Faculty of Pharmacy, Nursing and Health Professions

Master Program in Industrial Pharmaceutical Technology

Validation of Cleaning Procedures in the manufacture of different Tablets in Shared Facility (Diclofenac Potassium, Ibuprofen and Olanzapine)

التثبت من فعالية اجراءات التنظيف لماكنات التصنيع المشتركة في انتاج الحبوب للمستحضرات ذات الفعالية المختلفة

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Contents

List of Abbreviations

cGMP: Current Good Manufacturing Practices

FDA: Food and Drug Administration

PIC/S: Pharmaceutical Inspection Convention / Pharmaceutical Inspection Cooperation Scheme

CFR: Code of Federal Regulations

WHO: World Health Organization

SDI: Samih Darwazeh Institute

USP: United States Pharmacopeia

API: Active Pharmaceutical Ingredient

MOC: Material of Construction

MACO: Maximum Allowable Carryover

MAR: Maximum Allowable Residue

ADE: Acceptable Daily Exposure (operator)

OEL: Occupational Exposure Limits in µg of the material per cubic meter

ADI: acceptable daily intake (patient)

LD50: the dose that kill 50% of the animal community

SLS: Sodium Lauryl Sulfate

CIP: Clean In Place

COP: Clean out of place

TOC: Total organic carbon in ppm

TACT: Time, Action, Concentration and Temperature cleaning parameters

OTC: Over-The-Counter (without medical prescription)

SOP: Standard Operating Procedure

NOEL: No observed effect level

BW: Body weight in Kg

SF: Safety factor (Risk minimizing Factor)

MBSs: Minimum Batch Size of the subsequent product (Can be in weight units or in number of doses)

MDDs: Maximum daily dose of the subsequent product (Can be in weight units or in number of doses)

TDD: Therapeutic Daily Dose (in weight or number of doses)

LTDp: Lowest therapeutic daily dose of the previous product (worst case in weight of previous API in a single dose)

IFs: Intake frequency of the subsequent product

MDs: Mass of the dosage form of the subsequent product

HPLC: High performance liquid chromatography

GC: Gas chromatography

UV: Ultraviolet is an electromagnetic radiation with a wavelength from 100 nm to 400 nm, shorter than that of visible light but longer than X-rays.

MIR: mid-infrared spectroscopy

ppm: Parts per million (mg of substance per liter of solvent or mg of substance per kg of total formula)

LOD: Limit of Detection in concentration units

LOQ: Limit of Quantification in concentration units

PLC: Programmable Logic Controller

DEHT: Dirty Equipment Hold Time

CEHT: Cleaned Equipment Hold Time

SST: System Suitability Test

RSD: Relative Standard Deviation

SD: Standard deviation

T_f: Tailing Factor

K': Capacity factor

N: Theoretical plate number

TAMC: Total Aerobic Microbial Count

TAP: Transfer of Analytical Procedures

IPA: Isopropyl Alcohol

RODAC: Replicate Organism Detecting and Counting

Equipment Hot Spots: locations that are considered hard to clean or have complex geometries

Equipment Critical Spots: locations that may disproportionately contribute residue to the next process

TRS: Technical Report Series

AU: Arbitrary units

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Abstract

Diclofenac Potassium 50 mg, Ibuprofen 200 - 600 mg and Olanzapine 2.5 - 20 mg tablets were manufactured in a multi-product facility, where Diclofenac Potassium, Ibuprofen and Olanzapine could be possible cross-contaminants, may alter the safety, identity, strength, quality and purity of the subsequent drug product beyond the established requirements. Validation of cleaning processes provides documented evidence that the approved cleaning procedure will consistently provide clean equipment suitable for subsequent product processing. To achieve that, the worst-case product (using potency, cleanability and solubility criteria), difficult to clean locations of each equipment and the sampling methods (swab or rinse) for each sampling location were determined, in addition the acceptable limits for API residue was calculated, and an analytical method for estimation of the worst-case product was developed and validated. Simulation study using coupons from both sampling procedures and the surface of the equipment was accomplished. Finally, one batch of the worst-case product was manufactured and cleaned using the suggested procedures, and then the worst-case samples were analyzed to verify the effectiveness of the cleaning procedure for removal of product residues and cleaning agents to acceptable limits. Cleaned Equipment hold time (CEHT) was determined to control the potential of microbiological contaminants before equipment reuse or re cleaning.

Olanzapine tablets were the worst-case over the other products, since it had risk in its solubility and in pharmacology or potency. The maximum allowable Olanzapine

residue from the previous product using swab technique must be below 0.2273 ppm/ swab of 5 cm x 5 cm, while the acceptance criteria for Olanzapine residue using rinse technique for Bin Mixer, Tablet Press Punches and Dies and for Coating Pan equipment were 0.45453 ppm, 0.020980 ppm, 0.031817 ppm, 0.62489 ppm, respectively.

The average recovery for swab technique was found to be 76.73%, while it was 102.98% and 102.99% for rinse technique for Bin Mixer and Coating Pan, respectively. According to soak technique for Tablet Press Punches and Dies, the average recoveries were 89.03% and 89.19%, respectively. So depending on WHO _TRS_937 guidelines, the sampling technique is considered good.

Pilot scale Olanzapine Tablets were manufactured on SDI equipment, and cleaned using the suggested cleaning procedure. In addition, CEHT was studied for eleven days, giving good results for microbiological contamination during the period. The analytical results insure with documented evidence that the used cleaning procedure for the equipment, reduce the residues of the worst-case Olanzapine product and cleaning agent (15% SLS) from the equipment contact surface to acceptable limits and leave the equipment safe for manufacturing the subsequent product.

يتم تصنيع العديد من المستحضرات الصيدلانية على ماكينات تصنيع مشتركه في معهد سميح دروزة للأبحاث الصيدلانية في جامعة بيرزيت، وهذا ممكن ان يحدث تلوث متقاطع للمستحضرات اثناء عمليات التصنيع مما قد يؤثر على سلامة وهويه وتركيز وجودة ونقاوة المستحضر اللاحق تصنيعه عن المواصفات المطلوبة وبالتالي يؤثر على سلامة المريض.

التثبت من طريقة التنظيف تقدم دليلا علميا مدعوما بالنتائج بان طريقة التنظيف المعتمدة قادرة على تنظيف الماكينات من بقايا المستحضر السابق للحد الامن والمقبول والمثبت علميا بانه لا يشكل اية خطورة على جودة المستحضر وبالتالي تكون ماكينات التصنيع جاهزة لتصنيع المستحضر اللاحق.

للوصول الى الهدف المطلوب من الدراسة تم اختيار المركب الاصعب في التنظيف (بالاعتماد على الفاعلية والذائبية وصعوبة التنظيف)، والاماكن الاصعب تنظيفها في الماكينات مع تحديد طريقه اختبار نظافة كل منها (اما عن طريق اخذ مسحه او بأخذ كميه من الماء من اخر شطفه). اضافه الى ذلك تم حساب تركيز المادة الفعالة المسموح انتقالها، وتم تطوير طريقه تحليل لفحص المركب الاصعب في التنظيف والتحقق من فعاليتها. في هذه الدراسة تم التحقق من فعالية طريقة اخذ العينات بالإضافة الى سطح الماكينات على كوبون محاكيه لسطح ماكينات التصنيع. في النهاية تم تصنيع المركب الاصعب في التنظيف وبعدها تم تنظيف ماكينات التصنيع حسب طريقه التنظيف المقترحة. العينات الاصعب في التنظيف تم تحليلها للتأكد من فعالية طريقة التنظيف في ازاله رواسب المستحضر ومواد التنظيف الى الحد المسموح. على الجانب الاخر تم دراسة الزمن الاقصى لبقاء الماكينات نظيفة للتحكم باحتمالية التلوث الميكروبيولوجي.

الدراسة العلمية التي قمنا بها اثبتت ان مستحضر حبوب الاولانزابين هو الاصعب في التنظيف مقارنه بباقي المستحضرات، لان لديه صعوبة في الإذابة والفاعلية. الحد الاقصى من رواسب الاولانزابين المسموح انتقاله للمستحضر اللاحق باستخدام طريقة المسحة هو اقل من 0.2273 جزء من مليون لكل مسحه. بينما الحد المسموح انتقاله من رواسب الاولانزابين باستخدام طريقة الشطف لكل من الخلاط وماكينة الكبس (Punches and Dies) وماكنه التلبيس هو 0.45453، 0.02098، 0.031817، 0.62489 جزء من مليون على التوالي.

اثبتت طريقة التحليل ان معدل الاسترجاع الكمي باستخدام المسحة هو 76.73%، بينما معدل الاسترجاع باستخدام الشطف لكل من الخلاط وماكنه التلبيس هو 102.98% و102.99% على التوالي. اما بخصوص التنظيف باستخدام طريقة النقع لماكنة الكبس (Punches and Dies) فان معدل الاسترجاع هو 89.03% و89.19% على التوالي. لذلك بالاعتماد على المبادئ التوجيهية ل WHO _TRS_937، فان طريقة اخذ العينات تعتبر جيدة.

تم تصنيع خلطه تجريبيه من مستحضر الاولانزابين على الماكينات في معهد سميح دروزة، وتم تنظيفها باستخدام طريقة التنظيف المقترحة. بالإضافة الى ذلك تم دراسة الزمن الاقصى لبقاء الماكينات نظيفة لمدة أحد عشرة يوما، وكانت النتيجة هي غياب لجميع الملوثات المكروبيولوجية في هذه الفترة. نتائج الفحص تؤكد بدليل علمي موثوق بان طريق التنظيف قادرة على ازاله رواسب الاولانزابين وهو المركب الاصعب في التنظيف عن أسطح الماكينات بالإضافة لإزاله مسحوق التنظيف المستخدم وهو (SLS 15%) الى الحد المسموح به، بحيث تكون الماكينة امنه لتصنيع المستحضر اللاحق.

Part one: Introduction

1.1 Background Information of Cleaning Validation

Pharmaceutical products are manufactured using special facilities. Most facilities are being used to manufacture different products (common or multi-purpose equipment) and some facilities are specified to the production of a certain drug [1], so there is a potential that the subsequently manufactured products may be contaminated by a variety of substances such as contaminants associated with microbes, previous products (residues of both APIs and excipients, and API degradants), residues of cleaning agents, airborne materials, lubricants and ancillary materials [2,3]. Manufacturing processes have to be designed and carried out in a way that prevent cross contamination as much as possible [4]. That is because cleaning validation is required to comply with regulations. As the Code of Federal Regulations (CFR) states "Equipment and utensils, shall be cleaned, maintained and sanitized at appropriate intervals to prevent malfunctions or contamination that would alter the safety, identity, strength, quality, or purity of the drug product beyond the official or other established requirements" [5]. As indicated by the above FDA statement cleaning validation is necessary to fulfill customers and regulatory expectations as well as to ensure safety, identity, strength, and purity of the products, which are the basic requirements of cGMP (Current Good Manufacturing Practice), and to provide manufacturer with enough confidence that internal controls are established and monitored properly.

The awareness of cleaning validation beginning early in 1980s, Samuel Harder published an article, "The Validation of Cleaning Procedures," in 1984 [6]. Also in

1988 the Food and Drug Administration (FDA) had its first major experience with cross contamination due to a recall of a finished drug product, Cholestyramine Resin USP [6,7]. Then in 1992, FDA instituted an import alert for a foreign bulk pharmaceutical manufacturer who used common equipment [7]. After that, the FDA published in July 1993 "Guide to Inspection of Validation of Cleaning Process" whose increase the attention of cleaning validation [8].

The pharmaceutical inspection convention / Co-operation scheme (PIC/S) defined cleaning validation as "a documented evidence that an approved cleaning procedure will consistently provide equipment which is suitable for processing of pharmaceutical products or active pharmaceutical ingredients (APIs)" [2].

During the manufacturing of a commercial product, it is recommended to perform at least three levels of cleaning as in Table 1. 1 [4,9], however additional levels of cleaning might be necessary depending on the characteristics of the previous and subsequent products such as solubility, nature of residues, process step, recovery studies, etc.

Table 1. 1 Levels of cleaning during the manufacturing of commercial product.

Level	***Thoroughness of cleaning***	***Cleaning verification***		***Cleaning validation***
		Visual inspection	***Analytical verification***	
2	Carryover of the previous product is critical. Cleaning required until predetermined stringent carry over limits met.	Yes	Yes	Mandatory

	High risk			
1	Carryover of the previous product is less critical. Cleaning should reduce the potential carry over to a less stringent limit as required for level 2. Medium risk.	Yes	Yes	Recommended
0	Only gross cleaning if carryover of the previous is not critical. Low risk.	Yes	No	No

The cleaning verification can be done by visual inspection or visual inspection and analytical verification (e.g., direct by swabbing and/or indirect by rinsing). Visual inspection is usually applied in Level 0 where no cleaning validation is required. The analytical verification methods should be validated before use in cleaning validation and/or cleaning verification, unless they are compendial methods.

1.1.1 Lifecycle of Cleaning Validation Process

The Food and Drug Administration's (FDA's) defined process validation in: General Principles and Practices guidance as "the collection and evaluation of data, from the process design stage through commercial production, which establishes scientific evidence that a process is capable of consistently delivering quality product". In addition, it considered the cleaning validation as a special type of process validation

having three different stages: cleaning process design, cleaning process qualification and continued cleaning process verification [9–11] as shown in Figure 1. 1 below.

Figure 1. 1 Lifecycle of cleaning validation process.

1.1.1.1 Lifecycle-1: Cleaning Process Design

This stage aims to design, develop and understand the cleaning process residues and to set the strategy for the cleaning process control. The main activities in this stage are:

1. Evaluation of the chemical and physical properties of the residue
2. Determination of the most difficult to clean residue
3. Evaluation of residue solubility and stability.

1.1.1.2 Lifecycle-2: Cleaning Process Qualification

To prove that the cleaning procedure works as expected. The following activities are included:

1. Qualification of specific equipment used in the cleaning such as Clean In Place (CIP) systems
2. Cleaning operational parameters (e.g. temperature, flow rates, pressure, etc.)
3. Identification of the most difficult cleaning locations.

1.1.1.3 Lifecycle-3: Continued Cleaning Process Verification

To prove that the cleaning process remains in control throughout the product lifecycle. The following should be considered in this stage:

1. Post validation monitoring means that after cleaning validation the analytical verification can be replaced with non-specific test methods like TOC; pH; Conductivity; etc. in addition to the visual inspection.
2. Change control means that any change during the execution of the cleaning validation procedure after finishing the validation should be handling through the change control procedure and the impact on the cleaning validation process should be evaluated.
3. Periodic management review.

1.1.2 Types of Cleaning Methods

Several types of cleaning methods are followed in equipment cleaning such as:

- Manual cleaning methods including soaking, brushing, crumbling, etc.
- Semi-automated cleaning methods such as Clean Out of Place method (COP)
- Automated cleaning methods such as Clean In Place method (CIP)

Manual cleaning means “direct cleaning of equipment by a trained equipment operator using a variety of hand tools and cleaning agents” [12]. The advantage of the manual cleaning methods is that trained operator can report any changes in cleaning conditions. On the other hand these methods have many disadvantages, manual methods are expensive, time consuming, requiring dismantling, highly operator dependent which make their validation difficult and requiring high efforts from an experienced and highly trained staff [13].

Semi-automated cleaning method includes automatic controls. In this method, some parts removed and cleaned manually before automated CIP methods are applied. However, in Clean-Out-Of-Place (COP) method, the disassembled equipment cleaned in a central washing machine [13].

Clean-In-Place (CIP) method: The cleaning of the equipment performed in place without disassembling, and it may be controlled manually or by an automated program. The disadvantage of this method is the difficulty of visual inspection for a closed system.

1.1.3 Cleaning Agent Selection

Proper selection of a suitable cleaning agent and cleaning process parameters could simplify the cleaning validation process [14].

1.1.3.1 Cleaning Mechanisms

The mechanisms of product contaminant removal from the surface are depending on the type of cleaning agent selected. One or more of the following mechanisms can occur and explained:

1.1.3.1.1 Solubilization

This term involves removing the residue by dissolving it in a suitable cleaning agent and / or solvent. This can be achieved by adding surfactant or by doing change in the pH of the solution. Water is the preferred solubilizer that can be used to dissolve inorganic salts, organic residues can be removed by dissolving in organic solvents.

1.1.3.1.2 Wetting

The displacement of one fluid from a solid surface by another fluid. It depends on how well the cleaning solution will wet and penetrate into crevices and cracks which are difficult to clean locations of the equipment surfaces. Wetting can be improved by the addition of surfactants, since it improves penetration of the cleaning solution into cracks, which are usually difficult to clean locations.

1.1.3.1.3 Emulsification

The emulsification term used for breaking up an insoluble liquid residue, such as an oil, into smaller droplets and then suspending those droplets throughout the water. Emulsions are thermodynamically unstable, so agitation should continue until the time to discharge the cleaning solution to the drain to avoid redisposition of the cleaned residue back onto the equipment surface.

Emulsion = Mechanical energy + surfactants / polymers

1.1.3.1.4 Hydrolysis

Is the process of breaking the chemical bonds using acids or bases to make small molecules that are easier to be removed.

1.1.3.1.5 Oxidation

This process used to break down proteins and other organic compounds that are cannot be cleaned by other mechanisms.

1.1.3.1.6 Physical Removal

Is cleaning by some mechanical force. The objective is to remove residues physically.

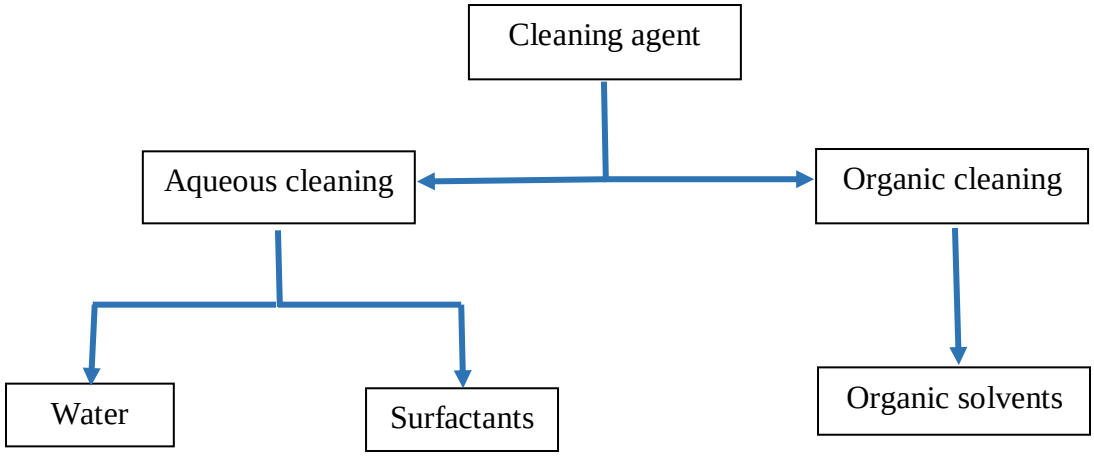


Figure 1. 2 Types of cleaning agents.

Cleaning agents used for cGMP process are three categories: aqueous cleaning, surfactants and organic cleaning [14] as shown in Figure 1. 2.

Organic cleaning like organic solvents class three (acetone, ethanol, ethyl acetate), are usually used in the bulk pharmaceutical manufacturing industry. They are mostly used for solubilization as the cleaning mechanism for residue removal. The advantage of these solvents that they can have simple analytical method and single component used as a cleaning agent. However, the disadvantages to use these solvents are their cost, environmental effect and safety. Therefore, manufacturers prefer aqueous cleaning agents when possible.

Aqueous cleaning like acids (glycolic acid, citric acid) or bases like Sodium Hydroxide (NaOH), potassium Hydroxide (KOH) or oxidants like Sodium Hypochlorite (NaOCl), or Hydrogen Peroxide (H_2O_2) are usually use solvation and hydrolysis or oxidation as cleaning mechanisms. The advantages of these agents are they are available, cheap and

simple since one component used as a cleaning agent. However, the disadvantage of these agents are the insufficient penetration into residue due to low wetting and the precipitation of water hardness.

The aqueous surfactants like sodium lauryl sulfate (SLS), fatty acid salts may provide better wetting, surface action, and emulsification, depending on the chemistry and concentrations used. The disadvantages of using surfactants are that their limited number of sources and their mechanism of action are not always well understood.

1.1.3.2 Cleaning Parameters

The most important parameters that determine cleaning effectiveness are the cleaning time, the action or impingement on the surface, the concentration of the cleaning agent and the temperature of the cleaning solution. These parameters- time, action, concentration and temperature- are closely related, and known by TACT [14] as in Figure 1. 3.

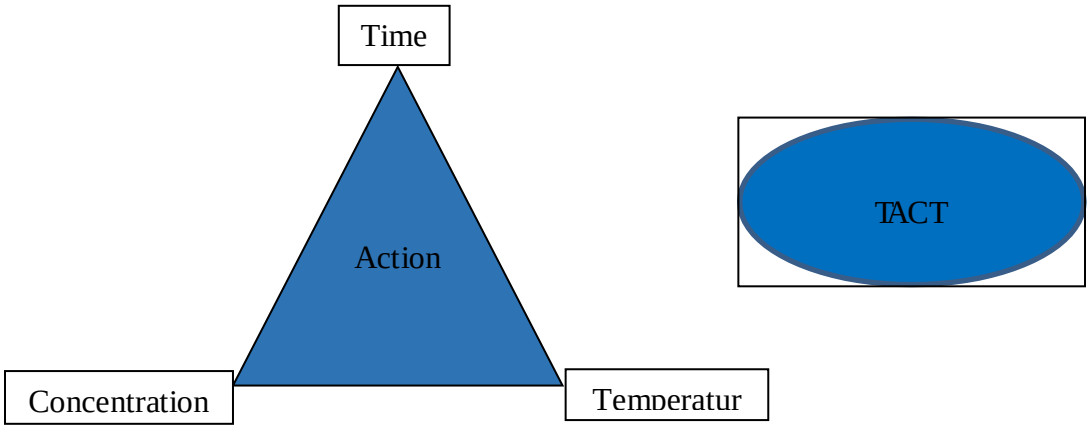


Figure 1. 3 Cleaning effectiveness parameters.

The chemistry and nature of the equipment surface also can affect cleaning effectiveness, it determines the range of adhesion of the residue to the surface as well as the range of wetting and residue removal by the cleaning solution. During the selection of the proper cleaning agent we should take in consideration its toxicity since it should be environmental friendly also it must be analytical detectable to measure its acceptable limit residue [3].

1.1.4 Principles of Grouping (Bracketing)

Similar cleaning procedures for products and processes do not need to be validated individually as stated in the PIC/S validation document [2]. Bracketing can be used by making groups and sub groups in order to select the worst-case [9]. The concept of grouping or bracketing accepted only if it mentioned in the company's cleaning validation master plan or cleaning validation policy and in grouping SOP [9,15]. In grouping, we have three criteria to consider; product grouping, equipment grouping and identical cleaning process. The first criteria product grouping, it is used when there are similar products (products that have same dosage form / drug delivery or same processing method) manufactured on the same equipment train using similar cleaning processes [3,15]. In this case, the validation protocol should include all types of equipment necessary for the production of every product. The disadvantage of this criteria is when changes occur to a specific type of production equipment and/or its

cleaning procedure, then all product listed in the product validation protocol must be checked and revalidated.

The second criteria equipment grouping, it is used when there are various products manufactured in the same equipment or equipment train [3], having the same material of construction, same design risks and the same worst-case positions (sampling sites). The validation protocol in equipment grouping should include all critical products manufactured on this equipment.

The third criteria is identical cleaning process, which means that all products in the group be cleaned with the same cleaning process, having the same cleaning agent and the same cleaning parameters TACT (Time, Action, Concentration and Temperature), in order to select the worst-case product [15].

