurekasante.vidal.fr/medicaments/vidal-famille/medicament-gp2968-DICLOFENAC-SANDOZ-injectable.html* (Accessed May 27, 2018).

[7] European pharmacopeia 9.0[th] edition, vol. 1. European Directorate for the Quality of Medicines & HealthCare (EDQM), France (2017) 2414.

[8] ICH Harmonised Triplicate Guideline. Validation of analytical Procedures, text and Methodology, International Conference on Harmonisation Q2 (R1), Geneva (2005) 1-13.

[9] Luciana Valéria P, Okasaki Fukumori N, Nakamura Matsuda M. Determination of the worst case for cleaning validation of equipment used in the radiopharmaceutical production of lyophilized reagents for 99mTc labeling. *BJPS*. (2016) 52 (1): 105-112.

[10] Walsh A. Cleaning validation for the 21st century: acceptance limits for active pharmaceutical ingredients (APIs): Part I. *Pharmaceutical Engineering* (2011) 31 (4): 74-83.

[11] United States Pharmacopeia (USP) 30-NF 25. USP Monographs, Rockville MD (2007) 1922.

[12] Fourman G. L, Mullen M.V. Determining cleaning validation acceptance limits for pharmaceutical manufacturing operations. *Pharm. Technol.* (1993) 17 (4) : 54-60.

[13] EMA European Medicine Agency. Guideline on setting health based exposure limits for use in risk identification in the manufacture of different

medicinal products in shared facilities, United Kingdom (2014) 3-8.

[14] ANSM Agence nationale de sécurité du médicament et des produits de santé. Guide des bonnes pratiques de fabrication, Commission européenne, locaux et matériel, France (2016) (3) 22-25.

[15] ANSM Agence nationale de sécurité du médicament et des produits de santé. Guide des bonnes pratiques de fabrication, Commission européenne, production, France (2016) (5) 34-43.

[16] ICH Harmonised Triplicate Guideline. Impurities: guideline for residual solvents, International Conference on Harmonisation Q3C, Geneva (1997) 1-16.

[17] Bourgois S. La détermination du taux de recouvrement est une étape préliminaire, nécessaire lors d'un exercice de validation du nettoyage, A3P. *La Vague N° 54 revue* (2017): 41-42.

[18] Rubashvili I, Karukhnishvili N, Loria K, Dvali N. Validation of swab sampling and HPLC methods for determination of Meloxicam residues on pharmaceutical manufacturing equipment surfaces for cleaning validation. *Turk. J. Pharm. Sci.* (2015) 12 (3): 287-298.

[19] *AZIERTA science to business*; *contract scientific support consulting*: *Diclofenac sodium ADE determination strategy LAB, Madrid* (2018). *https://azierta.eu/toxicologia/pde/#1459756928789-0d9f6100-77c9* (Accessed May 27, 2018).

[20] FDA Food and Drug Administration. Center for drug evaluation and research, public health service, pharmacology/toxicology NDA 22-396, Dyloject[®] Diclofenac sodium Intravenous injection, Washington DC (2014) 99-102.

[21] ICH Harmonised Triplicate Guideline. Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients, Guidance for Industry,

International Conference on Harmonisation Q7, Geneva (2016) 34-35.

[22] Klinkenberg R, Streel B, Ceccato A. Development and validation of a liquid chromatographic method for the determination of amlodipine residues on manufacturing equipment surfaces. *J. Pharm. Biomed. Anal.* (2003) 32 (2): 345-352.

[23] *Vidal: Gentamicine Panpharma 80 mg/2ml solution injectable, France.* *https://www.vidal.fr/Medicament/gentamicine_panpharma-7479.htm* (Accessed May 27, 2018).

[24] EMA European Medicine Agency. VICH GL 18 residual solvents in new veterinary medicinal products, active substances and excipients, United Kingdom (2010) 5-7.

[25] WHO World Health Organization. Quality assurance of pharmaceuticals: a compendium of guidelines and related materials, 2nd edition, Good manufacturing practices and inspection, Geneva, (2007) 128.

[26] *Cole-Parmer scientific experts: Effective Swabbing Techniques for Cleaning Validation, United States.* *https://www.coleparmer.com/tech-article/effective-swabbing-techniques* (Accessed May 21, 2018).

[27] Goti P.P, Savsani J.J, Patel P.B. Development and validation of analytical method for estimation of Diclofenac sodium in swab samples. *Int. J. Pharm. Sci. Res.* (2013) 4 (2): 741-744.

[28] Sindhur Nag N, Gouthami B, Madhuri L, Krishnaveni N, Meyyanathan S, Suresh B. Development and validation of a RP-HPLC method for the simultaneous determination of paracetamol and Diclofenac potassium on stainless steel surface of pharmaceutical manufacturing equipments. *JOCPR.* (2012) 4 (3): 1670-1675.

ONLINE SUBMISSION

www.ijps.ir