1.1.5 Selecting the Worst-Case

After determined the product groups, the next step is to determine the worst-case for each group. Below listed the scientific basis for selecting the worst-case [9]:

1.1.5.1 Cleanability

Cleanability (from the historical production data) difficult to clean or high risk to clean because of issues due to the nature of product (other than potency, toxicity and solubility). Examples: Coated tablets, extended release products.

Table 1. 2 Cleanability factor for selecting the worst case.

Cleanability Factor	***Description***	***Example***
1	Easiest to clean	V. Soluble tablets; products doesn't stick to surfaces
2	Average cleaning time/effort	Uncoated tablets, capsules
3	More difficult to clean	Coated tablets
4	Very difficult to clean	Insoluble actives in ointments/ creams
5	Most difficult to clean	Dyes that stain equipment, strong odors

1.1.5.2 Solubility Data (in the Selected Cleaning Solvent)

Solubility of active in water/solvent/cleaning agent used to clean equipment. Example:

Table 1. 3 Solubility factor for selecting the worst case.

Solubility (from USP)	***Solubility Factor***
Very soluble	1
Freely Soluble	2
Soluble	3
Sparingly Soluble	4
Slightly Soluble	5
V. Slightly Soluble	6
Practically Insoluble	7

1.1.5.3 Potency (ADE, ADI, OEL)

Potency can be defined based on the ADE: acceptable daily exposure (operator), ADI: acceptable daily intake (patient), OEL: occupational exposure level or it can be defined based on normal daily dose of the product, whatever data is available.

Table 1. 4 Potency factor based on acceptable daily exposure (ADE) for selecting the worst case.

Acceptable Daily Exposure (ADE)	***Potency Factor***
< 1 μg	5
1 -9 μg	4
10- 99 μg	3
100 – 500 μg	2
> 500 μg	1

Table 1. 5 Potency factor based on normal daily does for selecting the worst case.

Normal Daily Dose	***Potency Factor***
< 5 mg	5
5 -199 mg	4
200 - 400 mg	3
400 - 600 mg	2
600 - 800 mg	1

1.1.5.4 Lowest Therapeutic Dose (or Toxicity Data LD50)

Therapeutic dose data are usually for oral and/or parenteral dosage form. In the cases where the therapeutic doses data are not available, the toxicity data (LD50: the dose that kill 50% of the animal community) can be used [9].

Table 1. 6 Toxicity factor for selecting the worst case.

Product Grouping	***Toxicity Factor***
Prescription Products	3
OTC	2
Dietary Supplements	1

1.1.6 Acceptance Criteria

The acceptable limits of potential carryover residue for a piece of equipment must be calculated during cleaning validation. The Maximum Allowable Carryover (MACO) can be calculated based on different methods:

1.1.6.1 Acceptance Criteria Using Health-Based Data

The Maximum Allowable Carryover (MACO) can be calculated based on the Acceptable Daily Exposure (ADE) whenever this data is available [9]. Where the ADE can be calculated according to equation No: (1) and the result used for the calculation of the MACO as in equation No: (2).

$$\text{ADE [mg]} = \frac{\text{NOEL [mg/kg]} \times \text{BW [kg]}}{\text{SF}} \quad (1)$$

$$\text{MACO [mg]} = \frac{\text{ADE [mg]} \times \text{MBSs [g]}}{\text{MDDs [g]}} \quad (2)$$

Where;

NOEL: No observed effect level = LD50 [mg/kg] / 2000

BW: Body weight in Kg

SF: Safety factor (Risk minimizing factor)

MBS_s: Minimum Batch Size of the subsequent product

MDD_s: Maximum daily dose of the subsequent product

1.1.6.2 Acceptance Criteria Based on Therapeutic Daily Dose

The Maximum Allowable Carryover (MACO) for active pharmaceutical ingredient (API) can be calculated based on the toxicological data and the Therapeutic Daily Dose (TDD). To determine the acceptance limits for cleaning validation, there are three basic approaches [2,16] that suggested by Fourman and Mullen. Which then had adopted by "PDA 1998 TRS 29 Guideline, PIC/S 2007 Guideline (European), CEFIC/APIC 2000 Guideline (European), TPP 2008 CV Guidelines (Health Canada), and WHO Guide to GMPs TRS 937 2006" [16]. While the FDA guidelines does not determine an acceptance limits or methods for cleaning validation, since there is a wide variation in equipment and products used in industries, so simply they consider the ppm criterion,

dose criterion and visual criterion as alternative possibilities [17]. Approach 1 is based on dose criterion, where "no more than 0.001 of normal therapeutic daily dose of any product will appear in the maximum daily dose of the subsequent product" [2]. This dose criterion can be calculated using the mathematical equation No: (3) [9].

$$\text{MACO [mg]} = \frac{(\frac{1}{\text{SF}}) \times \text{LTDp [mg]} \times \text{MBSs [mg]}}{\text{IFs} \times \text{MDs [mg]}} \quad (3)$$

Where;

LTD_p: Lowest therapeutic daily dose of the previous product (worst-case)

MBS_s: Minimum Batch size of the subsequent product

IF_s: Intake frequency of the subsequent product

MD_s: Mass of the dosage form of the subsequent product

SF: Safety factor

MDD: Maximum therapeutic daily dose of the subsequent product = IF_s * MD_s [mg]

Approach 2 is based on 10 ppm criterion, where" no more than 10 ppm of any product will appear in another product" [2]. This 10-ppm criterion can be calculated using the mathematical equation No: (4) [9].

$$\text{MACO [mg]} = 10 \text{ ppm [mg/kg]} \times \text{MBSs [kg]} \quad (4)$$

Approach 3 is visually clean criterion, where "no quantity of residue should be visible on the equipment after cleaning procedures are performed. Spiking studies should determine the concentration at which most active ingredients are visible" [2]. The

visual criteria would always represent the strictest acceptance criterion if the limit in the subsequent product calculated according to ppm criterion or dose criteria leads to a surface concentration of more than 100 μg/25 cm^2 → 4 μg/25 cm^2 as acceptable residue in the sample. Where this visual limit of detection for most active pharmaceutical ingredients specified by Fourman and Mullen.

The safety factor (SF) is a measure of degree of risk for a particular situation. It is applied during calculation to ensure that the level of product carryover is low enough not to have pharmacological effect. The SF value depends on the route of administration, for topical application range 10 – 100, where for oral application 100 – 1000 and finally for parenteral application the SF range 1000 – 10000 [12].

1.1.7 Sampling Techniques

Sampling techniques are used to sample the equipment worst-case locations after cleaning. There are two types of sampling techniques for cleaning validation, the swab sampling (Direct Surface Sampling) and rinse sampling (Indirect Sampling). A combination of the two techniques is generally the most likable. Where FDA prefers swab sampling to rinse sampling [6].

1.1.7.1 Swab Sampling (Direct Surface Sampling)

The presence of residues on a cleaned and dried equipment are tested by physical removal using this method [12,13]. A swab saturated with a solvent (e.g., water,

alcohol), to increase the solubility of the residue or it may be used dry, to swab the pre-determined worst-case equipment surface of 5 cm x 5 cm firmly and evenly in a back-and-forth motion. The swab then extracted into known volume of solvent and examined using a suitable analytical method [9,13]. One of the advantages of this method is the physical removal of the dried out or insoluble residues. This method is suitable for API, microbiological and cleaning agent residues [13]. The disadvantages of this technique; that the swab used may release fibers, and the results are technique dependent, also the large, complex and hard to reach areas are difficult to be evaluated using this sampling technique [13].

1.1.7.2 Rinse Sampling (Indirect Sampling)

In cases where swab sampling is not possible, the rinse sampling is used to determine the amount of residue remaining on the equipment contact surfaces after cleaning [9]. This method can be evaluated by analytically examining the last rinse solvent collected (generally water). The volume of the collected rise samples should be considered in order to calculate the amount of remaining residues [13]. The disadvantages of this method are that the contaminant not physically removed, and the locations of residues cannot be determined since it is not homogeneously distributed. In addition, one of the disadvantages of this method is the danger of rinsing with organic solvents for water insoluble materials [13].

1.1.8 Analytical Test Methods

The samples taken by the sampling methods discussed in section 1.1.7 Sampling Techniques above should be analyzed, using an appropriate analytical test method (e.g. HPLC, GC, TOC, UV, conductivity or pH), to evaluate the cleanliness of the equipment. The selected analytical method should be sensitive to detect the determined residue limits [9,12]. There are two types of analytical methods: specific analytical methods and non-specific Analytical methods.

1.1.8.1 Specific Analytical Methods

Specific Analytical methods are methods that can quantitate an anticipated residue in presence of other residues such as GC, HPLC, NIR, UV / Visible etc. They are used to separate and selectively detect analytes since they are highly specific, highly sensitive, and quantitative. The disadvantages of these methods are their cost and time consuming [13].

1.1.8.2 Non-Specific Analytical Methods

Non-specific Analytical methods can detect a variety of residues in cleaning validation work, they are used to detect residues of cleaning agents during and after the cleaning process, also it is used to monitor the cleaning effectiveness by correlating the results from a specific method to the results from other non-specific methods [12]. An example of these methods are TOC, pH, Conductivity, Titration etc. These tests are moderate in

cost and much faster than specific tests. The disadvantage of these methods that they can detect more than one residue (not specific).

1.1.9 Analytical Test Method Validation [18–22]

The analytical methods used for testing cleaning samples must be validated according to [ICH Q2 (R1)] [18] for:

1.1.9.1 Specificity (Placebo Interference)

The ICH documents define specificity as “the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically, these might include impurities, degradants, matrix, etc.” This test to prove that the results obtained are not confounded or affected by the presence of other active drugs, degradants or placebo.

1.1.9.2 Limit of Detection (LOD)

ICH defines the detection limit of an individual analytical procedure as “the lowest amount of analyte in a sample which can be detected but not necessarily be quantitated as an exact value”.

1.1.9.3 Limit of Quantification (LOQ)

ICH defines the limit of quantitation (LOQ) of an individual analytical procedure as "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, and is used particularly for the determination of impurities and/or degradation products".

1.1.9.4 Linearity

ICH defines linearity of an analytical procedure as "its ability (within a given range) to obtain test results that are directly proportional to the concentration (amount) of analyte in the sample".

1.1.9.5 Range

ICH defines the range of an analytical procedure as "the interval from the upper to the 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".

1.1.9.6 Accuracy

ICH defines the accuracy of an analytical procedure as “the closeness of agreement between the conventional true value or an accepted reference value and the value found”.

1.1.9.7 Precision

The ICH documents define precision of an analytical procedure as “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 three levels: repeatability, intermediate precision and reproducibility. Where repeatability expresses the precision under the same operating conditions over a short interval of time. It is also termed intra-assay precision. Intermediate precision expresses variations within the same laboratory, such as different days, different analysts, different equipment, and so forth. And reproducibility expresses the precision between laboratories”.

1.1.9.8 Ruggedness

Ruggedness not addressed in the ICH documents. Its definition has replaced by Intermediate precision, which has the same meaning. Ruggedness is defined by the USP <1225> as “expresses variations within-laboratory variation, as on different days, or with different analysts or equipment within the same laboratory” [20].

1.1.9.9 Robustness

ICH defines the robustness of an analytical procedure as “a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters. It provides an indication of the procedure’s reliability during normal usage”.

1.1.9.10 System Suitability

System Suitability Test (SST) is one of the most important parts of HPLC method development and calibration [23]. The system suitability test is carried out to evaluate the entire chromatographic system suitability and efficacy before and during the analysis, since its performance may be changed during their regular usages, and that can affect the accuracy of the HPLC analytical results. For these reasons different parameters can be monitored to insure that the whole HPLC system is accurate and precise. The most important SST parameters are the Capacity factor (k'), Resolution (R), Relative retention (α), Precision/injection repeatability (RSD— relative standard deviations—of peak response and retention time), Theoretical plate number (N), and Tailing factor (T) [23,24].

1.1.10 Determination of Recovery

As in the PIC/S PI006 guideline, the recovery study is performed to determine the ability of the sampling technique to quantitatively remove the contaminant from the surface sampled [2]. This can be achieved by spiking a surface equivalent to the equipment surface (coupon) with different known concentrations of the API or impurity. The impurity can then be recovered and analyzed using the same sampling and analytical methods that will be used for the cleaning validation study [9,13]. Then the percentage Recovery calculated by divided the quantity found, over the actual quantity of product spiked multiplied by 100 as in equation No: (5).

$$\% \text{ Recovery } = \frac{\text{Quantity found}}{\text{Actual quantity of product spiked}} \text{ X } 100 \qquad (5)$$

According to WHO _TRS_937 guidelines [25], if the result of recovery factor is more than 80%, then the sampling technique is considered good, and if it is more than 50% then the technique considered reasonable, but if the recovery is less than 50%, then the sampling technique is questionable. The recovery factor must be taken into consideration while calculating the acceptable limit for residue [13].

1.1.11 Cleaning Hold Time / Dirty Hold Time

The concepts of "clean-hold time" and "dirty-hold time" are parts of cleaning validation. Cleaned Equipment Hold Time (CEHT) is “the time between end of cleaning and equipment reuse, prior to additional cleaning” [9], where Dirty Equipment Hold Time (DEHT) is “the time between the end of manufacturing and the beginning of the cleaning process” [26]. This is to study the stabilization period for hold time for equipment during storage, to avoid the potential formation of degradation products or microbiological contamination that are difficult to be cleaned by the standard cleaning procedure.

It is necessary to perform a risk assessment study if the dirty validated hold time exceeds and even it may also be necessary to evaluate the product or microbiological contamination of equipment following cleaning. If the clean hold-time exceeds, equipment should be cleaned again prior to use and verified as clean [4,26].

1.1.12 Microbiological Evaluation

Cleaning validation concerned not only in removing the ingredients of previous products and detergent residues used, but it is also concerned in reducing the microbiological contaminants to acceptable safe limits for manufacturing the subsequent product.

The cleaning validation techniques for microbiological evaluation include swab method, surface rinse method, contact plate method [4]. Swab method is useful for

checking the cleanliness of curved pieces of equipment, pipes and valves. Contact plates (or RODAC plates) method is suitable for checking flat surfaces and it accurately evaluates the microbiological status in site. Finally rinse method is suitable for irregular surfaces, particularly when the other two methods are difficult to be used.

1.2 Background Information of Three Products Manufactured in a Multi-Product Facility

Diclofenac Potassium, Ibuprofen and Olanzapine being donated by Jerusalem Pharmaceuticals Co. Ltd. and the certificate of analysis for raw materials were attached in Appendix I.

1.2.1 Diclofenac Potassium 50 mg Tablets

Table 1. 7 Information of Diclofenac Potassium 50 mg Tablets.

Information about:	***Diclofenac Potassium 50 mg Tablets***
API	Diclofenac Potassium
Pharmacological effect	Indicated for the acute treatment of migraine attacks with or without aura in adults (18 years of age or older).
Dosage form/Route	Tablet; oral
Strength	50 mg
Daily dose	100 - 150 mg in two or three divided doses
Solubility	Freely Soluble in 96% ethanol and methanol Sparingly soluble in water
Permeability	high permeability through the intestinal membrane
Appearance	White or slightly yellowish, slightly hygroscopic, crystalline powder.

1.2.2 Ibuprofen 200 - 600 mg Tablets

Table 1. 8 Information of Ibuprofen 200 - 600 mg Tablets.

Information about:	***Ibuprofen Tablets***
API	Ibuprofen
Pharmacological effect	The relief of symptoms of pain, inflammation and fever
Dosage form/Route	Tablet; oral
Strength	200 mg, 400 mg, 600 mg
Daily dose	800 - 3,200 mg/day*
Solubility	Practically insoluble in water
Permeability	Rapidly absorbed from the upper GI tract
Appearance	White or almost white, crystalline powder or colorless crystals

*: Ibuprofen became available without prescription for the treatment of acute minor pain in the UK in 1983 and in the United States in 1984; the licensed dose was 1200 - 1600 mg/day. The maximum daily prescription dose of ibuprofen for adults is 3200 mg [27,28].

1.2.3 Olanzapine 2.5 - 20 mg Tablets

Table 1. 9 Information of Olanzapine 2.5 - 20 mg Tablets.

Information about:	***Olanzapine Tablets***
API	Olanzapine
Pharmacological effect	Treatment of schizophrenia
Dosage form/Route	Tablet; oral
Strength	2.5 mg, 5 mg, 7.5 mg, 10 mg, 15 mg, 20 mg Tablets
Daily dose	20 mg/day
Solubility	Practically insoluble in water
Permeability	High permeability

1.2.3.1 Olanzapine Solubility Test

Solubility of 1 gm Olanzapine in various solutions according to USP solubility in "General Notices and Requirements" [29].

Table 1. 10 Solubility of Olanzapine determined in various solutions

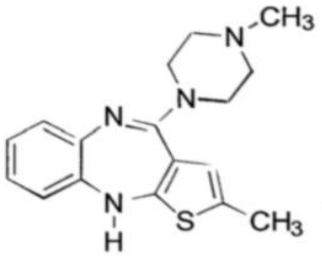
Solvent	***Volume of solvent (ml)***	***Descriptive term***
Acetonitrile	80	Sparingly soluble
Ethanol	140	Slightly soluble
0.1N HCl	80	Sparingly soluble
0.1N NaOH	More than 15,000	Practically insoluble
Isopropyl alcohol (IPA)	80	Sparingly soluble
Acetone	30	Soluble
1% SLS	410	Slightly soluble

1.2.3.2 Olanzapine Physical and Chemical Properties

The source of the following information is FDA [30].

Table 1. 11 Olanzapine physical and chemical properties.

IUPAC Name	2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno[3,2-b][1,5]benzodiazepine
Brand Name	Zyprexa
M. Formula	$C_{17}H_{20}N_4S$
M. Weight	312.44 g/mol
Chemical Structure	

	
Color	Yellow micronized powder
Olanzapine	weak base
Melting Point	195 °C
Dissociation Constants	pKa1 = 4.01; pKa2 = 7.24; pKa3 = 14.17

To have ionized analyte for basic Olanzapine, pH should be less than pKa by 2. So if pH = 2, then no analyte retained in the column since it is highly ionized. At pH = 12, it will damage or deteriorate the column since it is very basic.

1.2.3.3 Olanzapine 2.5 mg Formula

Table 1. 12 Olanzapine 2.5 mg Formula.

Component	***Function***	***Quantity per tablet (mg)***	***Quantity per Pilot Batch 35K tablets***	***% (w/w)***
Olanzapine 2.5 mg	API	2.5	87.5 g	2.38 %
Microcrystalline Cellulose pH-102	Filler	12.725	445.4 g	12.12 %
Spray-dried Lactose	Filler	79.25	2773.8 g	75.48 %

monohydrate				
Hydroxy Propyl Cellulose LH 11	Disintegrant	7.5	262.5 g	7.14 %
Magnesium Stearate	Lubricant	0.525	18.4 g	0.5 %
Opadry II*	coating agents	2.5	87.5 g	2.38 %
Total Weight		105.0 mg	3.6751 Kg	100 %

* : Opadry II White 85F28751 containing: 40.0% (w/w) Polyvinyl alcohol (E1203), 25.0% (w/w) Titanium dioxide (E171), 20.2% (w/w) Macrogol 3000 (E1521), 14.8% (w/w) Talc (E553b).

1.2.3.4 Manufacturing Procedure for Olanzapine Tablets

1. Sieve the following components on mesh No. (40) separately.
    - Olanzapine
    - Microcrystalline Cellulose (Avicel) PH-102
    - Lactose SD
    - Hydroxy Propyl Cellulose LH 11
2. Mix Olanzapine component with adequate amount of Avicel PH-102 in suitable polyethylene (PE) bag. Then mix the produced amount with another sufficient amount of Avicel PH-102.
3. Put the components in step No. 1 and No. 2 in the Bin Mixer, then mix for 15 minutes at 13 rpm.
4. Sieve the Magnesium Stearate components on mesh No. (100).

5. Add the sieved Magnesium Stearate to the Bin Mixer and mix for 5 minutes.
6. Set the Tablet Press Machine parameters as in Figure 1. 4 below to give tablets with weight: 102.5 mg ± 10% and hardness: 6 – 14 kg/cm^2. Where the mean pressure is equal 0.4 KN.

Figure 1. 4 Press machine parameters.

7. Press all Olanzapine powder and monitor the weight and hardness of the tablets every 15 minutes.
8. Make sure that the average weight of the compressed tablets is 102.5 mg ± 10%.
9. Prepare 15% Opadry II suspension before 45 minutes of use, by suspending 87.5 g of Opadry II in 495.5 ml of purified water with temperature not more than 30°C, then mix using Silverson Homogenizer for 45 minutes.
10. Make sure that the coating solution is homogeneous.
11. Put the Olanzapine tablets inside the Coating Pan and heat them up to 55°C.
12. Set the pan speed at 18.0 rpm and the spray speed at 15.0 ml/min.
13. After tablet coat the average tablet weight = 105.0 mg/tab ± 10%.

14. Switch off the heating system and allow coated tablets to cool to room temperature, then tablets transferred to PE bag and preserved in tight, light-resistant containers, and stored at controlled room temperature.

Part Two: Problems and Objectives

2.1 Research Problem

Production of tablets with Diclofenac Potassium 50 mg, Ibuprofen 200 - 600 mg and Olanzapine 2.5 - 20 mg in a multi-product facility, where Diclofenac Potassium, Ibuprofen and Olanzapine could be possible cross-contaminants, may alter the safety, identity, strength, quality and purity of the subsequent drug product beyond the official or other established requirements.

Adequate cleaning procedures play an important role in preventing contamination and cross-contamination as much as possible.

Validation of cleaning methods provides documented evidence with a high degree of assurance that the approved cleaning procedure will consistently provide cleaned equipment suitable for subsequent product processing.

2.2 Objective of the Thesis

The present study aims to provide documented evidence with a high degree of assurance that the cleaning procedures for the equipment used in production of the three target products will consistently reduce the residues of the previous product from the equipment contact surface to acceptable limits and leave the equipment safe for manufacturing the subsequent product.

The validation study steps of the cleaning procedures from the residues of Diclofenac Potassium, Ibuprofen and Olanzapine in multi-product facility manufactured on the same tablet production line are summarized in the following:

1. Determination of worst-case products (using potency, cleanability and solubility criteria).
2. Calculate the shared surface area of the equipment train.
3. Determine the difficult to clean locations of each equipment (hot and critical spots).
4. Determine the suitable sampling method (swab or rinse) for each sampling location.
5. Determine the type of residue to be tested for each sample with the rationale (API residue, cleaning agent residue or microbiological cleanliness status) depending on the sampled site.
6. Determine the acceptable limits for API residue using both sampling procedures (swab / rinse).
7. Development and validation of analytical method for estimation of the worst-case product.
8. Recommend cleaning solution and cleaning procedure.
9. Determine the analytical method for the cleaning agent residues (nonspecific method).
10. Study the recovery from both sampling procedures and the surface of the equipment (simulation study using coupons).

11. Manufacture of one batch of the worst-case product, cleaning using the validated procedures, take the worst-case samples as specified in the study, and analyze to determine the effectiveness of the cleaning procedures.
12. Determine cleaned Equipment hold time (CEHT).

Part Three: Cleaning Validation Protocol

Prepared By: Title and Date:	Reference:
Reviewed By: Title and Date:	
Reviewed By: Title and Date:	
Approved By: Title and Date:	

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3.1 Introduction

Pharmaceutical products are manufactured using special facilities. Most facilities are being used to manufacture different products (common or multi-purpose equipment), and some facilities are specified to the production of a certain drug. Consequently, they may be contaminated by a variety of substances such as contaminants associated with microorganisms, previous products (residues of both APIs and excipients), residues of cleaning agents, airborne materials, lubricants and ancillary materials. Manufacturing processes have to be designed and carried out in a way that prevent cross contamination as much as possible. Not only because cleaning validation is required to comply with regulations, but also it is necessary to fulfill customers' expectations as well as to ensure safety, identity, strength, and purity of the products which are the basic requirements of cGMP (Current Good Manufacturing Practices), and to provide manufacturer with enough confidence that internal control is established properly.

3.2 Purpose

To validate the cleaning procedures for equipment used for manufacturing of different products in shared facility, ensuring that Equipment cleaning procedures are effective to remove the potential residues of ingredients of previous products and detergents used and also to reduce the microbiological contamination to acceptable safe limits and leave the Equipment ready for manufacturing the subsequent product.

3.3 Objectives

- The objective of the cleaning validation is to verify the effectiveness of the cleaning procedure for removal of product residues and cleaning agents to acceptable limits as well as for the control of potential microbiological contamination.
- To establish a documented evidence of the cleaning effectiveness of the cleaning procedure to a predetermined residue level.
- To verify that the cleaning procedure is able to clean the defined Equipment consistently and reliably.

3.4 Responsibilities

3.4.1 Pilot Plant Responsible

- To ensure that the various pieces of equipment cleaned according to the relevant SOPs. (SOP No. QA121 with the following attachments):
- Cleaning Parts for Tablet Press Machine FMQA115
- Cleaning Parts for Coating machine FMQA118
- Cleaning Parts for Bin Mixer FMQA119
- Machine Log Book FMQA121
- To train and monitor the operator for collecting samples by final rinse/swab from the worst-case locations in the equipment.
- Responsible for checking of the protocol and final report.

3.4.2 Responsibility of Q.C Department

- Responsible for validating analytical methods used to analyze residues of active ingredients of the previous product and residues of cleaning agents used to acceptable predetermined levels.
- To test samples according to the validated analytical method.
- Responsible for reviewing of the protocol and final report.

3.4.3 Responsibility of Microbiology Department

- Responsible for testing the samples taken after cleaning for their viable content.

3.4.4 Responsibility of Q.A Department

- To supervise the operation to ensure that everything is executed according to the protocol
- Responsible for approving of the protocol and final report
- Responsible for monitoring the effectiveness of the cleaning process during product life cycle.

3.4.5 Responsibility of Engineering

- Responsible for supporting production, QC and QA personnel during cleaning validation.

3.5 Review of Cleaning Documents

3.5.1 Equipment to be Cleaned

This protocol will address the cleaning of the following equipment that have direct contact with products.

Table 3. 1 Equipment to be cleaned information.

Equipment	***Cleaning SOP***	***Criticality rating***	***Rationale***	***Difficult to clean locations***
Bin Mixer	FMQA119	Critical	Direct contact with the product	See section 3.7
Tablet Press	FMQA115	Critical	Direct contact with the product	See section 3.7
Coating Pan	FMQA118	Critical	Direct contact with the product	See section 3.7

Table 3. 2 Equipment to be cleaned surface area.

Equipment	***Surface Area [cm^2]***	***Surface area of sample (for swab) [cm^2]***
Bin Mixer	5,000	25
Tablet Press	3,600	25
Coating Pan	34,370	25
Total surface Area	**42,970 cm^2**	

3.5.2 Difficult to Clean Locations

Determine each equipment hot spots and critical spots for sampling locations such as:

- Beneath the mixing blades
- Dead spots in the tank
- Dead legs

3.5.3 Cleaning Procedure and Cleaning Equipment

3.5.3.1 Manual Cleaning Process or COP

The cleaning procedure SOP No. QA121 provides details of the procedure, equipment and material is required in order to conduct manual cleaning or clean out of place (COP) of the solid manufacture process equipment.

3.5.3.2 Automated Cleaning or CIP

The cleaning procedure SOP No. QA121 provides details of the procedure, equipment and materials required in order to conduct automated (CIP) cleaning of the solid manufacture process equipment.

3.5.4 Operator Training

Operator performing the cleaning program should be properly trained and assessed before, during and after they perform cleaning process. The training records and assessment should be preserved.

3.5.5 Holding Times

Clean Hold Time was approved to be 7 Days

3.5.6 Selection of Worst-Case Product

The 'Worst-Case' product has been determined by several factors including strength, toxicity, excipients and solubility.

Table 3. 3 Bracketing of products according to risk groups.

Product	***Solubility risk (Solubility Factor)***	***Pharmacology risk (Potency Factor)***	***Formulation risk (Cleanability Factor)***	***Risk Product***
Diclofenac Potassium	4	4	3	
Ibuprofen	7	1	3	
Olanzapine	7	4	3	Worst-Case

From the data in Table 3. 3, **Olanzapine** tablets is the worst-case over the other two products, since it possess risk groups in its solubility and in its therapeutic dose.

3.5.7 Cleaning Limits Selection Criteria for API Based on MACO Approach

$$MAR\ [mg] = \frac{(\frac{1}{1000})\ \times\ \text{LTDp [mg]}\ \times\ \text{MBSs [mg]}}{\text{IFs}\ \times\ \text{MDs [mg]}} \quad (6)$$

LTD_p: Lowest therapeutic dose of the previous product (worst-case) in mg

MBS_s: Minimum Batch size of the subsequent product in mg

IF_s: Intake frequency of the subsequent product

MD_s: Mass of the dosage form of the subsequent product in mg

Maximum daily dose of the subsequent product (MDD) = IF_s * MD_s [mg]

The worst-case product is Olanzapine tablets. Its MACO is **0.2273 ppm/swab.**

3.6 Sampling Procedure

- Cleaning will be carried out by production personnel after the manufacturing of drug product is complete. Each equipment has its own cleaning SOP that should be followed.
- The production supervisor inspects the equipment visually to assure that it is clean, and document the cleaning on the Equipment log.

3.6.1 Swab Sampling Procedure for Determining the Active Residue

- Remove a polyester large Alpha TX715 swab from its protective bag using a clean latex hand glove.
- Avoid touching the swab head to prevent its contamination.

- The polyester large Alpha TX715 swab was wetted with 0.5 ml acetonitrile solvent.
- Take a sterile swab to sampling point.
- Mark the swab vial with sampling point and date on outer cover.
- Swab the tested coupon surface of 5 cm * 5 cm firmly and evenly in a back-and-forth motion (three stroke backward and three strokes forward). Swab horizontally with one side of the swab and swab vertically with the other side of swab.
- Hold the stem of the swab without touching the head of the swab and let it drop into 10 ml vial then cut of the handle of the swab into the vial.
- Pipette out 10 ml of diluent into10 ml vial to extract the drug residue by sonication for 10 minutes.
- Filter the extracted sample.
- Analyze the sample by HPLC.
- Blank of coupon background was prepared at the same time the experimental sample taken by distributed evenly 100 µl of diluent directly over 5 cm * 5 cm coupon surface area. After drying the polyester large Alpha TX715 swab was wetted with 0.5 ml acetonitrile solvent. Then swab the tested coupon surface the same way for the samples.

3.6.2 Rinse Sampling Procedure for Determining the Active Residue and the Cleaning Agent Residue

- Rinse the clean equipment with purified water as in Table 3. 4.

Table 3. 4 Required rinse volume for each equipment.

Equipment	***Surface Area [cm^2]***	***Rinse volume – Water (liters)***
Bin Mixer	5,000	1
Soaking of upper and lower three Punches in Tablet Press	2.3079	0.01
Three Dies in Tablet Press	35	0.1
Coating Pan	34,370	5

- Collect approx. 200 ml from the final rinse into a clean pyrex bottle.
- Filter the rinse sample in HPLC vial, and then analyze them to determine the active residue.
- Then the Conductivity was measured for pure water as a standard reference and for the final rinse water to determine cleaning agent residue.

3.6.3 Procedure for Determining the Microbiological Contaminants

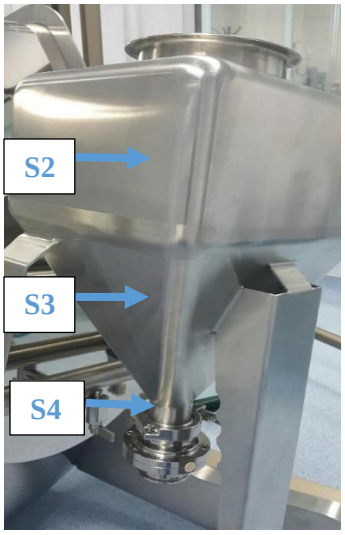
Contact Plate Method

- Prepare contact plate by pour enough amount of Plate Count Agar from OXOID in sterile empty contact Petri dishes to determine total aerobic count, and another contact plate was prepared by pour Yeast Extract Chloramphenicol Agar from Himedia to determine yeast and mold counts.
- Open the cover of the contact plate.
- Pressed on to the area to be sampled for approximately 5-10 seconds and immediately sealed.
- Disinfect the sampled area with 70% Ethanol in order to prevent microbial growth as a result of residual media on the surface.
- Then the samples are incubated for 2 days at 30° - 35°C to detect total aerobic microbial count (TAMC), followed by an additional incubation of 3 - 5 days at 20° - 25°C to detect yeast and mold counts.
- Apply this method immediately after cleaning (zero time), then after 2-days, 4-days, 7-days and 10-days to study the stabilization period for clean hold time for equipment during storage.

3.7 Sampling Locations

3.7.1 Swab and Rinse Sampling Locations for Bin Mixer

Table 3. 5 Swab and rinse sampling locations for Bin Mixer.

Swab #	***Swab Location (5 cm x 5 cm)***	***Detection for***	***Justification***	***Location photo***
S1	Door inlet surface	API & Micro	More contact surface	
S2 S3 S4	Internal surfaces	API	More contact surfaces	

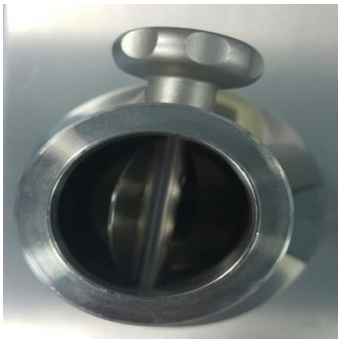
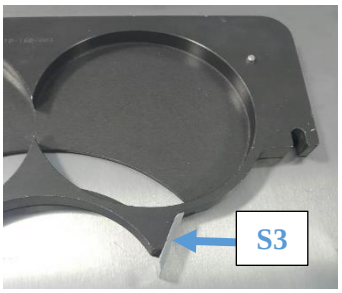
S5	External surface	API	More contact surface	
S6	Outlet door	API & Micro	Difficult to clean area	

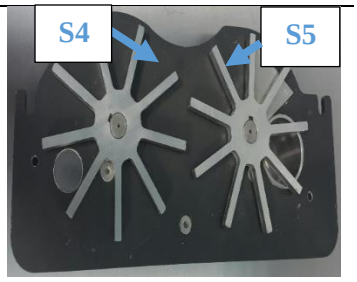
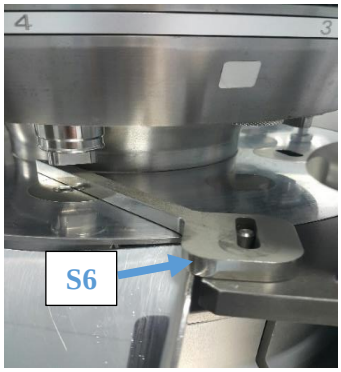
Rinse #	***Rinse location***	***Detection for***	***Justification***	***Rinse volume***
R1	Drain line from bulk tank	API & C. A.*	To ensure no residue	1000 ml of rinse purified water

*: For cleaning agent

3.7.2 Swab and Soak Sampling Locations for Tablet Press

Table 3. 6 Swab and rinse sampling locations for Tablet Press.

Swab #	***Swab Location (5 cm x 5 cm)***	***Detection for***	***Justification***	***Location photo***
S1	Internal surface of hopper	API	More contact surface	
S2	Hopper door	API	More contact surface	
S3	Feeding scraper	API	More contact surface	
S4	Internal surface of force feeder	API	More contact surface	

S5	Impeller force feeder			
S6	Tablet ejector	API	More contact surface	
S7	Scraper for powder	API	More contact surface	
S8	Tablets track	API	More contact surface	

S9	Rotary table	API & Micro	More contact surface	

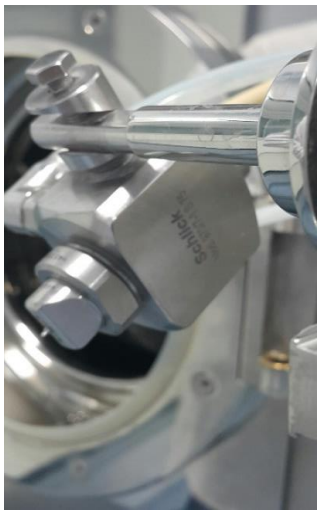
Soak #	***Soak location***	***Rinse volume***	***Detection for***	***Justification***	***Location photo***
SK1 SK2	Upper & lower Punches Dies	10 ml 100 ml	API & C.A.*	To ensure no residues	

*: Cleaning agent

3.7.3 Swab and Rinse Sampling Locations for Coating Pan

Table 3. 7 Swab and rinse sampling locations for Coating Pan.

Swab #	***Swab Location (5 cm x 5 cm)***	***Detection for***	***Justification***	***Location photo***
S1 S2	Two paddles inside coating machine (one swab from each)	API	More contact surface	

S3	Paddles arm	API	More contact surface	
S4	Spray nozzle	API	More contact surface	
S5 S6	Inside door (two swabs)	API	More contact surface	

S7	Baffles	API & Micro	More contact surface	
S8	Internal surface of coating machine	API	More contact surface	

Rinse #	***Rinse location***	***Detection for***	***Justification***	***Rinse volume***
R1	Drain line from bulk tank	API & C.A.*	To ensure no residue	5000 ml of rinse purified water

*: Cleaning agent

3.8 Testing Procedure

3.8.1 Physical Testing

Along with taking samples, it is important to perform visual inspection as well to ensure the process acceptability.

3.8.2 Chemical Testing

3.8.2.1 Method of Analysis for Olanzapine Residue

HPLC Chromatographic Conditions

Chromatographic separation was achieved using 150-mm C_{18} Luna Phenomenex column, I.D: 4.6 mm, packed with 5µm particles. The mobile phase was a mixture of Acetonitrile and 10 mM disodium hydrogen phosphate Buffer (55:45. v/v, pH 7.4). The diluent was a mixture of water and Acetonitrile (55:45. v/v). The UV detection was set at 254 nm, with a flow rate of 1.0 ml/min, injection volume was 20 µL and the column temperature was adjusted to 40˚C.

Buffer Preparation

10 mM of disodium hydrogen phosphate, pH of 7.4**:** 1.42 g of disodium hydrogen phosphate were dissolved in 1 L of water. 200 µl of triethylamine were also added. The buffer was adjusted with orthophosphoric acid to pH of 7.4 then filtered using a 0.45 µm Nylon membrane filter. Triethylamine was used as an organic modifier to reduce

peak tailing caused by the strong interaction of basic analytes with acidic surface silanol groups in the stationary phase.

3.8.2.2 Method of Analysis for Cleaning Agent (SLS) Residues

- Rinse the clean equipment with purified water, same volumes as in Table 3. 4.
- Collect approx. 200 ml from the final rinse into a clean pyrex bottle.
- Then the Conductivity was measured for purified water as a standard reference and for the final rinse water to determine cleaning agent residues.

3.9 Acceptance Criteria

The maximum allowable residue (MAR) for active pharmaceutical ingredients (APIs) can be calculated using dose criterion or 10 ppm criterion. Where dose criterion means that in a daily dose of the subsequent product a maximum of 1/1000 of the single dose of the API of the previous product may be contained. While the 10-ppm criterion means that no more than 10-ppm of any product will appear in another product. Where the non-specific method / conductivity test is used to detect residues of cleaning agents during cleaning validation.

Table 3. 8 The testing parameters to calculate MACO for API and cleaning agent and its acceptance criteria.

S. #	***Testing Parameter***	***Acceptance Criteria***
1.	Physical determination	No residues shall be visible by naked eyes on the equipment surface after performing the cleaning procedures.
2.	Chemical Determination	a) For API: Dose criteria is the appropriate acceptance criteria with MAR for Olanzapine found in the individual samples lie below 0.2273 ppm/swab. b) For cleaning agent the difference in conductivity shall not be more than ± 0.2 µs/cm.
3.	Microbiological Contaminants	a) Total Bacterial Counts = NMT 50 CFU/ 25 cm^2. b) Absence of indicator microorganisms (E.Coli, Staph.aureus, Pseudomonas and Salmonella). c) Absence of yeast and mold.

3.10 Validation Program

- Equipment cleaning validation may be performed concurrently with actual production steps during process development and bulk manufacturing. Validation programs should be continued through full-scale commercial production.

- The concept “Test-Until-Clean” will not be applied instead of cleaning validation. This concept involves cleaning, sampling and testing with repetition of this sequence until an acceptable residue limit is attained.
- A validation program generally encompasses at least three consecutive successful replicates of the cleaning procedure to establish that the procedure is reproducible and consistent in removing residues to acceptable limits.
- If the equipment of the similar size, design and construction cleaned by the same procedure, studies need not to be conducted on each unit as long as three successful replicates were performed on similar piece of equipment; this concept known as equipment grouping.

3.11 Change Control / Corrective Action (If Required)

Any of the following proposed changes are evaluated fully and investigated for their impact on the validated state of the procedure. Changes may include (but not limited) to the following:

- Changing in Machine
- Changes in cleaning agents used (if applicable)
- Changes in cleaning procedures
- Changes in products formulation
- Adding new products

- Changes in critical cleaning parameters (TACT)

If any of the above-cited changes occurred, it should be dealt in accordance with the Quality Management System change control procedure.

After the approval of any change according to procedure, it is required to assess the risks of the change and then evaluate if revalidation of the Cleaning Procedure is required.

3.12 Inspection Criteria: (For Three Consecutive Batches)

Previous Product:	**Batch No.:_____**
While taking samples from **Machine Name (Machine No. _____)**, note down the following points. ● Description of machine/equipment/area: ● Major Product contact components: ● Product Contact Area: ● Previous Batch completed on: ● Equipment cleaned on: ● Detergent / Solvent used: ● Composition of the detergent used: ● Cleaning Tools: ● Ancillary Utilities:	

- Cleaning Cycles:
- Cleaned by:
- Supervised by:
- Sampled by (Chemical):
- Sampled by (Microbiological):
- After cleaning the Equipment used on:
- Subsequent Product:

- Batch No.:
- Name of API:
- Batch Size of the subsequent product:
- Maximum daily dose of the subsequent Product:

Part Four: Methodology, Strategy of Research and Experiments

4.1 Project Outline

- Determination of worst-case product.
- Determination of worst-case equipment sampling locations.
- Determination of sampling method (Rinse/Swab/soak?).
- MACO Calculation.
- Development of cleaning procedure.
- Development and validation of analytical test method.
- Recovery from coupons and samples.
- Implementation of cleaning procedure using pilot scale product & equipment.
- Sample collection & testing.
- Data analysis.
- Thesis writing & finalization.

4.2 Materials and Reagents

Olanzapine working standard was obtained from LEE Pharma, its in house lot number is 201703135 and it was certified to be 99.2% on dried basis. Disodium hydrogen phosphate and Orthophosphoric acid were from Merck, where HPLC-grade Acetonitrile and Triethylamine were from J. T. Baker. Olanzapine tablet placebo according to the formulation procedure of Olanzapine tablets which contain Microcrystalline Cellulose, Lactose, Hydroxypropyl cellulose (HPC), Magnesium

stearate, Opadry II were all obtained from Jerusalem Pharmaceuticals Co. Ltd. Table 4. 1 presents the information of each excipient manufacturer, their lot numbers and their expiry dates in addition to the other materials and reagents used in this study.

Table 4. 1 Materials and reagents used in this thesis.

Material and Reagents	***Supplier Name***	***Lot #***	***Exp. Date***
Diclofenac Potassium	AARTI	201708123	05/2022
Ibuprofen	Hubei	201710202	09/2022
Olanzapine	LEE Pharma	201703135	08/2021
Microcrystalline Cellulose 102	JRS	5610270803	01/2022
Lactose S.D	MEGGLE	L101502017A535	10/2018
Hydroxypropyl cellulose (HPC) LH11	Shin-Etsu	6043088	04/2019
Magnesium stearate	Magnesia	16002157/0	10/2018
Opadry II *	Colorcon	201703182	02/2019
Disodium hydrogen phosphate	Merck	F1955786 714	30/09/2021
Triethylamine	J. T. Baker	0000164841	01/12/2023
Orthophosphoric acid	Merck	K47453273 602	31/01/2021
Acetonitrile	J. T. Baker	0000177974	29/06/2022
Ethanol	Merck	K48484427 647	30/11/2019
Acetone	Merk	K48168020 633	31/08/2019
Isopropyl Alcohol	J. T. Baker	K14B08	-

* : Opadry II White 85F28751 containing: 40.0% (w/w) Polyvinyl alcohol (E1203), 25.0% (w/w) Titanium dioxide (E171), 20.2% (w/w) Macrogol 3000 (E1521), 14.8% (w/w) Talc (E553b).

4.3 Tools and Equipment

Tools and instruments used in this study were supplied by Jerusalem Pharmaceuticals Company and SDI. All tools and equipment used are tabulated in Table 4. 2 below.

Table 4. 2 Tools and Equipment used in this study.

Tools, Instruments, Equipment and Materials	***Source/Model***
HPLC 1 (method validation)	High performance liquid chromatography HPLC system Waters e2695. Photodiode array detector. Software: Empower. Version: 3.
HPLC 2 (recovery study)	High performance liquid chromatography HPLC system DIONEX UltiMate 3000. Diode Array detector. Software: Chromeleon. Version: 7.2.
HPLC 3 (Implementation of cleaning procedure)	High performance liquid chromatography HPLC system Agilant 1200. Variable Wavelength detector. Software: ChemStation Rev. B.04.03[16].
Balances	AS 60/220.R2 Analytical balance and Precisa XT 220A Analytical balance
pH Meter	Metrohm 691 pH meter
Centrifuge	HERMLE Z300 Centrifuge
Hotplate Magnetic Stirrer	Freed Electric
Sonicator	Elmasonic
Refrigerator	L.G.
Freezer	L.G.
Coupon	Stainless steel 316 coupons

Conductivity Meter	OHAUS
Silverson Homogenizer	Silverson L5M-A
Columns	150-mm C_{18} Luna column, I.D: 4.6 mm, packed with 5µm particles.
Swabs	Polyester large Alpha TX715
Filters	PTFE filters 0.22 microns and Nylon filter 0.45 microns
Magnetic Bars	Freed Electric
Spatulas	Stainless steel spatulas
Micropipette and tips	Eppendorf research pipette
Contact plate	JePharm
Plate Count Agar	OXOID
Yeast Extract Chloramphenicol Agar	Himedia
Glassware (Volumetric Flasks, beakers, pipettes …etc.)	Glass grade A
Computer	Lenovo
Ben Mixer	In SDI
Tablet Press	In SDI
Coating Pan	In SDI

4.4 Methodology

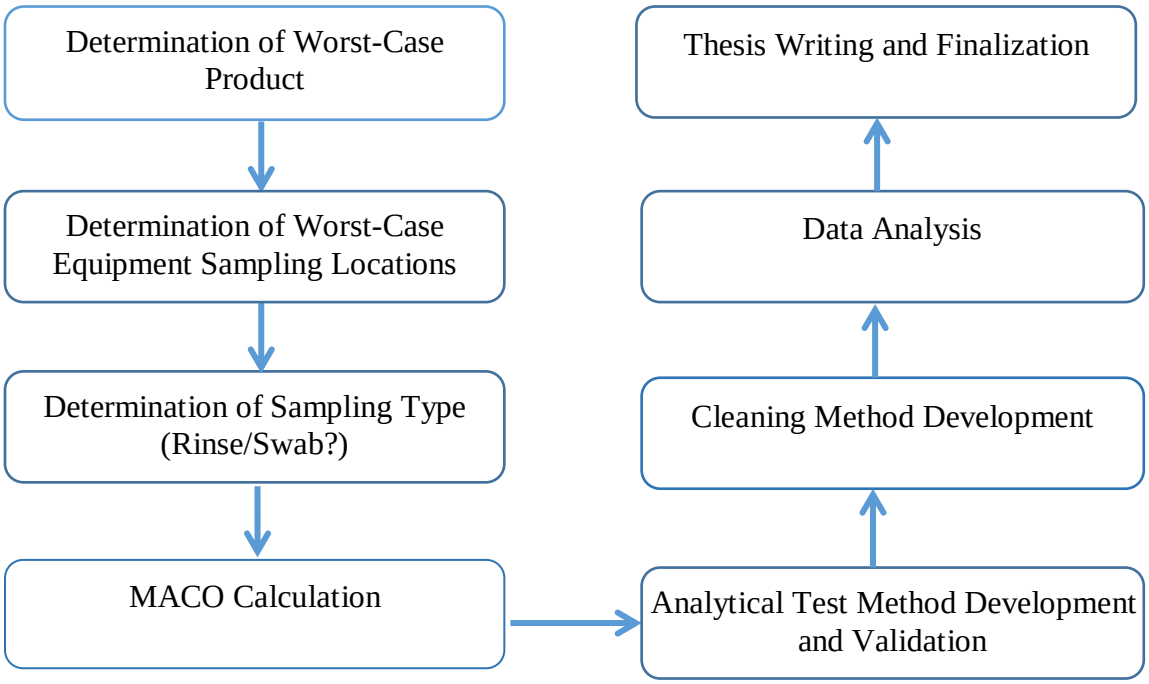


Figure 4. 1 Methodology diagram.

4.5 Determination of Worst-Case Product

Product matrix approach are bracketing of products according to risk groups.

As clarified in Table 3. 3, **Olanzapine** tablets is the worst-case over the other products, since it has risk in its solubility and in pharmacology or potency.

4.6 Determination of Worst-Case Equipment Sampling Locations

4.6.1 Flow Charting of Manufacturing Procedure.

General manufacture production method by direct compression for the three products

Flow chart: Equipment:

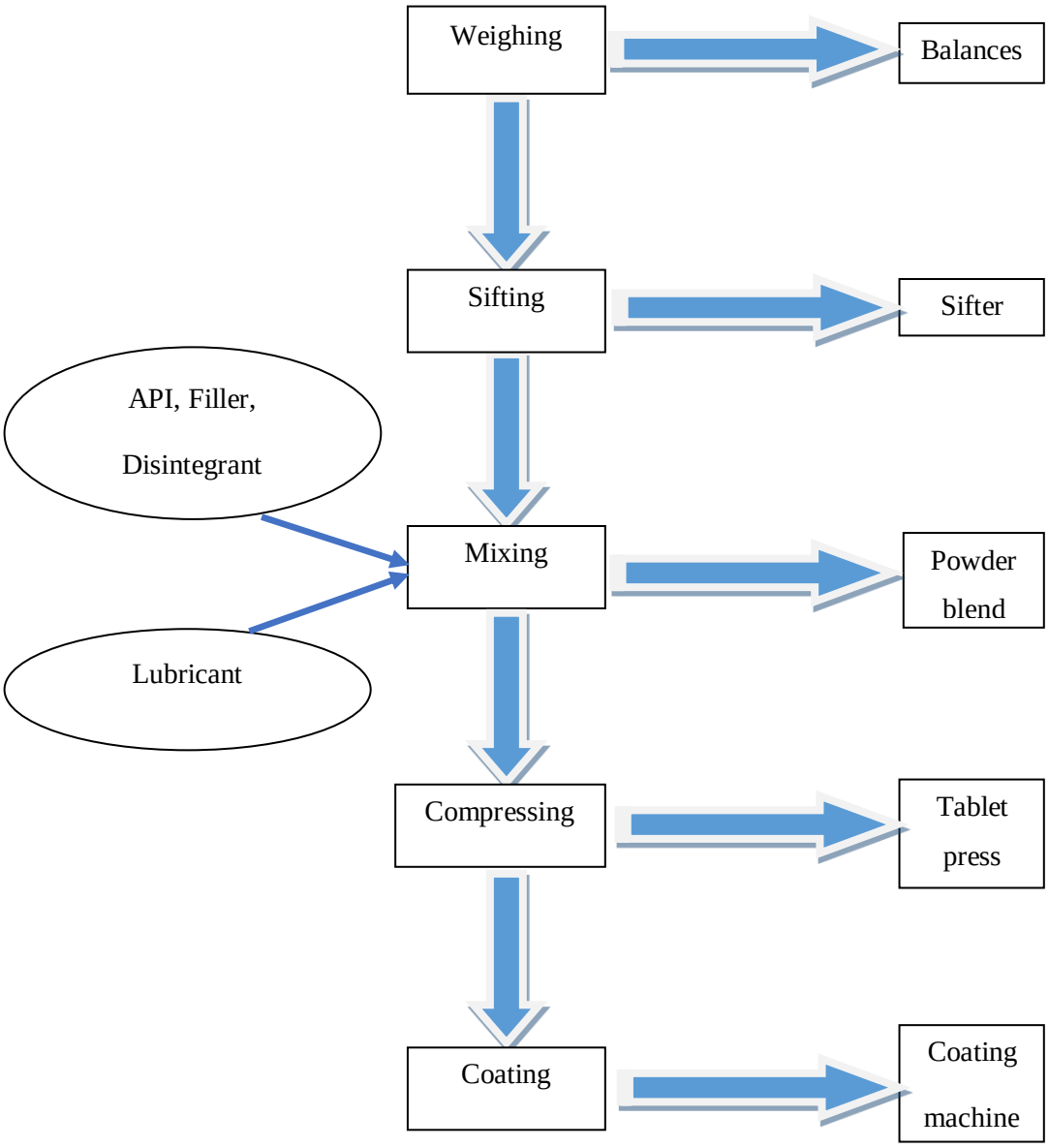


Figure 4. 2 Flow chart and Equipment used in the production of the three products.

4.6.2 Characterization of Manufacturing Equipment.

Bin Mixer, Tablet Press and Coating Pan were the three main Equipment that are concerned in this study. Table 3. 1 and Table 3. 2 clarified the characterization of manufacturing Equipment and their surface area.

4.7 Calculation of MACO for Olanzapine

The critical or worst-case product is Olanzapine tablet.

4.7.1 Selection of Appropriate Acceptance Criteria

4.7.1.1 Dose Criterion

Calculation of the maximum allowable residue (MAR):

$$\text{MAR [mg]} = \frac{(\frac{1}{1000}) \times \text{LTDp [mg]} \times \text{MBSs [mg]}}{\text{IFs} \times \text{MDs [mg]}} \quad (7)$$

LTD_p: Lowest therapeutic dose of the previous product (worst-case) in mg

MBS_s: Minimum batch size of the subsequent product in mg

IF_s: Intake frequency of the subsequent product

MD_s: Mass of the dosage form of the subsequent product in mg

Maximum daily dose of the subsequent product (MDD):

$$\text{MDD} = \text{IFs} \times \text{MDs [mg]} \quad (8)$$

In our study:

LTD_p = 2.5mg, MBS_s = 5Kg, MDD = 3200mg for Ibuprofen (to make it more stringent).

Ibuprofen was assumed as smallest batch size in our facility = 5Kg.

MAR [mg] = (1/1000 * 2.5 mg * 5,000,000 mg) / 3200 mg

MAR [mg] = 3.90625 mg

The maximum allowable residue for dose criterion = 3.90625 mg

4.7.1.2 10-ppm Criterion

Calculation of the maximum acceptable residue (MAR):

$$MAR\ [mg] = \ 10\ \text{ppm}\ [\text{mg/Kg}]\ \times\ \text{MBSs}\ [\text{Kg}] \qquad (9)$$

MBS_s: Minimum batch size of the subsequent product in Kg

In our study:

MBS_s= 5Kg

MAR [mg] = 10 ppm [mg/Kg] * 5 Kg= 50 mg

Then the maximum acceptable residue from 10 ppm Criterion = 50 mg

From the above calculations, we conclude that **the MAR [mg] for dose criteria that is equals to 3.90625 mg** must be used as appropriate acceptance criteria since it is **the most stringent limit**.

The acceptance residue in the subsequent product is based on the entire product contact surface of the production equipment used for manufacture and from this; the surface-related limit is calculated.

4.7.2 Calculation of the Acceptance Criteria for API Using Swab Test

Surface-related limits = the acceptance residue in the subsequent product divided by the product contact surface area multiplied by the sample surface area for the swab test over the volume of solvent used to dispense swab.

$$\text{Surface} - \text{related Limit [ppm]} = \frac{\text{MAR [mg]}}{\text{SA [cm2]}} \times \frac{\text{ST area [cm2]}}{\text{V [ml]}} \times 1000 \quad (10)$$

MAR: Maximum acceptable residue according to dose criterion in mg

SA: Total surface area for train of equipment in cm^2

ST area: Area of swab test in cm^2

V: Volume of solvent used to dispense swab in ml

1000: Conversion factor from mg to µg

In our study, we used the equipment and its surface area as in Table 3. 2:

Total surface area= 42,970 cm^2

The volume of solvent used to dispense swab = 10 ml

Then,

Surface-related Limit [ppm] = (3.90625 mg / 42,970 cm^2) * (25 cm^2 / 10 ml)* 1000

Surface-related Limit [ppm] = 0.09091 µg/cm^2 * (25 cm^2 / 10 ml)

Surface-related Limit [ppm] = 0.2273 ppm/swab

The above calculation showed that the maximum allowable residue is 0.09091 µg /cm^2, which is considered below the visual limit, since the minimum visual limit is 4µg/cm^2. If the Olanzapine residue quantities found in the individual samples lie **below 0.2273 ppm/swab**, then the **acceptance criteria have been fulfilled**.

4.7.3 Calculation of the Acceptance Criteria for API Using Rinse Test

The required rinse volume for each equipment to calculate the acceptance criteria for Olanzapine using rinse test was clarified in Table 3. 4.

$$\text{MACO (Equipment) [mg]} = \frac{\text{MAR [mg]} \times \text{SA Eq. [cm2]}}{\text{Total SA [cm2]}} \quad (11)$$

MACO (Equipment): Maximum acceptance carry over for each Equipment in mg

SA Eq.: Surface area for each Equipment in cm^2

Then,

MAR [mg] = 3.90625 mg, Total surface area= 42,970 cm^2

MACO (**Bin Mixer**) [mg] = (3.90625 * 5,000) / 42,970 = **0.45453 mg**

MACO (**Tablet Press Punches**) [mg] = (3.90625 * 2.3079) / 42,970 = **0.20980 µg**

MACO (**Tablet Press Dies**) [mg] = (3.90625 * 35) / 42,970 = **0.0031817 mg**

MACO (**Coating Pan**) [mg] = (3.90625 * 34,370) / 42,970 = **3.12445 mg**

$$\text{MACO (Equipment) [ppm]} = \frac{\text{MACO Eq. [mg]}}{\text{Rinse Volume Eq. [Liters]}} \quad (12)$$

Rinse Volume Eq.: Rinse volume for each equipment in Liters

MACO (**Bin Mixer**) [ppm] = 0.45453 mg / 1 L = **0.45453 ppm**

MACO (**Tablet Press Punches**) [ppm] = 0.20980 µg / 10 ml = **0.020980 ppm**

MACO (**Tablet Press Dies**) [ppm] = 0.0031817 mg / 0.1 L = **0.031817 ppm**

MACO (**Coating Pan**) [ppm] = 3.12445 mg / 5 L = **0.62489 ppm**

4.8 Visual Criteria (Visually Clean)

The maximum allowable residue is 0.09091 µg /cm^2 which is considered below the visual limit, since the minimum visual limit is 4 µg/cm^2 = 100 µg / 25 cm^2. Depending on that, we cannot correlate both the specific method with other nonspecific methods for cleaning verification.

4.9 Cleaning Procedure

The Standard Operating Procedure SOP No.: QA121 (attached in Appendix I) for full and dry cleaning for machines and equipment, illustrate the steps that should be taken during cleaning procedure for machines, equipment and rooms. Below are the steps needed to clean machines and equipment only, since they are our concern in this study (direct contact with the product).

1. Switch off the power.
2. Dry clean the entire equipment by vacuum.
3. Disassemble Machine parts and clean them by vacuum pump.
4. Clean the machine parts and surface, electrical panel and motor using the vacuum pump.
5. Follow the steps in FMQA115, FMQA118 and FMQA119 and move the movable parts to the washing room.

   Note: These files were attached in Appendix I.

6. Spray and clean the outer surface of the machine well using potable water with cleaning solution. Please take attention to avoid damage of the mechanical and electrical parts.
7. Using purified water to spray the outer surface of the machine and dry it with compressed air.
8. Spray of all parts and machine surface with 70% Ethanol solution.
9. Clean the movable parts in FMQA115, FMQA118 and FMQA119 using the following steps:
    - Hot water
    - Hot water with cleaning solution
    - Hot water
    - Purified water
    - 70% Ethanol

Note: For Coating machine cleaning do not use hot water, since Opadry II can cause gelation of certain polymers used in coating formulations [31].

4.10 Cleaning Agent

15% Sodium Lauryl Sulfate (SLS) was used as a cleaning agent. The action of cleaning on equipment surface is wetting mechanism by adding surfactants to reduce water surface tension to remove the dirt from the contact surface of the equipment.

4.10.1 Cleaning Agent Preparation

15 % Sodium Lauryl Sulfate (SLS) was prepared by dissolve 15 g of sodium lauryl sulfate (SLS) in 100 ml of purified water. Adjust with Sodium hydroxide (NaOH) to a pH of 6-8.

4.10.2 Cleaning Agent Detection Test

Cleaning solutions should be completely removed before the next processing run or campaign. Conductivity analysis can be used to monitor the cleaning steps and the final rinse, since the various cleaning solutions are more conductive than the water used for rinse. Therefore, the Conductivity was measured for purified water as a standard reference and for the final rinse water.

4.11 Cleaning Parameters

The cleaning parameters that were used in cleaning the machines and equipment to determine cleaning effectiveness are the cleaning time, the action or impingement on the surface, the concentration of the cleaning agent and the temperature of the cleaning

solution (TACT). These parameters were set in our study as five minutes for cleaning time, wetting mechanism as the action on surface, 15% is the concentration of SLS and 70°C is the temperature of water used during the cleaning process.

4.12 Test Method Validation

4.12.1 Preparation Method

4.12.1.1 Buffer Preparation

10 mM disodium hydrogen phosphate, pH of 7.4**:** a quantity of 1.42 g of disodium hydrogen phosphate was dissolved in 1 L of water. 200 µl of triethylamine were also added. The buffer was adjusted with orthophosphoric acid to a pH of 7.4 then filtered using a 0.45 µm Nylon membrane filters. Triethylamine was used as an organic modifier to reduce peak tailing caused by the strong interaction of basic analytes with acidic surface silanol groups in the stationary phase.

4.12.1.2 Mobile Phase

The mobile phase is a filtered and degassed mixture of Acetonitrile and Buffer (55:45, v/v).

4.12.1.3 Diluent

The diluent was a mixture of water and Acetonitrile (55:45, v/v).

4.12.1.4 The Nominal Concentration 0.2273 ppm

This HPLC method development and validation was part of cleaning validation study for three products manufactured in multi-product facility. Where the Olanzapine tablets was the worst-case product in cleaning of the Equipment train, with maximum allowable residue of **0.2273 ppm/swab** from previous product to the subsequent product as approved by this study. Therefore, this concentration was used as the nominal concentration during our validation study.

4.12.1.5 Stock Olanzapine Standard Solution Preparation (2.273 ppm)

An accurately weighed quantity of about 22.73 mg of Olanzapine standard was dissolved into a 100- ml volumetric flask with diluent then mixed for 15 minutes using magnetic stirrer. The concentration was 0.2273 mg/ml. 1 ml of this solution was withdrawn and taken in to another 100- ml volumetric flask. The volume was adjusted with diluent up to the mark. The concentration was 0.002273 mg/ml or 2.273 ppm.

Stock Olanzapine standard conc. = (22.73 mg / 100 ml Diluent) * (1 ml / 100 ml Diluent) * 1000 = 2.273 ppm

4.12.1.6 Nominal Olanzapine Standard Solution Preparation (0.2273 ppm)

An accurately measured 5 ml of stock Olanzapine standard solution (2.273 ppm) were transferred to a 50- ml volumetric flask and diluted with diluent to volume and mixed, to obtain a solution having a known concentration of 0.2273 ppm of Olanzapine.

Nominal Olanzapine standard conc. = (22.73 mg / 100 ml Diluent) * (1 ml / 100 ml Diluent) * (5 ml / 50 ml Diluent) * 1000 = 0.2273 ppm

4.12.1.7 Olanzapine Placebo Tablet Preparation

Olanzapine placebo tablets were prepared according to the formulation procedure of Olanzapine tablets that contain Microcrystalline Cellulose, Lactose, Hydroxypropyl cellulose (HPC), Magnesium stearate, Opadry II. The equivalent concentration of placebo in the analyzed sample (nominal concentration 0.2273 ppm of Olanzapine) expected to be about 9.3193 ppm.

(According to the formula, the 2.5 mg of Olanzapine standard need 102.5 mg excipient. So for 0.2273 mg of Olanzapine standard, it is expected to need an equivalent of 9.393 mg excipient).

4.12.1.8 Stock Placebo Solution Preparation (93.2 ppm)

An accurately weighed amount of about 931.93 mg of Olanzapine placebo tablet was transferred into a 100- ml volumetric flask and dissolved in Acetonitrile using magnetic stirrer. 1 ml of this solution was withdrawn and taken into another 100- ml volumetric flask. The volume was adjusted with Acetonitrile up to the mark and mixed. The concentration was 0.0932 mg/ml or 93.2 ppm.

Stock placebo conc. = (931.93 mg / 100 ml ACN) * (1 ml / 100 ml ACN) * 1000 = 93.2 ppm

4.12.1.9 Nominal Placebo Solution Preparation (9.32 ppm)

An accurately measured 5 ml of stock placebo solution (93.2 ppm) were transferred to a 50- ml volumetric flask and diluted with diluent to volume and mixed, to obtain a solution having a known concentration 9.32 ppm of Olanzapine placebo tablet.

Nominal placebo conc. = (931.93 mg / 100 ml ACN) * (1 ml / 100 ml ACN) * (5 ml / 50 ml Diluent) * 1000 = 9.32 ppm

4.12.1.10 Nominal Spiked Sample Solution Preparation

An accurately measured 5 ml of both stock Olanzapine standard solution and stock placebo solution were transferred to the same 50 ml volumetric flask and diluted with diluent to volume and mixed. The concentration was 0.2273 ppm Olanzapine and 9.32 ppm Olanzapine placebo tablet.

4.12.2 Procedure

4.12.2.1 Specificity (Placebo Interference)

Specificity was evaluated by injecting the nominal Olanzapine standard solution (0.2273 ppm), the nominal Olanzapine Placebo tablet solution (9.32 ppm), the nominal spiked sample solution and the diluent, to insure that there were no peaks appear at the Olanzapine retention time.

Data analysis:

The nominal Olanzapine standard solution (0.2273 ppm), the nominal Olanzapine placebo tablet solution (9.32 ppm), the nominal spiked sample solution and the diluent were injected.

Acceptance criteria:

No peaks appear at the Olanzapine retention time.

4.12.2.2 Limit of Detection and Limit of Quantitation (LOD & LOQ)

Different concentrations of Olanzapine standard solution were prepared using the stock standard solution (2.273 ppm) to prepare separate standards covering the range between (2 to 20%) of the nominal concentration (0.2273 ppm) according to Table 4. 3:

Table 4. 3 Preparation of LOD & LOQ solutions.

Conc. (ppm)	*% of MACO conc. (0.227266 ppm)*	*Volume (ml) from stock standard needed (2.273 ppm)*	*Flask volume (ml)*
0.004546	2	1	500
0.006819	3	3	1000
0.009092	4	2	500
0.013638	6	3	500
0.022730	10	1	100
0.045460	20	2	100

Data analysis:

Area versus standard concentration, prepared for LOD & LOQ over the range of standard solutions were plotted, then all of the following were calculated:

- The least squares linear regression analysis of the linearity data.
- The RSD for replicates of each concentration over the range.
- Determine slope (S) and Y-intercept.
- Determine the standard error SE value using Excel software.
- Calculate the standard deviation value SD (σ) = standard error SE * √n, where n is the number of points in the linearity curve.
- Calculate the LOD & LOQ values, where LOD = 3.3 σ/S and LOQ = 10 σ/S.

Acceptance criteria:

- RSD not more than 10.0% for all levels.

4.12.2.3 Linearity and Range

Separate Olanzapine standards were prepared using the stock standard solution (2.273 ppm) covering the range between (3 to 200%) of the nominal concentration (0.2273 ppm) according to Table 4. 4:

Table 4. 4 Preparation of linearity solutions.

Conc. (ppm)	***% of MACO conc. (0.2273 ppm)***	***Volume (ml) from stock standard needed (2.273 ppm)***	***Flask volume (ml)***
0.006819	3	3	1000
0.009092	4	2	500
0.013638	6	3	500
0.022730	10	1	100
0.045460	20	2	100
0.113650	50	5	100
0.181840	80	4	50
0.227300	100	5	50
0.454600	200	10	50

Data analysis:

Area versus standard concentration, prepared for linearity over the range of standard solutions were plotted, then all of the following were calculated:

- The least squares linear regression analysis of the linearity data.
- The RSD for replicates of each concentration over the range.
- Determine slope (S) and Y-intercept.

Acceptance criteria:

Correlation coefficient (R^2) not less than 0.990 for the least squares method of analysis of the line.

4.12.2.4 Accuracy

A mixture of the drug product components (Placebo) was spiked with known amounts of Olanzapine (10%, 100%, and 200%) of the nominal concentration (0.2273 ppm) named spiked samples were prepared. By dilution of the required placebo volume into the analysis volumetric flask. Into the same flask, known amount from the stock Olanzapine standard solutions were added according to Table 4. 5. In addition, three different standard solutions of Olanzapine with concentrations (10%, 100%, and 200%) of the nominal concentration (0.2273 ppm) were prepared as in Table 4. 6, to calculate percentage accuracy (recovery).

Table 4. 5 Preparation of accuracy test sample solutions.

Conc. (ppm)	***% of MACO conc. (0.2273 ppm)***	***Volume (ml) from stock standard needed (2.273 ppm)***	***Volume (ml) from stock placebo needed (93.2 ppm)***	***Flask volume (ml)***
0.02273	10	1	1	100
0.22730	100	5	5	50
0.45460	200	10	10	50

Table 4. 6 Preparation of accuracy test standard solutions.

Conc. (ppm)	***% of MACO conc. (0.2273 ppm)***	***Volume (ml) from stock standard needed (2.273 ppm)***	***Flask volume (ml)***
0.02273	10	1	100
0.22730	100	5	50
0.45460	200	10	50

Data analysis:

- Olanzapine solutions were analyzed according to the chromatographic HPLC test method of Olanzapine.
- Recovery data for each determination, the average of recovery data and the RSD for each level were calculated.

Acceptance criteria:

The mean recovery should be within 85-115% at each concentration level over the range of 10% - 200% of the nominal concentration. In addition, the RSD not more than 10.0%.

4.12.2.5 Precision (System Repeatability)

The nominal Olanzapine standard solution (0.2273 ppm) were injected six times into the HPLC for determination of system precision, and six replicate injections were analyzed for the nominal sample for determination of method precision during the same day (intraday precision).

Data analysis:

RSD of the replicate injections for the nominal standard and sample concentrations were calculated.

Acceptance criteria:

RSD not more than 10%.

4.12.2.6 Ruggedness (Intermediate Precision)

Ruggedness was studied through the analysis of six replicate injections for the nominal Olanzapine standard solution (0.2273 ppm) under a variation of analyst, instrument and analysis days within the same lab.

Data analysis:

RSD of the replicate injections for the nominal standard concentration was calculated.

Acceptance criteria:

RSD not more than 10.0%.

4.12.2.7 Robustness

Robustness of the method was investigated by making small deliberate changes in the chromatographic conditions for the nominal Olanzapine standard solution (0.2273 ppm) as in Table 4. 7:

Table 4. 7 The small deliberate changes in the chromatographic conditions for robustness test.

Chromatographic Conditions	***Variation***
Flow rate	0.9 ml/min
± 10%	1.1 ml/min
Wavelength	252 nm
± 2 nm	256 nm
Mobile phase composition	50:50
± 5%	(ACN:Buffer)
	60:40
Temperature	35 ˚C
± 5˚C	45 ˚C
Mobile phase pH	pH 7.3
± 0.1 units	pH 7.5
Mobile phase pH	pH 7.2
± 0.2 units	pH 7.6

Data analysis:

The influence of variations in method parameters must be within the previous acceptance criteria, the variations said to be within the method robustness range.

Acceptance criteria:

RSD for the replicate injections not more than 10.0%, and the tailing factor not more than 2.

4.12.2.8 Technology Transfer (Comparative Analysis)

Technology transfer or transfer of analytical procedures (TAP), was the documented process that qualifies a laboratory (the receiving unit) to use an analytical test procedure that originated in another laboratory (the transferring unit), thus ensuring that the receiving unit has the procedural knowledge and ability to perform the transferred analytical procedure as intended [32].

In our study although the personnel who developed and validated the analytical test method of Olanzapine in transferring unit (JePharm) were moved to the receiving unit (SDI), in this case transfer waiver type of the analytical method was achieved, we also used the comparative study transfer type between both laboratories.

To qualify this analytical method for comparative transfer, the following Olanzapine standard solutions and samples were prepared.

Different concentrations of Olanzapine standard solutions were prepared using the stock Olanzapine standard solution (2.273 ppm) to prepare 10%, 100%, 200% of the nominal concentration (0.2273 ppm). The volumes were adjusted with diluent up to the mark according to Table 4. 8:

Table 4. 8 Preparation of technology transfer standard solutions.

Conc. (ppm)	*% of MACO conc. (0.2273 ppm)*	*Volume (ml) from stock standard needed (2.273 ppm)*	*Flask volume (ml)*
0.02273	10	1	100
0.22730	100	5	50
0.45460	200	10	50

- **Stock sample solution preparation (2.273 ppm Olanzapine and 93.193 ppm Olanzapine placebo tablet)**

An accurately weighed quantity of 22.73 mg of Olanzapine standard and 931.93 mg of Olanzapine placebo tablet were transferred into a 100- ml volumetric flask and dissolved with Acetonitrile. The concentration was 0.2273 mg/ml for Olanzapine and 9.3193 mg/ml for Olanzapine tablet placebo. 1 ml of this solution was withdrawn and taken into a 100- ml volumetric flask. The volume was adjusted with Acetonitrile up to the mark. The final concentration was 2.273 ppm Olanzapine and 93.193 ppm Olanzapine tablet placebo.

Olanzapine Sample Conc. = ([22.73 mg Olanzapine + 931.93 mg Placebo] / 100 ml ACN) * (1 ml / 100 ml ACN) * 1000 = 2.273 ppm Olanzapine + 93.193 ppm Placebo

Different concentrations of sample solutions were prepared using the stock sample solution (2.273 ppm Olanzapine and 93.193 ppm Olanzapine tablet placebo) to prepare 10%, 100%, 200% of the nominal concentration (0.2273 ppm). Volumes were adjusted with diluent up to the mark according to Table 4. 9:

Table 4. 9 Preparation of technology transfer sample solutions.

Conc. (ppm)	*% of MACO conc. (0.2273 ppm)*	*Volume (ml) from stock sample needed*	*Flask volume (ml)*
0.02273	10	1	100
0.22730	100	5	50
0.45460	200	10	50

Data analysis:

- Olanzapine solutions were analyzed according to the validated chromatographic HPLC test method.
- Recovery data were calculated for each determination.

Acceptance criteria:

- The recovery should be within 85-115% over the range of 10 to 200% of the nominal concentration. In addition, the result in the receiving unit should not differ than that in the transferring unit by more than 15%.

4.12.2.9 Solution Stability

Solutions stability was studied through analysis of nominal Olanzapine standard solution (0.2273 ppm) of freshly prepared at zero time, after 24 hours and 48 hours at 25˚C, 5˚C and at - 21.5˚C.

Data analysis:

The assay of the analyte in each solution was calculated.

Acceptance criteria:

The average assay should be within 85-115%, and RSD not more than 10.0%.

4.12.2.10 Filter Compatibility

Filter compatibility test was studied through analysis of nominal Olanzapine standard solution (0.2273 ppm) and nominal spiked sample solution (0.2273 ppm Olanzapine and 9.32 ppm Olanzapine placebo tablet), using nylon and PTFE filters.

Data analysis:

The assay of the analyte in each solution was calculated.

Acceptance criteria:

The average assay should be within 85-115%, and RSD not more than 10.0%.

4.12.2.11 System Suitability

The system suitability test was carried out to evaluate the entire chromatographic system suitability and efficacy before and during the analysis, it was studied through the analysis of six replicate injections for the nominal Olanzapine standard solution (0.2273 ppm).

Data analysis:

- RSD of the peak areas and retention times were calculated.
- The tailing factor, capacity factor and theoretical plate number parameters were documented.

Acceptance criteria [24]**:**

- The relative standard deviation for replicate injections of the nominal Olanzapine standard solution is not more than 10.0%
- The tailing factor (T) ≤ 2
- The capacity factor (k') > 2
- The theoretical plate number (N) > 2000

4.13 Recovery Test from Coupons

4.13.1 Execution During Swab Test

4.13.1.1 Choosing the Optimum Solvent for Swab Wetting

The polyester large Alpha TX715 swabs, for HPLC sampling/cleaning validation were wetted with 0.5 ml of one of four different solvents (Ethanol, Isopropyl alcohol IPA, Acetone and Acetonitrile) before swabbing the coupon, then swabs were dropped in 10 ml diluent.

Preparation:

- **Olanzapine standard preparation (0.2273 ppm)** were prepared as nominal Olanzapine standard solution preparation. See section 4.12.1.6.

- **Olanzapine sample preparation (0.02273 mg/ml)**

An accurately measured 22.73 mg of Olanzapine standard and 931.93 mg of Olanzapine placebo tablet were transferred into 100 ml volumetric flask and dissolved with Acetonitrile. The concentration was 0.2273 mg/ml for Olanzapine and 9.3193 mg/ml for Olanzapine tablet placebo. 5 ml of this solution was withdrawn and taken in 50 ml volumetric flask. The volume was adjusted with Acetonitrile up to the mark. The concentration is 0.02273 mg/ml Olanzapine and 0.93193 mg/ml Olanzapine placebo tablet.

Olanzapine sample conc. = ([22.73 mg Olanzapine + 931.93 mg Placebo] / 100 ml ACN) * (5 ml / 50 ml ACN) = 0.02273 mg/ml Olanzapine + 0.93193 mg/ml Placebo

Procedure:

- 100 µl of 0.02273 mg/ml Olanzapine sample solution were distributed evenly directly over 5 cm * 5 cm coupon surface area.
- After drying, the tested coupon surface was swabbed firmly and evenly in a back-and-forth motion (three strokes backward and three strokes forward). It was swabbed horizontally with one side of the swab sampler and swabbed vertically with the other side of the swab sampler that were wetted with 0.5 ml

of one of four different solvents (Ethanol, Isopropyl alcohol IPA, Acetone and Acetonitrile) before swabbing the coupon.

- Finally the head of the swab was dropped into vial containing 10 ml of diluent. Then sonicated for 10 minutes for extracting the residues, then the extracted samples were filtered for analysis.

  Olanzapine sample conc. = ([22.73 mg Olanzapine + 931.93 mg Placebo] / 100 ml ACN) * (5 ml / 50 ml ACN) * (0.1 ml / 10 ml Diluent) * 1000 = 0.2273 ppm

- 0.2273 ppm of Olanzapine standard and sample solutions were injected into the HPLC in triplicates.

4.13.1.2 Choosing the Optimum Swab Samplers

Three different swabbing samplers were used, cotton swab, kimwipes swab and polyester large Alpha TX715 swab sampler named normal swab.

Procedure:

- 100 µl of 0.02273 mg/ml Olanzapine sample solution were distributed evenly directly over 5 cm * 5 cm coupon surface area.
- After drying, the tested coupon surface was swabbed firmly and evenly in a back-and-forth motion (three strokes backward and three strokes forward). It swabbed horizontally with one side of the swab sampler and swabbed vertically

with the other side of the swab sampler using the three different swab samplers that were wetted with 0.5 ml Acetonitrile.

- Finally the head of the swab samplers were dropped into a vial containing 10 ml of diluent. Then sonicated for 10 minutes for residues extraction, after that the extracted samples were filtered for analysis.
- 0.2273 ppm of Olanzapine standard and sample solutions were injected into the HPLC in triplicates.

4.13.1.3 Recovery from Swab Test

Preparation and Procedure:

Swab recovery standard curve preparation

- From the stock Olanzapine standard solution prepared in section 4.12.1.5 different diluted solutions of Olanzapine standards were prepared with concentrations of (0.02273, 0.09092, 0.18184, 0.2273, 0.4546 ppm equivalent to 10%, 40%, 80%, 100% and 200% of the MACO concentration during swab test = 0.2273 ppm respectively) as in Table 4. 10:

Table 4. 10 Preparation of swab recovery standard solutions.

Conc. (ppm)	***% of MACO conc. (0.2273 ppm)***	***Volume (ml) from stock solution needed (2.273 ppm)***	***Diluted to***
0.02273	10	1	100
0.09092	40	2	50
0.18184	80	4	50
0.2273	100	5	50
0.4546	200	10	50

- Each standard solution was injected into the HPLC in triplicates.

Swab recovery samples preparation over 5 cm * 5 cm coupon

- Different sample solutions of Olanzapine standard with placebo were prepared with concentration 40%, 80%, 100% and 200% of the MACO concentration = 0.2273 ppm to spike on the coupon as in Table 4. 11:

Table 4. 11 Preparation of swab recovery sample solutions.

	Solution-1 40%	***Solution-2 80%***	***Solution-3 100%***	***Solution-4 200%***
Conc. Of analyzed sample from coupon	0.09092 ppm	0.18184 ppm	0.2273 ppm	0.4546 ppm
Weight of Olanzapine	22.73 mg / 100 ml*	22.73 mg / 100 ml*	22.73 mg / 100 ml*	22.73 mg / 100 ml*
Weight of placebo	0.93193 g*	0.93193 g*	0.93193 g*	0.93193 g*
Dilution	2 ml / 50 ml	4 ml / 50 ml	5 ml / 50 ml	10 ml / 50 ml

Test area	25 cm^2	25 cm^2	25 cm^2	25 cm^2
Spiking volume on coupon	0.1 ml	0.1 ml	0.1 ml	0.1 ml
Swab diluted volume	10 ml	10 ml	10 ml	10 ml

*: For each solution, the Olanzapine standard and placebo were weighed in the same volumetric flask.

- Then 100 µl from each sample solution were distributed evenly in triplicates directly over 5 cm * 5 cm coupon surface area. After drying, the polyester large Alpha TX715 swab samplers were wetted with 0.5 ml Acetonitrile solvent. Then the tested coupon surface of 5 cm x 5 cm was swabbed firmly and evenly in a back-and-forth motions (three strokes backward and three strokes forward). It was swabbed horizontally with one side of the swab sampler and swabbed vertically with the other side of swab sampler. Finally the head of the swab sampler was dropped into a vial containing 10 ml of diluent. Then sonicated for 10 minutes to extract the residues, and then the extracted samples were filtered for analysis using the validated test method.
- Blank of coupon background was prepared by distributed evenly 100 µl of diluent directly over 5 cm * 5 cm coupon surface area. After drying, the polyester large Alpha TX715 swab sampler was wetted with 0.5 ml of Acetonitrile solvent. Then the tested coupon surface was swabbed in the same way for the samples.
- Blank and samples solutions were injected into the HPLC in triplicates.

4.13.2 Execution During Rinse Test

4.13.2.1 Bin Mixer

The maximum allowable carry over for Bin Mixer during the rinse test = **0.45453 ppm**

Preparation and Procedure:

Rinse recovery standard curve preparation for Bin Mixer

- 45.453 mg of Olanzapine standard were weighed into 100 ml volumetric flask and dissolved with enough amounts of diluent [Mixture of water and acetonitrile (55:45, v/v)] using magnetic stirrer for 15 minutes. The volume was completed up to 100 ml and the concentration was 0.45453 mg/ml.
- 1 ml of the above solution was withdrawn and taken into 100 ml volumetric flask. The volume was adjusted with diluent up to 100 ml. The concentration was 0.0045453 mg/ml or 4.5453 ppm. “Title this diluent as stock standard solution”.
- Different diluted solutions of Olanzapine standards were prepared from the stock standard solution (0.045453, 0.181812, 0.363624, 0.45453, 0.90906 ppm equivalent to 10%, 40%, 80%, 100% and 200% of the MACO concentration of Olanzapine during rinse test for Bin Mixer = 0.45453 ppm respectively) as in Table 4. 12:

Table 4. 12 Preparation of rinse recovery standard solutions for Bin Mixer.

Conc. (ppm)	***% of MACO conc. (0.45453 ppm)***	***Volume (ml) from stock solution needed (4.5453 ppm)***	***Diluted to***
0.045453	10	1	100
0.181812	40	2	50
0.363624	80	4	50
0.45453	100	5	50
0.90906	200	10	50

- Each standard solution was injected into the HPLC instrument in triplicates.

Rinse recovery samples preparation for Bin Mixer over 5 cm * 5 cm coupon

- Different sample solutions of Olanzapine standard with placebo were prepared with concentrations of 40%, 80%, 100% and 200% of the MACO concentration of Olanzapine = 0.45453 ppm to be used for rinse test as in Table 4. 13:

Table 4. 13 Preparation of rinse recovery sample solutions for Bin Mixer.

	Solution-1 40%	***Solution-2 80%***	***Solution-3 100%***	***Solution-4 200%***
Conc. of analyzed sample from coupon	0.181812 ppm	0.363624 ppm	0.45453 ppm	0.90906 ppm
Weight of Olanzapine	45.453 mg / 100 ml*	45.453 mg / 100 ml*	45.453 mg / 100 ml*	45.453 mg / 100 ml*
Weight of placebo	1.863573 g*	1.863573 g*	1.863573 g*	1.863573 g*
Dilution	2 ml / 50 ml	4 ml / 50 ml	5 ml / 50 ml	10 ml / 50 ml

Spiking volume on coupon	0.1 ml	0.1 ml	0.1 ml	0.1 ml
Rinse diluted volume	10 ml	10 ml	10 ml	10 ml

*: For each solution, the Olanzapine standard and placebo were weighed in the same volumetric flask.

- Then 100 µl from each sample solution was distributed evenly in triplicates directly over 5 cm * 5 cm coupon surface area. The coupon was directly rinsed with 10 ml diluent, and then the collected samples were filtered for analysis using the validated analytical method.
- Blank of coupon background was prepared by distributed evenly 100 µl of diluent directly over 5 cm * 5 cm coupon surface area. The coupon was directly rinsed with 10 ml diluent, and then the collected sample was filtered for analysis.
- Blank and samples solutions were injected into the HPLC in triplicates.

4.13.2.2 Coating Pan

The maximum allowable carry over for Coating Pan during the rinse test = **0.62489 ppm**

Preparation and Procedure:

Rinse recovery standard curve preparation for Coating Pan

- 62.489 mg of Olanzapine standard were accurately weighed into a 100- ml volumetric flask and dissolved with enough amounts of diluent [Mixture of water and acetonitrile (55:45, v/v)] using magnetic stirrer for 15 minutes. The volume was completed and adjusted up to 100 ml, the concentration of the solution was 0.62489 mg/ml.
- 1 ml of 0.62489 mg/ml solution was withdrawn and taken into 100- ml volumetric flask. The volume was adjusted with diluent up to 100 ml. The concentration was 0.0062489 mg/ml or 6.2489 ppm. “Title this diluent as stock solution”.
- Different diluted solutions of Olanzapine standards were prepared from the stock standard solution (0.062489, 0.249956, 0.499912, 0.62489, 1.24978 ppm equivalent to 10%, 40%, 80%, 100% and 200% of the MACO concentration of Olanzapine during rinse test for Coating Pan = 0.62489 ppm respectively) as in Table 4. 14:

Table 4. 14 Preparation of rinse recovery standard solutions for Coating Pan.

Conc. (ppm)	***% of MACO conc. (0.62489 ppm)***	***Volume (ml) from stock solution needed (6.2489 ppm)***	***Diluted to***
0.062489	10	1	100
0.249956	40	2	50
0.499912	80	4	50
0.62489	100	5	50
1.24978	200	10	50

- Each standard solution was injected into the HPLC instrument in triplicates.

Rinse recovery samples preparation for Coating Pan over 5 cm * 5 cm coupon

- Different sample solutions of Olanzapine standard with placebo were prepared with concentrations of 40%, 80%, 100% and 200% of the MACO concentration of Olanzapine = 0.62489 ppm to be used for rinse test as in Table 4. 15:

Table 4. 15 Preparation of rinse recovery sample solutions for Coating Pan.

	Solution-1 40%	***Solution-2 80%***	***Solution-3 100%***	***Solution-4 200%***
Conc. of analyzed sample from coupon	0.249956 ppm	0.499912 ppm	0.62489 ppm	1.24978 ppm
Weight of Olanzapine	62.489 mg / 100 ml*	62.489 mg / 100 ml*	62.489 mg / 100 ml*	62.489 mg / 100 ml*
Weight of placebo	2.562049 g*	2.562049 g*	2.562049 g*	2.562049 g*
Dilution	2 ml / 50 ml	4 ml / 50 ml	5 ml / 50 ml	10 ml / 50 ml
Spiking volume on coupon	0.1 ml	0.1 ml	0.1 ml	0.1 ml
Rinse diluted volume	10 ml	10 ml	10 ml	10 ml

*: For each solution, the Olanzapine standard and placebo were weighed in the same volumetric flask.

- Then 100 µl from each sample solution were distributed evenly in triplicates directly over 5 cm * 5 cm coupon surface area. The coupon was directly rinsed with 10 ml diluent, and then the collected samples were filtered for analysis.

- Blank of coupon background was prepared by distributed evenly 100 µl of diluent directly over 5 cm * 5 cm coupon surface area. The coupon was rinsed with 10 ml diluent, and then the collected sample was filtered for analysis.
- Blank and samples solutions were injected into the HPLC system in triplicates

4.13.3 Execution During Soak Test

4.13.3.1 Tablet Press Punches

The maximum allowable carry over for Tablet Press for the three Punches during the soak test = **0.020980 ppm**.

In tablet press the upper and lower punches were soaked in 10 ml of diluent, for 10 minutes on sonicator. The percentage recovery for this method was calculated.

Preparation and Procedure:

Soak recovery standard curve for Tablet Press Punches and Dies were prepared as swab recovery standard curve. See section 4.13.1.3.

Soak recovery samples preparation for Tablet Press Punches

- Different sample solutions of Olanzapine standard with placebo were prepared with concentrations of 40%, 80%, 100% and 200% of the MACO concentration of Olanzapine = 0.2273 ppm to use for soak test as in Table 4. 16:

Table 4. 16 Preparation of soak recovery sample solutions for Tablet Press Punches.

	Solution-1 40%	***Solution-2 80%***	***Solution-3 100%***	***Solution-4 200%***
Conc. of analyzed sample from coupon	0.09092 ppm	0.18184 ppm	0.2273 ppm	0.4546 ppm
Weight of Olanzapine	22.73 mg / 100 ml*	22.73 mg / 100 ml*	22.73 mg / 100 ml*	22.73 mg / 100 ml*
Weight of placebo	0.93193 g*	0.93193 g*	0.93193 g*	0.93193 g*
Dilution	2 ml / 50 ml	4 ml / 50 ml	5 ml / 50 ml	10 ml / 50 ml
Spiking volume on coupon	0.1 ml	0.1 ml	0.1 ml	0.1 ml
Soaking volume	10 ml	10 ml	10 ml	10 ml

*: For each solution, the Olanzapine standard and placebo were weighed in the same volumetric flask.

- Then 100 µl from each sample solution were distributed evenly in triplicate directly over the upper and lower tablet press punches (16.7 µl on each one). After drying, the punches were soaked with 10 ml of diluent using sonicator for 10 minutes, and then the extracted samples were filtered for analysis.
- Blank of coupon background was prepared by distributing evenly 100 µl of diluent directly over the upper and lower tablet press punches (16.7 µl on each one). After drying, the punches were soaked with 10 ml of diluent using sonicator for 10 minutes, and then the extracted sample was filtered for analysis.

- Blank and samples solutions were injected into the HPLC instrument in triplicates.

4.13.3.2 Tablet Press Dies

The maximum allowable carry over for Tablet Press of the three Dies during the soak test = **0.031817 ppm**.

In tablet press the three dies were soaked in 100 ml of diluent, for 10 minutes on a sonicator. The percentage recovery for this method was calculated.

Preparation and Procedure:

Soak recovery samples preparation for Tablet Press Dies

- Different sample solutions of Olanzapine standard with placebo were prepared with concentrations of 40%, 80%, 100% and 200% of the MACO concentration of Olanzapine = 0.2273 ppm to be used for soak test as in Table 4. 17:

Table 4. 17 Preparation of soak recovery sample solutions for Tablet Press Dies.

	Solution-1 40%	***Solution-2 80%***	***Solution-3 100%***	***Solution-4 200%***
Conc. of analyzed sample from coupon	0.09092 ppm	0.18184 ppm	0.2273 ppm	0.4546 ppm
Weight of Olanzapine	227.3 mg / 100 ml*	227.3 mg / 100 ml*	227.3 mg / 100 ml*	227.3 mg / 100 ml*
Weight of placebo	9.3193 g*	9.3193 g*	9.3193 g*	9.3193 g*
Dilution	2 ml / 50 ml	4 ml / 50 ml	5 ml / 50 ml	10 ml / 50 ml

Spiking volume on coupon	0.1 ml	0.1 ml	0.1 ml	0.1 ml
Soaking volume	100 ml	100 ml	100 ml	100 ml

*: For each solution, the Olanzapine standard and placebo were weighed in the same volumetric flask.

- Then 100 µl from each sample solution were distributed evenly in triplicates directly over the three dies (33.3 µl on each one). After drying, the dies were soaked with 100 ml of diluent using sonicator for 10 minutes, and then the extracted samples were filtered for analysis.
- Blank of coupon background was prepared by distributing evenly 100 µl of diluent directly over the dies (33.3 µl on each one). After drying, the dies were soaked with 100 ml of diluent using a sonicator for 10 minutes, and then the extracted sample was filtered for analysis.
- Blank and samples solutions were injected into the HPLC in triplicates.

Acceptance criteria for recovery test from coupons:

According to WHO _TRS_937 guidelines[25], if the result of recovery factor is more than 80%, then the sampling technique is considered good, and if it is more than 50% then the technique considered reasonable, but if the recovery is less than 50%, then the sampling technique is questionable.

4.14 Implementation of Cleaning Procedure Using Pilot Scale Product and Equipment

In our study, pilot scale of Olanzapine product was manufactured on equipment in SDI in Birzeit University. Then cleaning procedure was implemented on Bin Mixer, Press Machine and Coating Pan. To validate the effectiveness of cleaning procedure, swab and rinse samples were taken from worst-case positions as mentioned in section 3.7.

4.14.1 Bin Mixer

Olanzapine standard curve for both swab and rinse sampling techniques was prepared as following.

4.14.1.1 Execution During Swab Test

Olanzapine standard curve preparation for swab technique was prepared as swab recovery standard curve. See section 4.13.1.3.

Swab technique was applied over 5 cm * 5 cm of Bin Mixer surface

Swab technique was applied on the worst-case Bin Mixer sampling locations as clarified in section 3.7. The filtered samples were injected into the HPLC instrument, then the concentration of Olanzapine in swabbed samples were calculated from the equation of Olanzapine standard curve for swab sampling technique. Finally, the actual quantity for Olanzapine residues found on the machine surface was obtained by dividing the result by the swab recovery factor for the swab sampling technique.

Acceptance criteria for Bin Mixer swab test:

Olanzapine residue should not be more than the MACO concentration of the swab sampling technique that equal 0.2273 ppm/swab of 5 cm x 5 cm as determined by our study.

4.14.1.2 Execution During Rinse Test

The maximum allowable carry over for Bin Mixer during the rinse sampling test using our study = **0.45453 ppm**

Olanzapine standard curve preparation for rinse technique was prepared as rinse recovery standard curve preparation for Bin Mixer. See section 4.13.2.1.

Rinse technique was applied on Bin Mixer surface to detect Olanzapine and SLS residue

Rinse technique was applied on Bin Mixer surface by washing the internal surface of the Bin Mixer with 1000 ml of purified water as clarified in section 3.7. The filtered sample was injected in HPLC instrument, and then the concentration of Olanzapine in the final rinse sample was calculated from the equation of Olanzapine standard curve for Bin Mixer rinse test. Finally, the actual quantity for Olanzapine residue found on the machine surface was obtained by dividing the result by the rinse recovery factor for the rinse sampling technique. The SLS cleaning agent residue was determined by measuring the conductivity of the final rinse water from the Bin Mixer and compare

the reading with water conductivity before been used for rinsing. The difference shall not be more than 0.2 µs/cm.

Acceptance criteria for Bin Mixer rinse test:

Olanzapine residue should not be more than the MACO concentration of rinse sampling technique for Bin Mixer that equal 0.45453 ppm as determined by our study. In addition, the conductivity of purified water should be equal to that for the final rinse ± 0.2 µs/cm, to ensure that the cleaning agent (SLS) was completely removed.

4.14.2 Tablet Press

In Tablet Press, swab and soak sampling techniques were used, and both have the same Olanzapine standard curve.

4.14.2.1 Execution During Swab and Soak Sampling Techniques

Olanzapine standard curve preparation for swab and soak sampling techniques was prepared as swab recovery standard curve. See section 4.13.1.3.

Swab sampling technique was applied over 5 cm * 5 cm of Tablet Press surface

Swab sampling technique was applied on the worst-case Tablet Press sampling locations as clarified in section 3.7. The filtered samples were injected into the HPLC instrument, then the concentration of Olanzapine in swab samples were calculated from

the equation of Olanzapine standard curve for swab and soak technique. Finally, the actual quantity for Olanzapine residue found on the machine surface was obtained by dividing the result by the swab recovery factor for the swab sampling technique.

Soak sampling technique was applied on Tablet Press Punches and Dies surfaces to detect Olanzapine residue

Punches and Dies of Tablet Press machine were soaked in 10 ml and 100 ml diluent respectively as clarified in section 3.7. The filtered samples were injected into the HPLC instrument, then the concentration of Olanzapine in soak samples were calculated from the equation of Olanzapine standard curve for swab and soak techniques. Finally, the actual quantity for Olanzapine residue found on the machine surface was obtained by dividing the result by the soak recovery factor for the soak sampling technique.

Soak sampling technique was applied on Tablet Press Punches and Dies surfaces to detect SLS residues

To detect the cleaning agent (SLS) residues, the soak technique was repeated by soak separately the tablet press Punches and Dies in 100 ml purified water for 10 minutes. In addition, the conductivity for this soak water was measured and the reading was compared with water conductivity before been used for soaking to determine the SLS cleaning agent residues. The difference shall not be more than ± 0.2 µs/cm.

Acceptance criteria for Tablet Press swab and soak sampling tests:

Olanzapine residue for swab sampling test should not be more than the MACO concentration of swab sampling technique that equal 0.2273 ppm/swab of 5 cm x 5 cm, and for soak test it also not more than 0.2273 ppm as determined by our study. In addition, the conductivity of purified water should be equal to that for the soak water ± 0.2 µs/cm, to ensure that the cleaning agent (SLS) was completely removed.

4.14.3 Coating Pan

Olanzapine standard curve for both swab and rinse sampling techniques were prepared as following.

4.14.3.1 Execution During Swab Test

Olanzapine standard curve preparation for swab technique was prepared as swab recovery standard curve. See section 4.13.1.3.

Swab sampling technique was applied over 5 cm * 5 cm of Coating Pan surface

Swab sampling technique was applied on the worst-case Coating Pan sampling locations as clarified in section 3.7. The filtered samples were injected into the HPLC instrument, then the concentration of Olanzapine in swab samples were calculated from the equation of Olanzapine standard curve for swab sampling technique. Finally, the

actual quantity for Olanzapine residue found on the machine surface was obtained by dividing the result by the swab recovery factor for the swab sampling technique.

Acceptance criteria for Coating Pan swab test:

Olanzapine residue should not be more than the MACO concentration of swab sampling technique that equal 0.2273 ppm/swab of 5 cm x 5 cm as determined by our study.

4.14.3.2 Execution During Rinse Sampling Test

The maximum allowable carry over for Coating Pan during the rinse test using our study = **0.62489 ppm**

Olanzapine standard curve preparation for rinse sampling technique were prepared as rinse recovery standard curve preparation for Coating Pan. See section 4.13.2.2.

Rinse sampling technique was applied on Coating Pan surface to detect Olanzapine and SLS residue

Rinse sampling technique was applied on Coating Pan surface by washing the internal surface of the Coating Pan with 5000 ml of purified water as clarified in section 3.7. The filtered sample was injected into the HPLC instrument, and then the concentration of Olanzapine in the final rinse sample was calculated from the equation of Olanzapine

standard curve for Coating Pan rinse test. Finally, the actual quantity for Olanzapine residue found on the machine surface was obtained by dividing the result by the rinse recovery factor for the rinse sampling technique. The SLS cleaning agent residue was determined by measuring the conductivity of the final rinse water from the Coating Pan and compare the reading with water conductivity before been used for rinsing. The difference shall not be more than 0.2 µs/cm.

Acceptance criteria for Coating Pan rinse sampling test:

Olanzapine residue should not be more than the MACO concentration of rinse sampling technique for Coating Pan that equal 0.62489 ppm as determined by our study. In addition, the conductivity of purified water should be equal to that for the final rinse ± 0.2 µs/cm, to ensure that the cleaning agent (SLS) was completely removed.

4.15 Microbiological Contamination Test

In our cleaning validation study we used contact plate or agar RODAC plates (for flat surfaces) to detect microbial contaminants from Bin Mixer, Tablet Press and Coating Pan worst-case locations as clarified in section 3.7.

4.15.1 Contact Plate Preparation Method

Contact Plate preparation method was mentioned in procedure for determining the microbial contaminants. See section 3.6.3.

Acceptance criteria for microbiological contaminants test:

- Total Bacterial Counts should be NMT 50 CFU/ 25 cm^2.
- Absence of indicator microorganisms (E.Coli, Staph.aureus, Pseudomonas and Salmonella).
- Absence of yeast and mold.

Part Five: Results and Discussions

5.1 Test Method Validation

5.1.1 Specificity (Placebo Interference)

The specificity was evaluated by injecting the nominal Olanzapine standard solution (0.2273 ppm), the nominal Olanzapine placebo tablet solution (9.32 ppm), the nominal spiked sample solution and the diluent, to insure that there are no peaks appear at the Olanzapine retention time.

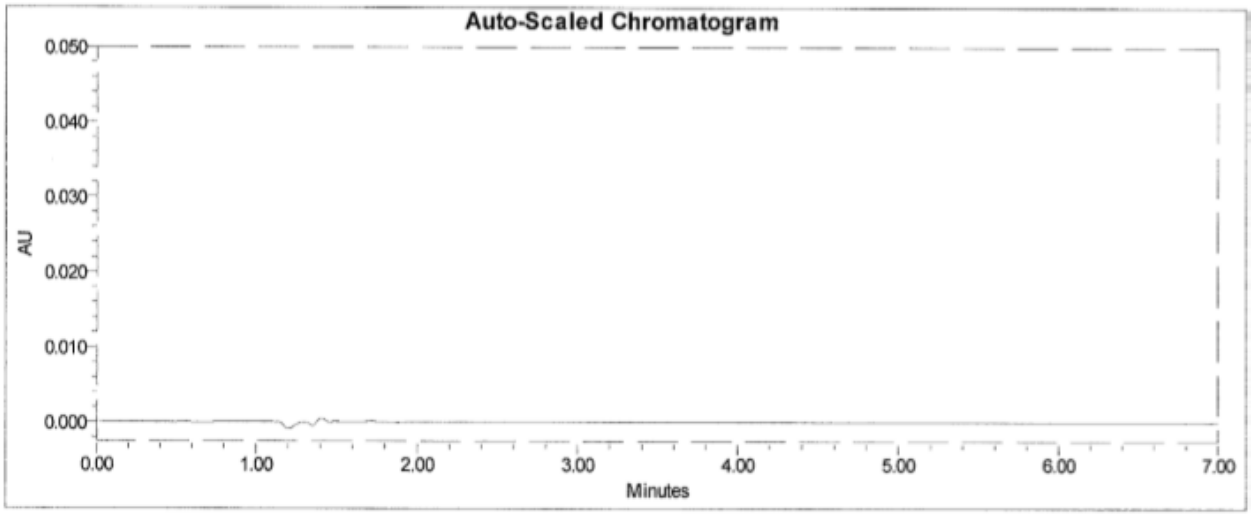


Figure 5. 1 Chromatograph for diluent, a mixture of water and Acetonitrile (55:45, v/v).

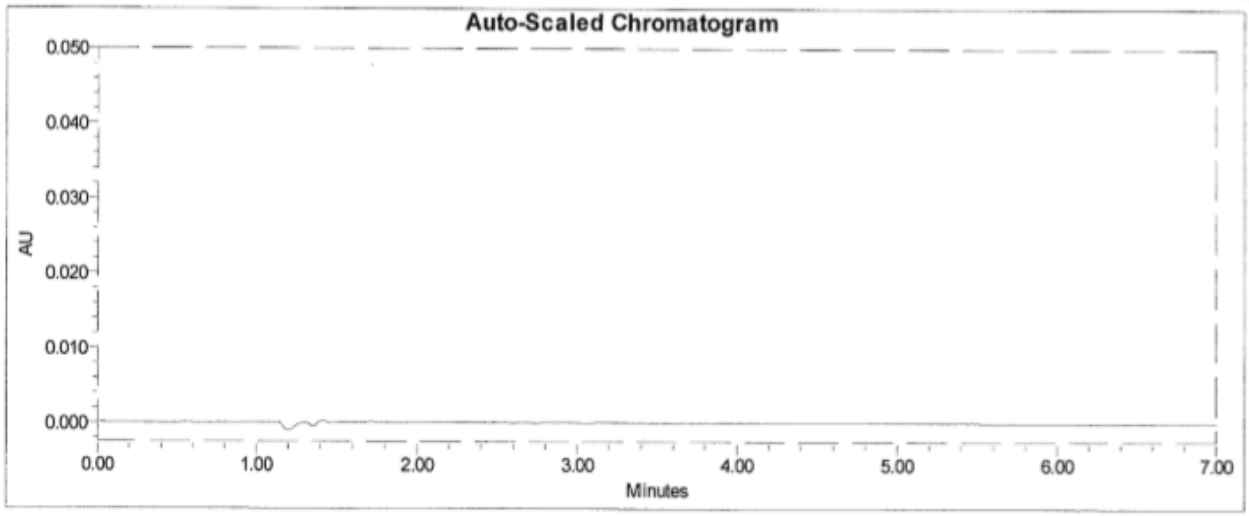


Figure 5. 2 Chromatograph for Olanzapine placebo tablets.

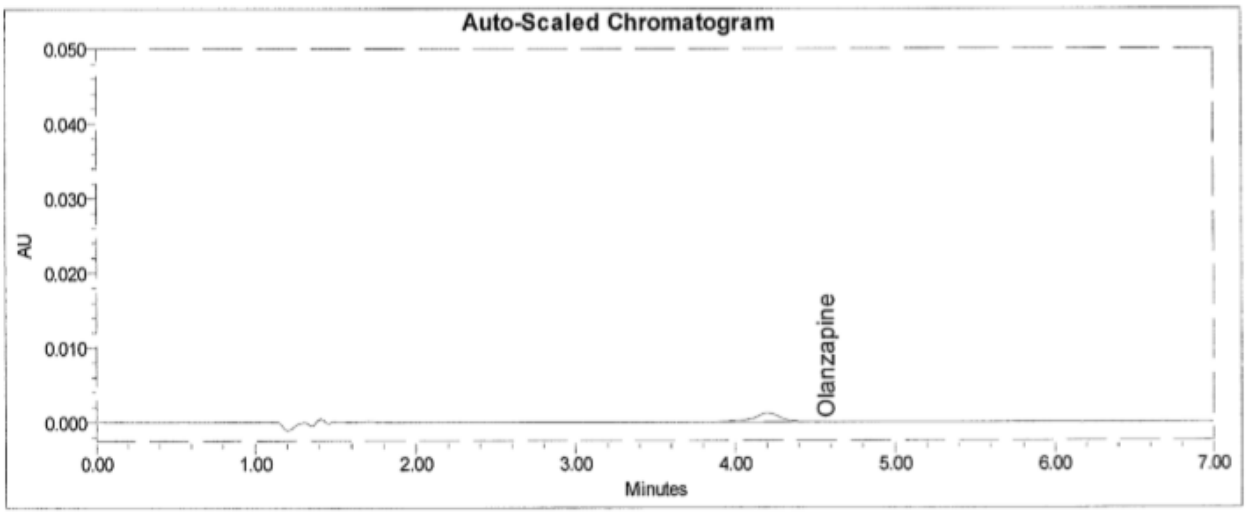


Figure 5. 3 Chromatograph for Olanzapine standard solution (0.2273 ppm).

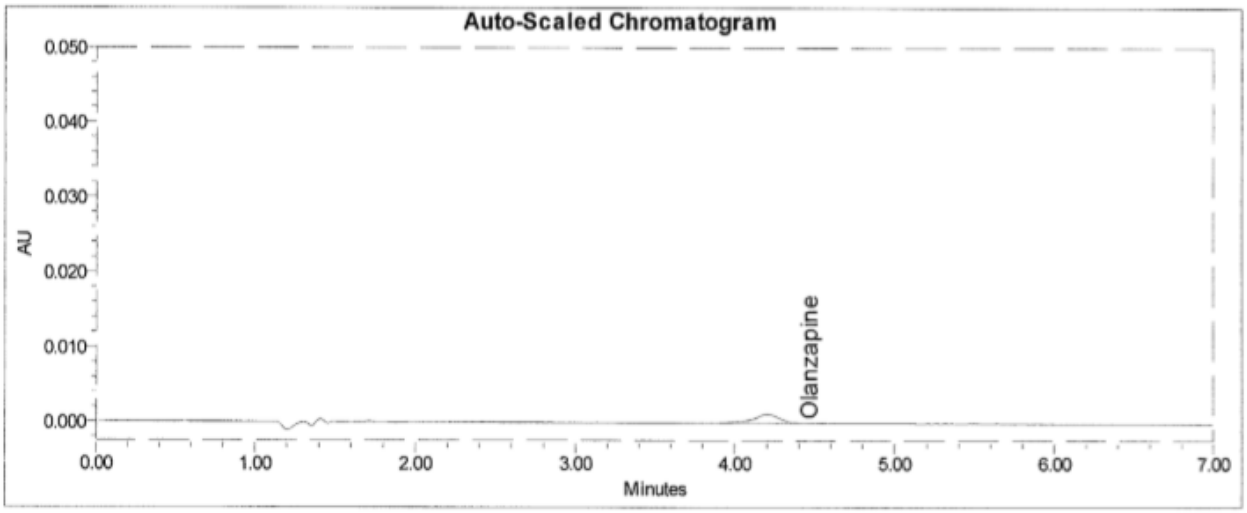


Figure 5. 4 Chromatograph for Olanzapine spiked sample solution.

From the above chromatograms (Figure 5. 1 – Figure 5. 4) for the nominal Olanzapine standard solution (0.2273 ppm), the nominal Olanzapine Placebo tablet solution (9.32 ppm), the nominal spiked sample solution and the diluent, it is clear that the method is specific for Olanzapine since there are no interfering peaks appear at the Olanzapine retention time.

5.1.2 Limit of Detection and Limit of Quantitation (LOD & LOQ)

Different concentrations of standard solution were prepared using the stock standard solution (2.273 ppm) to prepare separate standards covering the range between (2 to 20%) of the nominal concentration of Olanzapine. Data and results are summarized in Table 5. 1:

Table 5. 1 LOD & LOQ data and results.

Conc. (ppm)	*Area (AU)*	*Average Area*	*SD*	*RSD (%)*
0.004546	293, 265, 255	271.00	19.70	7.27
0.006819	434, 426, 415	425.00	9.54	2.24
0.009092	564, 526, 554	548.00	19.70	3.59
0.013638	845, 799, 748	797.33	48.52	6.09
0.02273	1227, 1357, 1410	1331.33	94.16	7.07
0.04546	2407, 2677, 2529	2537.67	135.21	5.33

After drawing Area VS. STD concentration curve Figure 5. 5, the equation was: $y = 55177x + 44.433$, where $R^2 = 0.9995$, slope = 55177 and Y-intercept = 44.433. The repetitions of standard solutions over the range of linearity are precise with RSD less than 10% for all levels.

The LOD and LOQ values were calculated by the following equations:

LOD = 3.3 σ/S, where σ is the standard deviation of the response, and S is the slope of the calibration curve.

Standard deviation σ was calculated.

Standard deviation SD = standard error SE * √n, where n is the number of points in the linearity curve

SE = 14.0603, SD = 34.441

Then the limit of detection LOD = (3.3 * 34.441) / 55177 = 0.002 ppm.

LOQ = 10 σ/S

Then the limit of quantification LOQ = (10 * 34.441) / 55177 = 0.006 ppm.

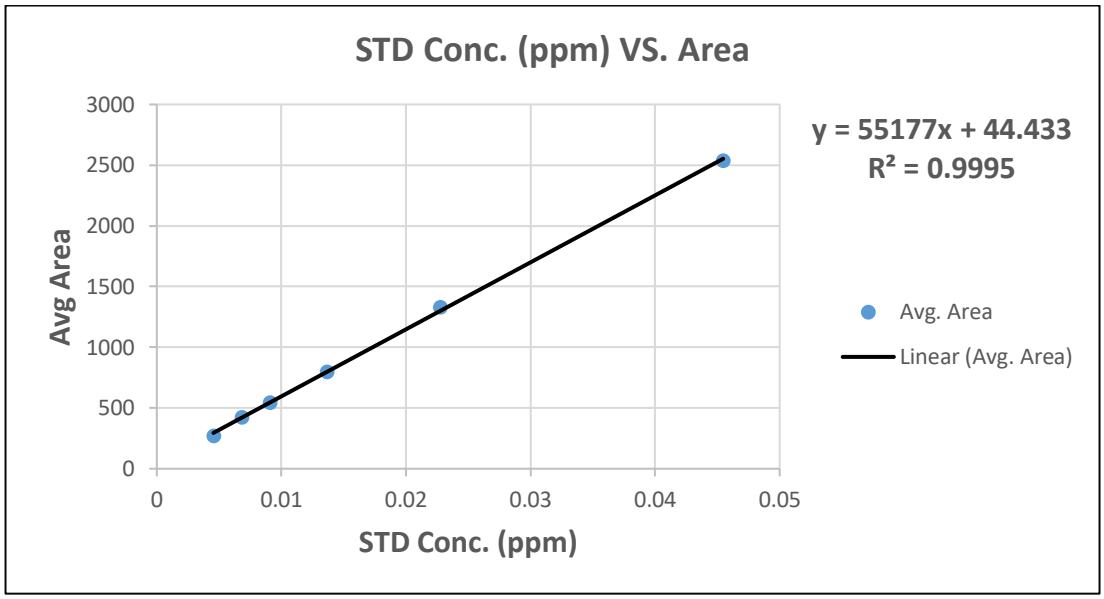


Figure 5. 5 LOD & LOQ data plot.

5.1.3 Linearity and Range

Different concentrations of standard solution were prepared using the stock standard solution (2.273 ppm) to prepare separate standards covering the range between (3 to 200%) of the nominal concentration of Olanzapine. Data and results are summarized in Table 5. 2:

Table 5. 2 Linearity and range data and results.

Conc. (ppm)	*Area(AU)*	*Average Area*	*SD*	*RSD (%)*
0.006819	434, 426, 415	425.00	9.54	2.24
0.009092	564, 526, 554	548.00	19.70	3.59
0.013638	788, 799, 748	778.33	26.84	3.45
0.02273	1227, 1357, 1301	1295.00	65.21	5.04
0.04546	2407, 2677, 2529	2537.67	135.21	5.33

0.11365	6689, 6522, 6712	6641.00	103.70	1.56
0.18184	10398, 10346, 10437	10393.67	45.65	0.44
0.2273	13386, 13540, 13481	13469.00	77.70	0.58
0.4546	27809, 27758, 27729	27765.33	40.50	0.15

After drawing Area VS. STD concentration plot Figure 5. 6, the equation was: $y = 60769x - 164.59$, where $R^2 = 0.9993$ that more than 0.980, slope = 60769, Y-intercept = - 164.59.

From this equation, we can conclude that the current method is linear with range between (3 to 200%) of the nominal concentration.

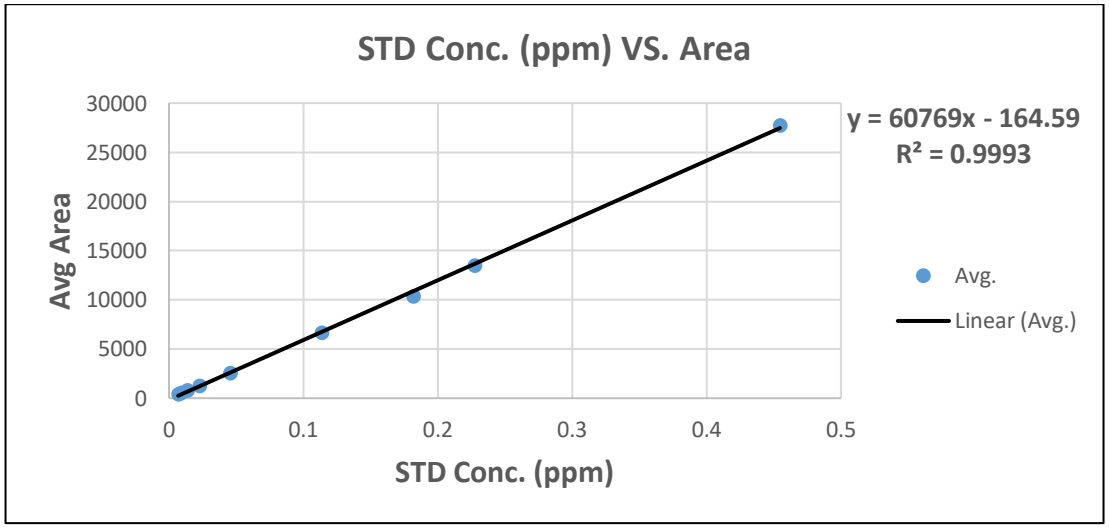


Figure 5. 6 Linearity and range data plot.

5.1.4 Accuracy

A mixture of the drug product components (Placebo) was spiked with known amounts of Olanzapine (10%, 100%, and 200%) of the nominal concentration (0.2273 ppm) named spiked samples were prepared. 3-solutions were prepared for each concentration, and then the recovery was calculated, by dividing sample area by standard area, multiplied by 100% as in Table 5. 3:

Table 5. 3 Accuracy test data and results.

Average standard area for 10% of nominal concentration (0.02273 ppm) = (1440 + 1472)/2 = 1456				
Vial #	***Conc. (ppm)***	***Sa. Area (AU)***	***% Accuracy (Recovery)***	***Average & RSD***
1	0.02273	1423	97.7	97.0% & 0.8
2	0.02273	1401	96.2	
3	0.02273	1412	97.0	
Average standard area for 100% of nominal concentration (0.2273 ppm) = (14397 + 14110)/2 = 14253.5				
1	0.2273	14205	99.7	99.7% & 0.0
2	0.2273	14216	99.7	
3	0.2273	14206	99.7	
Average standard area for 200% of nominal concentration (0.4546 ppm) = (27315 + 27113)/2 = 27214				
1	0.4546	27188	99.9	100.1% & 0.3
2	0.4546	27362	100.5	
3	0.4546	27176	99.9	

Olanzapine solutions with concentrations 10%, 100% and 200% of the nominal concentration were analyzed according to the chromatographic HPLC test method of analysis for Olanzapine, and then the recovery % was calculated for each level of Olanzapine. The average recovery results are 97.0%, 97.7%, 100.1% respectively and are within limits 85-115%. In addition, the RSD are less than 10.0%, so we can conclude that the method is accurate.

5.1.5 Precision (System Repeatability)

Six replicate injections were analyzed for the nominal standard concentration 0.2273 ppm of Olanzapine for determination of system precision, and six replicate injections were analyzed for the nominal sample for determination of method precision during the same day (intraday precision) as in Table 5. 4:

Table 5. 4 Precision test data and results.

System precision at nominal standard concentration 0.2273 ppm		
Vial #	***Retention time (Minutes)***	***Area (AU)***
1	4.206	14229
2	4.205	14462
3	4.202	14106
4	4.211	14237
5	4.207	14541
6	4.205	14145
Average	4.206	14286.67
RSD	0.071%	1.228%
Method precision at nominal sample concentration 0.2273 ppm		

1	4.202	14602
2	4.201	14299
3	4.202	14439
4	4.203	14376
5	4.199	14325
6	4.201	14224
Average	4.201	14377.5
RSD	0.033%	0.916%
Limit	**RSD NMT 10.0%**	

The system precision and method precision in the cleaning validation method was evaluated with the relative standard deviation not more than 10.0%. So from the RSD results in Table 5. 4, the method is precise.

5.1.6 Ruggedness (Intermediate Precision)

Ruggedness was studied through the analysis of six replicate injections for the nominal standard concentration 0.2273 ppm of Olanzapine under a variation of analyst, instrument and analysis days within the same lab as in Table 5. 5:

Table 5. 5 Ruggedness test data and results.

Vial #	***Retention time (Minutes)***	***Area (AU)***
1	4.707	0.224
2	4.713	0.228
3	4.720	0.222
4	4.720	0.228
5	4.727	0.228

6	4.730	0.230
Average	4.720	0.227
RSD	0.181%	1.328%
Limit	**RSD NMT 10.0%**	

Ruggedness was studied through the analysis of six replicate injections for the nominal standard concentration 0.2273 ppm of Olanzapine under a variation of analyst, instrument (HPLC instrument: Dionex Ultimate 3000 system) and analysis days. The RSD result is about 0.18% and 1.33% for retention time and Area respectively. The results are within the limit (RSD NMT 10.0%). This indicate that the method is precise within laboratory variation.

5.1.7 Robustness

The robustness of the method was investigated by making small deliberate changes in the chromatographic conditions for the nominal concentration 0.2273 ppm of Olanzapine as in Table 5. 6:

Table 5. 6 Robustness test data and results.

Chromatographic Conditions	***Variation***	***Peak area precision***		***Retention time precision***		***Avg. Tailing factor (NMT 2.0)***
		Avg. Area*	***RSD (%)***	***Avg. R. Time****	***RSD (%)***	
Normal condition	-	13469.00	0.577	4.353	0.000	0.9877
Flow rate	0.9 ml/min	15233.50	1.086	5.129	0.082	1.0382
± 10%	1.1 ml/min	12255.33	0.768	4.218	0.083	1.0288
Wavelength	252 nm	13603.17	0.703	4.616	0.052	0.9733
± 2 nm	256 nm	13708.83	1.447	4.616	0.029	0.9690
M.Ph. composition	50:50 (ACN:Buffer)	13314.83	1.225	6.181	0.126	1.0349
± 5%	60:40	13463.33	1.163	4.154	0.360	1.0606
Temperature	35 ˚C	13631.17	0.231	4.654	0.084	1.0815
± 5˚C	45 ˚C	13651.17	0.173	4.655	0.046	1.0674
pH of the M.Ph.	pH 7.3	13216.67	0.719	5.019	0.109	1.0189
± 0.1 units	pH 7.5	13459.50	0.807	4.869	0.091	1.0523
pH of the M.Ph.	pH 7.2**	0.2255	0.830	4.727	0.099	1.0383
± 0.2 units	pH 7.6**	0.2252	1.471	4.928	0.102	1.0267

*: Average value of six injections.

**: Robustness at pH of the M.Ph. ± 0.2 units measured using different HPLC instrument (Dionex Ultimate 3000 system)

Robustness was studied through analysis of nominal standard solution 0.2273 ppm of Olanzapine under a variation of method parameters (flow rate, wavelength, M. Ph.

Composition, temperature and pH of the mobile phase). Referring to the results in Table 5. 6, the method is robust to all changes in the chromatographic conditions, since the RSD value less than 10.0% and the tailing factor in all parameters were less than 2.

5.1.8 Technology Transfer (Comparative Analysis)

To qualify this analytical method for comparative transfer, three separate standard and sample solutions of 10%, 100%, 200% of the nominal concentration of Olanzapine (0.2273 ppm) were prepared then the recovery data was calculated for each determination. Data and results are summarized in Table 5. 7:

Table 5. 7 Technology Transfer data and results.

Average standard area for 10% of nominal concentration (0.02273 ppm) = (1.310 + 1.307 + 1.312) / 3 = 1.310					
Conc. (ppm)	***Area Sample (AU)***	***Avg. Area***	***% Accuracy (Recovery) in receiving unit***	***% Accuracy (Recovery) in transferring unit***	***Differ NMT 15%***
0.02273	1.408, 1.437, 1.397	1.414	107.9	97.7	10.2
Average standard area for 100% of nominal concentration (0.2273 ppm) = (13.958 + 13.901 + 13.948) / 3 = 13.936					
0.2273	14.693,14.623, 14.593	14.636	105.0	99.7	5.3
Average standard area for 200% of nominal concentration (0.4546 ppm) = (28.213 + 28.268 + 28.216) / 3 = 28.232					
0.4546	29.538,	29.481	104.4	99.9	4.5

	29.413, 29.492

Technology Transfer by comparative analysis was studied through analysis of three separate standard and sample solutions of 10%, 100%, 200% of the nominal concentration of Olanzapine (0.2273 ppm). The recovery results for each determination was within 85-115% at each concentration. In addition, results in the receiving unit differ than that in the transferring unit with NMT than 15% as in Table 5. 7.

5.1.9 Solution Stability

Solutions stability was studied through analysis of nominal standard solution 0.2273 ppm of Olanzapine at freshly prepared time, after 24 hours and 48 hours at 25˚C in HPLC vial tray, at 5˚C in the refrigerator and at - 21.5˚C in freezer. Assays of the analyte in each solution were calculated as in Table 5. 8:

Table 5. 8 Stability test data and results.

STD conc.	***50%***	***100%***	***200%***
Freshly STD avg. area 27/03/2018	6732.67	13113.00	28177.33
Freshly STD avg. area 28/03/2018	6631.00	13447.33	27922.33
Avg. area after 24 hrs. @ 25˚C in HPLC vial tray 28/03/2018	6683.00	13040.00	27595.67
Degree of stability after 24 hrs.	**100.8%**	**97.0%**	**98.8%**

Freshly STD avg. area 29/03/2018	6687.00	13239.33	28021.33
Avg. area after 48 hrs. @ 25˚C in HPLC vial tray 29/03/2018	6346.67	12464.00	27305.33
Degree of stability after 48 hrs.	**94.9%**	**94.1%**	**97.4%**
Freshly STD avg. area	6641.00	13469.00	27765.33
Avg. area after 24 hrs. @ 5˚C in the refrigerator	3741.67	13371.00	23670.67
Degree of stability after 24 hrs. in the refrigerator	**56.3%**	**99.3%**	**85.3%**
Freshly STD avg. area	6641.00	13469.00	27765.33
Avg. area after 24 hrs. @ -21.5˚C in freezer	3374.33	10499.00	17324.67
Degree of stability after 24 hrs. in freezer	**50.8%**	**78.0%**	**62.4%**

The acceptance criteria for average assay is 85-115% with RSD ≤ 10.0%, so from the results in Table 5. 8, we can conclude that Olanzapine standard solution was stable for 48 hours if stored at about 25 ˚C. However, it is not stable in refrigerator and in freezer.

5.1.10 Filter Compatibility

Filter compatibility test was studied through analysis of nominal Olanzapine standard solution (0.2273 ppm) and nominal spiked sample solution (0.2273 ppm Olanzapine and 9.32 ppm Olanzapine placebo tablet), using centrifuge (as a standard), nylon and PTFE filters as in Table 5. 9:

Table 5. 9 Filter compatibility test data and results.

	Average standard area* (AU)	***Average sample area* (AU)***	***Assay (%)***
Centrifuge	13483.67	13718.33	101.7
Nylon filter	13615.00	13630.33	100.1
PTFE filter	13598.00	13616.67	100.1
Average	13565.56	13655.11	100.63%
RSD	0.527%	0.404%	0.918%

*: Average value of three injections.

Referring to the results in Table 5. 9, it is clear that nylon and PTFE filters are both suitable to use in Olanzapine analysis for cleaning validation. Since the average assay for all solutions are within 85-115% and the RSD percent are less than 10.0%.

5.1.11 System Suitability

The system suitability test was studied through the analysis of six replicate injections for the nominal standard concentration 0.2273 ppm of Olanzapine as in Table 5. 10:

Table 5. 10 System suitability test data and results.

Vial #	***Retention time (Minutes)***	***Area (AU)***	***T***	***K'***	***N***
1	4.206	14229	0.84	41.1	4288
2	4.205	14462	0.83	41.1	4321
3	4.202	14106	0.87	41.0	4336
4	4.211	14237	0.86	41.1	4249
5	4.207	14541	0.85	41.1	4086
6	4.205	14145	0.86	41.1	4213
Average	4.206	14286.67	0.85	41.1	4248.8
RSD	0.071%	1.228%	1.728%	0.099%	2.161%

The relative standard deviation for peak areas were 1.228 < 2.0% and for peak retention time were 0.071 < 1.0%. The average tailing factor was 0.85 which less than 2. Also the average capacity factor was 41.1 which more than 2. Finally the average theoretical plates was 4248.8 which is also higher than 2000.

The following system suitability parameters recorded in Table 5. 11 were recommended for routine analysis:

Table 5. 11 Routine analysis system suitability parameters.

	RSD for Area	***RSD for Retention time***	***Tailing Factor (T)***	***Capacity Factor (K')***	***Theoretical Plates (N)***
Exact result	1.228	0.071	0.85	41.1	4248.8
Accepted result	NMT* 2.0%	NMT* 1.0%	NMT* 2.0	NLT** 2	NLT** 2000

*: Not more than.

**: Not less than.

5.2 Recovery Test from Coupons

5.2.1 Execution During Swab Test

5.2.1.1 Choosing the Optimum Solvent for Swab Wetting

The polyester large Alpha TX715 swab sampler for cleaning validation sampling was wetted with 0.5 ml of each of the four different solvents (Ethanol, Isopropyl alcohol IPA, Acetone and Acetonitrile) before performing the coupon swabbing. Then after coupon sampling the swab was dropped into an accurately measured volume of 10 ml of the diluent.

- **For 0.2273 ppm Olanzapine standard**

Table 5. 12 Results for 0.2273 ppm Olanzapine standard solution.

Conc. (ppm)	*Area (AU)*	*Avg. Area*	*SD*	*RSD (%)*
0.2273	0.211, 0.211, 0.211	0.211	0	0.0

- **For 0.2273 ppm Olanzapine sample with swab sampler wetted with Ethanol**

Table 5. 13 Results for 0.2273 ppm Olanzapine sample with swab sampler wetted with Ethanol.

Conc. (ppm)	*Area (AU)*	*Avg. Area*	*SD*	*RSD (%)*	*Recovery (%)*
0.2273	0.088, 0.086, 0.085	0.0863	0.0015	1.7693	40.90

- **For 0.2273 ppm Olanzapine sample with swab wetted with IPA**

Table 5. 14 Results for 0.2273 ppm Olanzapine sample with swab sampler wetted with IPA.

Conc. (ppm)	*Area (AU)*	*Avg. Area*	*SD*	*RSD (%)*	*Recovery (%)*
0.2273	0.079, 0.080, 0.079	0.0793	0.0006	0.7278	37.58

- **For 0.2273 ppm Olanzapine sample with swab sampler wetted with Acetone**

Table 5. 15 Results for 0.2273 ppm Olanzapine sample with swab sampler wetted with Acetone.

Conc. (ppm)	***Area (AU)***	***Avg. Area***	***SD***	***RSD (%)***	***Recovery (%)***
0.2273	0.063, 0.062, 0.061	0.0620	0.0010	1.6129	29.38

- **For 0.2273 ppm Olanzapine sample with swab sampler wetted with Acetonitrile**

Table 5. 16 Results for 0.2273 ppm Olanzapine sample with swab wetted with Acetonitrile.

Conc. (ppm)	***Area (AU)***	***Avg. Area***	***SD***	***RSD (%)***	***Recovery (%)***
0.2273	0.163, 0.159, 0.159	0.1603	0.0023	1.4404	75.97

From the results observed in (Table 5. 13 - Table 5. 16), it can be concluded that when the swab wetted with 0.5 ml Acetonitrile the % recovery was 75.97% which more than 70%. So the Acetonitrile is the optimum solvent for swab wetting.

5.2.1.2 Choosing the Optimum Swab Sampler Tools

We use three different tools for swabbing, the cotton swab, the Kimwipes swab and the polyester large Alpha TX715 swab sampler named normal swab.

- **For 0.2273 ppm Olanzapine sample using the cotton swab sampler**

Table 5. 17 Results for 0.2273 ppm Olanzapine sample using a cotton swab sampler.

Conc. (ppm)	***Area (AU)***	***Avg. Area***	***SD***	***RSD (%)***	***Recovery (%)***
0.2273	0.036, 0.035, 0.037	0.0360	0.0010	2.7778	17.06

- **For 0.2273 ppm Olanzapine sample using Kimwipes swab sampler**

Table 5. 18 Results for 0.2273 ppm Olanzapine sample using Kimwipes swab sampler.

Conc. (ppm)	***Area (AU)***	***Avg. Area***	***SD***	***RSD (%)***	***Recovery (%)***
0.2273	0.049, 0.045, 0.048	0.0473	0.0021	4.3979	22.42

- **For 0.2273 ppm Olanzapine sample using polyester large Alpha TX715 swab sampler named normal swab**

Table 5. 19 Results for 0.2273 ppm Olanzapine sample using polyester large Alpha TX715 swab sampler.

Conc. (ppm)	***Area (AU)***	***Avg. Area***	***SD***	***RSD (%)***	***Recovery (%)***
0.2273	0.169, 0.169, 0.164	0.1673	0.0029	1.7252%	79.29%

From the results observed in (Table 5. 17 - Table 5. 19), it can be concluded that the optimum swab sampling tool is the polyester large Alpha TX715 swab sampler named normal swab since it gives the higher recovery value which is equal to 79.29%.

5.2.1.3 Recovery from Swab Sampling Test

Swab recovery standard curve

Different concentrations of standard solution were prepared using the stock standard solution (2.273 ppm) to prepare (0.02273, 0.09092, 0.18184, 0.2273, 0.4546 ppm that are equivalent to 10%, 40%, 80%, 100% and 200% of the MACO concentration during swab sampling test of Olanzapine = 0.2273 ppm). The data and results are shown in Table 5. 20.

Table 5. 20 Swab recovery standard curve data and results.

Conc. (ppm)	***% of MACO conc. (0.2273 ppm)***	***Area (AU)***	***Avg. Area***	***SD***	***RSD (%)***
0.02273	10	0.024, 0.022, 0.021	0.0223	0.0015	6.8397
0.09092	40	0.078, 0.071, 0.073	0.0740	0.0036	4.8724
0.18184	80	0.155, 0.158, 0.157	0.1567	0.0015	0.9750
0.2273	100	0.211, 0.211, 0.211	0.211	0.0	0.0
0.4546	200	0.393, 0.407, 0.405	0.4017	0.0076	1.8851

After drawing Area VS. STD concentration curve Figure 5. 7, the equation was: $y = 0.8897x - 0.0008$, where $R^2 = 0.9982$ that more than 0.980, slope = 0.8897 and Y-intercept = - 0.0008.

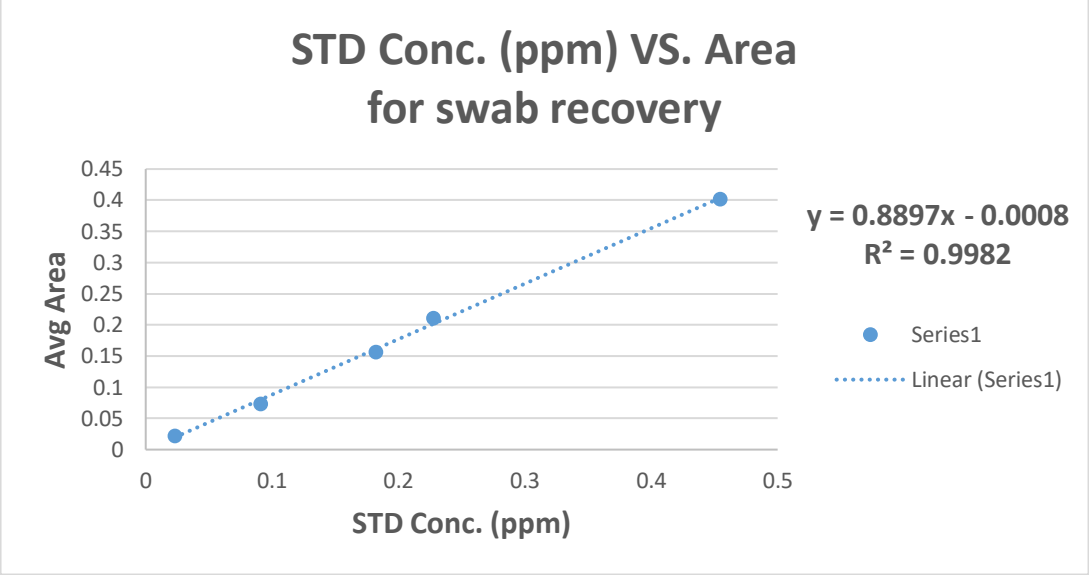


Figure 5. 7 Swab sampling recovery standard curve data plot.

Swab recovery samples preparation over 5 cm * 5 cm coupon

Different sample solutions of Olanzapine standard with placebo were prepared to be spiked on the coupon with concentrations 40%, 80%, 100% and 200% of the MACO concentration of Olanzapine = 0.2273 ppm as determined by our study. The data and results are in Table 5. 21.

Table 5. 21 Swab recovery samples over 5 cm * 5 cm coupon data and results.

Conc. (ppm)	***% of MACO conc. (0.2273 ppm)***	***Area (AU)***	***Avg. Area***	***RSD (%)***	***Recovery (%)***
0.09092	40	Sample 1: 0.058, 0.060, 0.055 Sample 2: 0.056, 0.059, 0.053 Sample 3: 0.052, 0.053, 0.053	0.0554	5.3436	74.86

0.18184	80	Sample 1: 0.122, 0.123, 0.119 Sample 2: 0.108, 0.108, 0.104 Sample 3: 0.111, 0.111, 0.108	0.1127	6.1172	71.92
0.2273	100	Sample 1: 0.165, 0.166, 0.164 Sample 2: 0.163, 0.163, 0.160 Sample 3: 0.169, 0.170, 0.166	0.1651	1.8776	78.25
0.4546	200	Sample 1: 0.341, 0.340, 0.344 Sample 2: 0.325, 0.320, 0.327 Sample 3: 0.321, 0.322, 0.321	0.3290	2.9781	81.90

From the results in Table 5. 21, it was observed that the % recovery for all solutions are above 70% and the average % recovery for swab test is equal 76.73%.

5.2.2 Execution During Rinse Test

5.2.2.1 Bin Mixer

Rinse recovery standard curve for Bin Mixer

Different concentrations of standard solution were prepared using the stock standard solution (4.5453 ppm) to prepare (0.045453, 0.181812, 0.363624, 0.45453, 0.90906 ppm that are equivalent to 10%, 40%, 80%, 100% and 200% of the MACO concentration of Olanzapine during rinse test for Bin Mixer = 0.45453 ppm). The data and results are in Table 5. 22.

Table 5. 22 Rinse recovery standard curve for Bin Mixer data and results.

Conc. (ppm)	*% of MACO conc. (0.45453 ppm)*	*Area (AU)*	*Avg. Area*	*SD*	*RSD (%)*
0.045453	10	0.050, 0.049, 0.049	0.0493	0.0006	1.1703
0.181812	40	0.179, 0.180, 0.177	0.1787	0.0015	0.8550
0.363624	80	0.364, 0.362, 0.365	0.3637	0.0015	0.4200
0.45453	100	0.477, 0.478, 0.476	0.4770	0.0010	0.2096
0.90906	200	0.990, 0.993, 0.985	0.9893	0.0040	0.4085

After drawing Area VS. STD concentration plot Figure 5. 8, the equation was: $y = 1.097x - 0.0172$, where $R^2 = 0.9986$ that more than 0.980, slope = 1.097 and Y-intercept = - 0.0172.

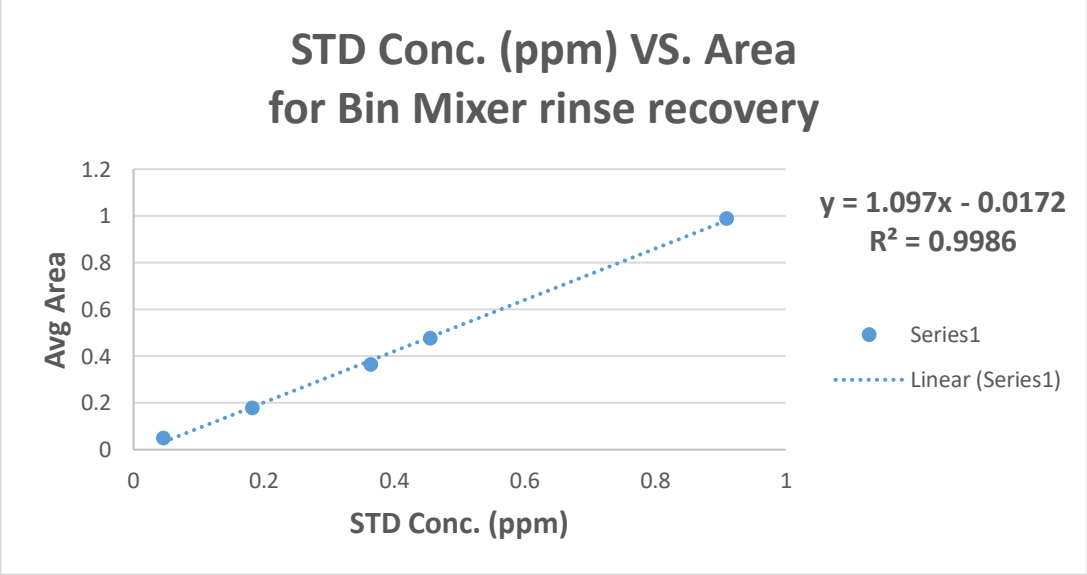


Figure 5. 8 Rinse recovery standard curve for Bin Mixer data plot.

Rinse recovery samples for Bin Mixer over 5 cm * 5 cm coupon

Different solutions of Olanzapine standard with placebo were prepared with concentration 40%, 80%, 100% and 200% of the MACO concentration of Olanzapine for Bin Mixer = 0.45453 ppm to be used for rinsing test. The data and results are in Table 5. 23.

Table 5. 23 Rinse recovery samples for Bin Mixer over 5 cm * 5 cm coupon data and results.

Conc. (ppm)	***% of MACO conc. (0.45453 ppm)***	***Area (AU)***	***Avg. Area***	***RSD (%)***	***Recovery (%)***
0.181812	40	Sample 1: 0.190, 0.191, 0.190 Sample 2: 0.184, 0.180, 0.183 Sample 3: 0.193, 0.193, 0.194	0.1887	2.6765	105.60
0.363624	80	Sample 1: 0.388, 0.385, 0.383 Sample 2: 0.356, 0.357, 0.359 Sample 3: 0.353, 0.353, 0.356	0.3656	4.1036	100.52
0.45453	100	Sample 1: 0.489, 0.487, 0.493 Sample 2: 0.480, 0.479, 0.475 Sample 3: 0.477, 0.476, 0.476	0.4813	1.3742	100.90
0.90906	200	Sample 1: 1.041, 1.047, 1.051 Sample 2: 1.044, 1.040, 1.046 Sample 3: 1.023, 1.013, 1.033	1.0376	1.1981	104.88

From the results in Table 5. 23, it was observed that the % recovery for all solutions are above 70% and the average % recovery for Bin Mixer is equal 102.98%.

5.2.2.2 Coating Pan

Rinse recovery standard curve for Coating Pan

Different concentrations of standard solution were prepared using the stock standard solution (6.2489 ppm) to prepare (0.062489, 0.249956, 0.499912, 0.62489, 1.24978 ppm that are equivalent to 10%, 40%, 80%, 100% and 200% of the MACO concentration of Olanzapine during rinse test for Coating Pan = 0.62489 ppm). The data and results are in Table 5. 24.

Table 5. 24 Rinse recovery standard curve for Coating Pan data and results.

Conc. (ppm)	***% of MACO conc. (0.62489 ppm)***	***Area (AU)***	***Avg. Area***	***SD***	***RSD (%)***
0.062489	10	0.068, 0.067, 0.065	0.0667	0.0015	2.2913
0.249956	40	0.250, 0.253, 0.252	0.2517	0.0015	0.6070
0.499912	80	0.509, 0.511, 0.513	0.5110	0.0020	0.3914
0.62489	100	0.632, 0.630, 0.629	0.6303	0.0015	0.2423
1.24978	200	1.291, 1.288, 1.292	1.2903	0.0021	0.1613

After drawing Area VS. STD concentration plot Figure 5. 9, the equation was: $y = 1.032x - 0.0046$, where $R^2 = 0.9998$ that more than 0.980, slope = 1.032 and Y-intercept = - 0.0046.

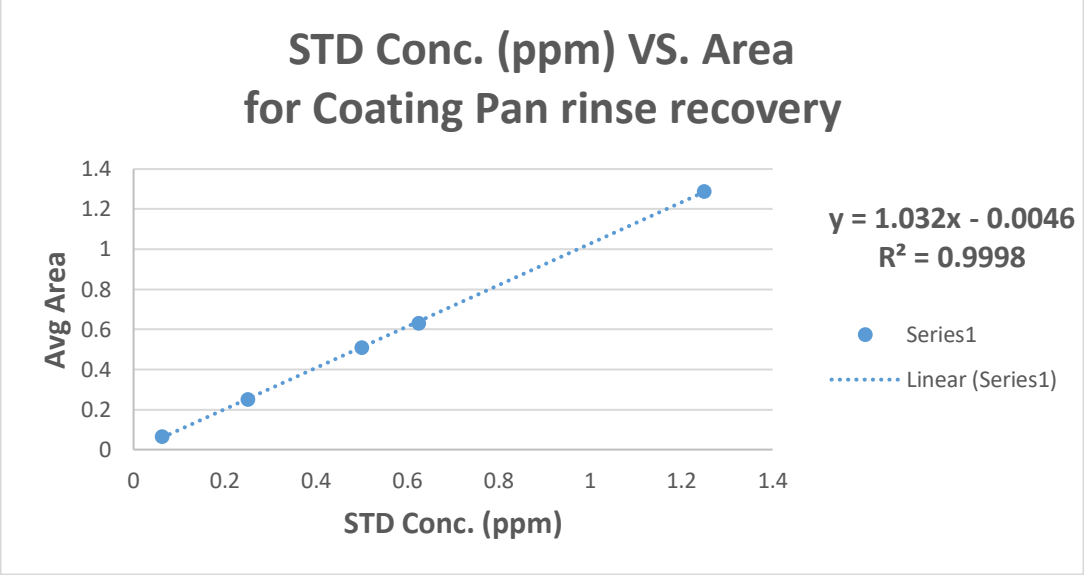


Figure 5. 9 Rinse recovery standard curve for Coating Pan data plot.

Rinse recovery samples for Coating Pan over 5 cm * 5 cm coupon

Different solutions of Olanzapine standard with placebo were prepared with concentration 40%, 80%, 100% and 200% of the MACO concentration of Olanzapine = 0.62489 ppm to be used for rinsing test. The data and results are in Table 5. 25.

Table 5. 25 Rinse recovery samples for Coating Pan over 5 cm * 5 cm coupon data and results.

Conc. (ppm)	***% of MACO conc. (0.62489 ppm)***	***Area (AU)***	***Avg. Area***	***RSD (%)***	***Recovery (%)***
0.249956	40	Sample 1: 0.248, 0.250, 0.246 Sample 2: 0.256, 0.258, 0.259 Sample 3: 0.251, 0.248, 0.252	0.2520	1.8507	100.12

0.499912	80	Sample 1: 0.519, 0.517, 0.515 Sample 2: 0.527, 0.525, 0.527 Sample 3: 0.513, 0.513, 0.516	0.5191	1.1085	101.59
0.62489	100	Sample 1: 0.653, 0.651, 0.650 Sample 2: 0.659, 0.661, 0.659 Sample 3: 0.684, 0.684, 0.683	0.6649	2.1917	105.49
1.24978	200	Sample 1: 1.338, 1.334, 1.334 Sample 2: 1.336, 1.331, 1.335 Sample 3: 1.385, 1.386, 1.386	1.3517	1.8916	104.76

From the results in Table 5. 25, it was observed that the % recovery for all solutions are above 70% and the average % recovery for Coating Pan is equal 102.99%.

5.2.3 Execution During Soak Test

5.2.3.1 Tablet Press Punches

Soak recovery standard curve for Tablet Press Punches and Dies

Different concentrations of standard solution were prepared using the stock standard solution (2.273 ppm) to prepare (0.02273, 0.09092, 0.18184, 0.2273, 0.4546 ppm that are equivalent to 10%, 40%, 80%, 100% and 200% of the MACO concentration during swab test of Olanzapine = 0.2273 ppm). The data and results are in Table 5. 26.

Table 5. 26 Soak recovery standard curve for Tablet Press Punches and Dies data and results.

Conc. (ppm)	*% of MACO conc. (0.2273 ppm)*	*Area (AU)*	*Avg. Area*	*SD*	*RSD (%)*
0.02273	10	0.024, 0.025, 0.024	0.0243	0.0006	2.3727
0.09092	40	0.099, 0.096, 0.091	0.0953	0.0040	4.2393
0.18184	80	0.169, 0.169, 0.169	0.1690	0	0.0
0.2273	100	0.229, 0.224, 0.223	0.2253	0.0032	1.4266
0.4546	200	0.433, 0.431, 0.428	0.4307	0.0025	0.5844

After drawing Area VS. STD concentration plot Figure 5. 10, the equation was: $y = 0.9376x + 0.0056$, where $R^2 = 0.9987$ that more than 0.980, slope = 0.9376 and Y-intercept = 0.0056.

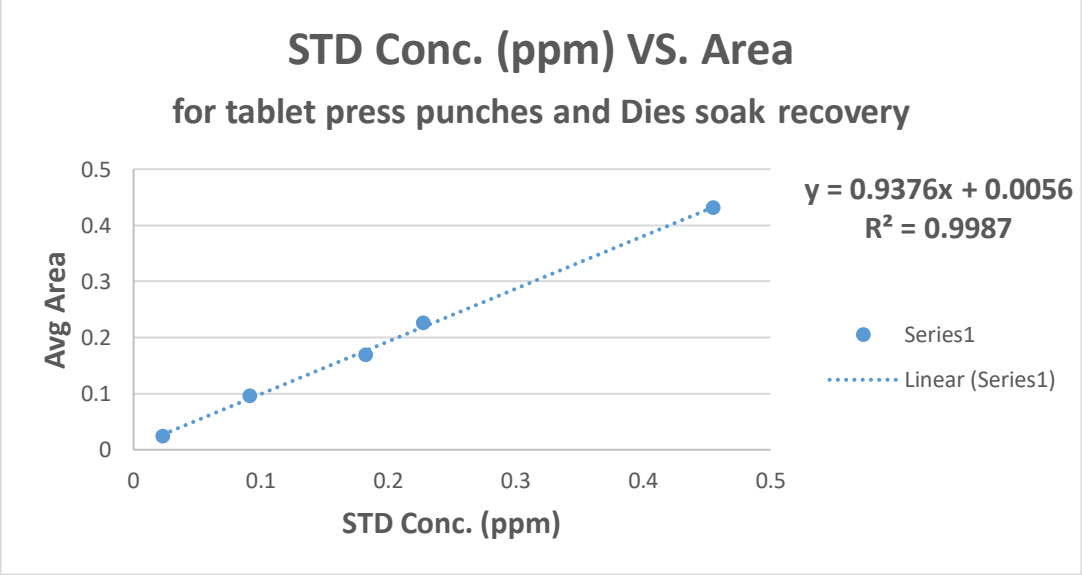


Figure 5. 10 Soak recovery standard curve for Tablet Press Punches and Dies plot.

Soak recovery samples for Tablet Press Punches

Different solutions of Olanzapine standard with placebo were prepared with concentration 40%, 80%, 100% and 200% of the MACO concentration of Olanzapine = 0.2273 ppm to be used for soaking test. The data and results are in Table 5. 27.

Table 5. 27 Soak recovery samples for Tablet Press Punches data and results.

Conc. (ppm)	***% of MACO conc. (0.2273 ppm)***	***Area (AU)***	***Avg. Area***	***RSD (%)***	***Recovery (%)***
0.09092	40	Sample 1: 0.078, 0.078, 0.079 Sample 2: 0.068, 0.070, 0.071 Sample 3: 0.080, 0.081, 0.082	0.0763	6.8386	80.06
0.18184	80	Sample 1: 0.142, 0.145, 0.143 Sample 2: 0.158, 0.158, 0.156 Sample 3: 0.150, 0.150, 0.149	0.1501	4.0991	88.82
0.2273	100	Sample 1: 0.202, 0.203, 0.204 Sample 2: 0.212, 0.210, 0.215 Sample 3: 0.205, 0.200, 0.201	0.2058	2.5689	91.34
0.4546	200	Sample 1: 0.437, 0.436, 0.433 Sample 2: 0.429, 0.423, 0.427 Sample 3: 0.380, 0.374, 0.378	0.4130	6.5711	95.89

From the results in Table 5. 27, it was observed that the % recovery for all solutions are above 70% and the average % recovery for soak Tablet Press Punches is equal 89.03%.

5.2.3.2 Tablet Press Dies

Soak recovery samples for Tablet Press Dies

Different solutions of Olanzapine standard with placebo were prepared with concentrations 40%, 80%, 100% and 200% of the MACO concentration of Olanzapine = 0.2273 ppm to be used for soaking test. The data and results are in Table 5. 28.

Table 5. 28 Soak recovery samples for Tablet Press Dies data and results.

Conc. (ppm)	***% of MACO conc. (0.2273 ppm)***	***Area (AU)***	***Avg. Area***	***RSD (%)***	***Recovery (%)***
0.09092	40	Sample 1: 0.066, 0.067, 0.068 Sample 2: 0.077, 0.076, 0.076 Sample 3: 0.078, 0.077, 0.076	0.0734	6.6742	77.02
0.18184	80	Sample 1: 0.158, 0.158, 0.156 Sample 2: 0.160, 0.161, 0.160 Sample 3: 0.158, 0.157, 0.158	0.1584	1.0034	93.73
0.2273	100	Sample 1: 0.199, 0.195, 0.194 Sample 2: 0.199, 0.199, 0.199 Sample 3: 0.194, 0.195, 0.194	0.1964	1.2498	87.17
0.4546	200	Sample 1: 0.431, 0.431, 0.429 Sample 2: 0.421, 0.421, 0.424 Sample 3: 0.422, 0.426, 0.426	0.4257	0.9397	98.84

From the results in Table 5. 28, it was observed that the % recovery for all solutions are above 70% and the average % recovery for soak Tablet Press Dies is equal 89.19%.

5.3 Implementation of Cleaning Procedure Using Pilot Scale Product and Equipment

5.3.1 Bin Mixer

5.3.1.1 Execution During Swab Test

Olanzapine standard curve for swab technique

Different concentrations of standard solution were prepared using the stock standard solution (2.273 ppm) to prepare (0.02273, 0.09092, 0.18184, 0.2273, 0.4546 ppm that are equivalent to 10%, 40%, 80%, 100% and 200% of the MACO concentration during swab test of Olanzapine = 0.2273 ppm). The data and results are in Table 5. 29.

Table 5. 29 Olanzapine standard curve for Bin Mixer swab technique data and results.

Conc. (ppm)	***% of MACO conc. (0.2273 ppm)***	***Area (AU)***	***Avg. Area***	***SD***	***RSD (%)***
0.02273	10	1.002, 1.020, 1.017	1.013	0.0096	0.9520
0.09092	40	5.343, 5.391, 5.257	5.3303	0.0679	1.2737
0.18184	80	11.217, 11.138, 11.119	11.1580	0.0520	0.4658
0.2273	100	13.780, 13.865, 13.806	13.8170	0.0436	0.3152
0.4546	200	25.907, 25.931, 25.838	25.8920	0.0483	0.1865

After drawing Area VS. STD concentration plot Figure 5. 11, the equation was: $y = 57.41x + 0.2197$, where $R^2 = 0.9972$ that more than 0.980, slope = 57.41 and Y-intercept = 0.2197.

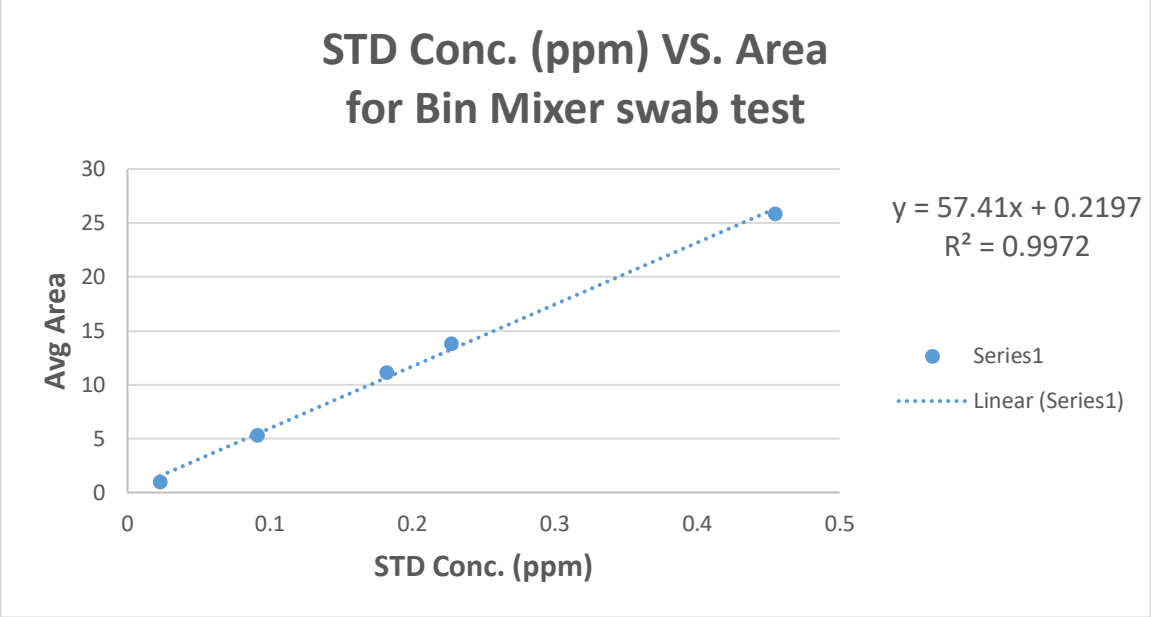


Figure 5. 11 Olanzapine standard curve for Bin Mixer swab test data plot.

Swab samples applied over 5 cm * 5 cm of Bin Mixer surface

Swab sampling technique was applied on the worst-case Bin Mixer sampling locations as clarified in section 3.7. The data and results are in Table 5. 30.

Table 5. 30 Swab samples over 5 cm * 5 cm of worst-case Bin Mixer sampling locations data and results.

Swab #	***Area (AU)***	***Avg. Area***	***RSD (%)***	***Olanzapine Conc.* (ppm)***	***Actual Olan. Conc.** (ppm)***	***Limit (NMT 0.2273 ppm)***
S1	2.655, 2.613, 2.564	2.6107	1.7446	0.0416	0.0542	Pass
S2	1.930, 1.902, 1.916	1.9160	0.7307	0.0295	0.0384	Pass
S3	1.535, 1.513, 1.516	1.5213	0.7842	0.0227	0.0296	Pass
S4	1.323, 1.330, 1.320	1.3243	0.3875	0.0192	0.0250	Pass

S5	1.167, 1.178, 1.165	1.1700	0.5983	0.0166	0.0216	Pass
S6	0.956, 0.950, 0.915	0.9403	2.3549	0.0126	0.0164	Pass

*: Concentration of Olanzapine in swab samples that are calculated from Olanzapine standard curve equation for Bin Mixer swab test.

**: The actual quantity for Olanzapine residue found on the machine surface was obtained by dividing Olanzapine concentration by the swab recovery factor for the swab sampling technique that equal 76.73%.

From the results in Table 5. 30, it was observed that Olanzapine concentration in all swab samples over 5 cm * 5 cm of worst-case Bin Mixer sampling locations were below the MACO concentration which is 0.2273 ppm. Which indicate that the suggested cleaning procedure is sufficient and effective for Olanzapine residue

5.3.1.2 Execution During Rinse Test

Olanzapine standard curve preparation for rinse technique

Different concentrations of standard solution were prepared using the stock standard solution (4.5453 ppm) to prepare (0.045453, 0.181812, 0.363624, 0.45453, 0.90906 ppm that are equivalent to 10%, 40%, 80%, 100% and 200% of the MACO concentration of Olanzapine during rinse test for Bin Mixer = 0.45453 ppm). The data and results are in Table 5. 31.

Table 5. 31 Olanzapine standard curve for Bin Mixer rinse technique data and results.

Conc. (ppm)	*% of MACO conc. (0.45453 ppm)*	*Area (AU)*	*Avg. Area*	*SD*	*RSD (%)*
0.045453	10	2.770, 2.777, 2.823	2.7900	0.0288	1.0320
0.181812	40	11.278, 11.329, 11.272	11.2930	0.0313	0.2773
0.363624	80	22.603, 22.613, 22.627	22.6143	0.0121	0.0533
0.45453	100	28.349, 28.296, 28.291	28.3120	0.0321	0.1135
0.90906	200	56.329, 56.337, 56.314	56.3267	0.0117	0.0207

After drawing Area VS. STD concentration plot Figure 5. 12, the equation was: $y = 61.986x + 0.0373$, where $R^2 = 1$ that more than 0.980, slope = 61.986 and Y-intercept = 0.0373.

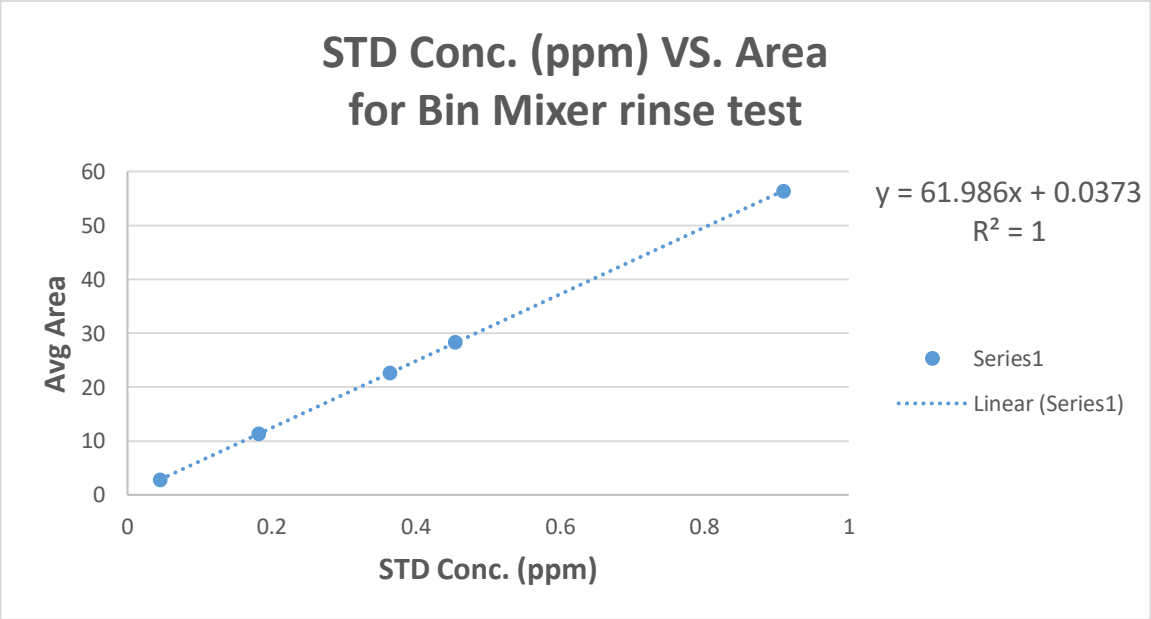


Figure 5. 12 Olanzapine standard curve for Bin Mixer rinse test data plot.

Rinse technique applied on Bin Mixer surface to detect Olanzapine and SLS residue

Rinse sampling technique was applied on Bin Mixer surface by washing the internal surface of the Bin Mixer with 1000 ml of purified water as clarified in section 3.7. In addition, the conductivity of this rinse was measured to detect cleaning agent (SLS) residue. The data and results for Olanzapine and SLS residue are in Table 5. 32 and Table 5. 33, respectively.

Table 5. 32 Rinse sample for Bin Mixer data and results.

Rinse #	***Area (AU)***	***Avg. Area***	***RSD (%)***	***Olanzapine Conc.* (ppm)***	***Actual Olan. Conc.** (ppm)***	***Limit (NMT 0.45453 ppm)***
R1	0.203, 0.239, 0.214	0.2187	8.4366	0.003	BQL***	Pass

*: Concentration of Olanzapine in rinse sample that are calculated from Olanzapine standard curve equation for Bin Mixer rinse test.

**: The actual quantity for Olanzapine residue found on the machine surface was obtained by dividing Olanzapine concentration by the rinse recovery factor for the rinse sampling technique that equal 102.98% for Bin Mixer.

***: Below quantitation limit.

Table 5. 33 Determination of cleaning agent residue in Bin Mixer using Conductivity test.

Conductivity of purified water as standard reference	***Conductivity of the final rinse water***	***Limit (should be NMT ± 0.2 µs/cm)***
1.06 about 1.1	1.09 about 1.1	Pass

From the results in Table 5. 32, it was observed that Olanzapine concentration is below the MACO concentration that is 0.45453 ppm, also it is below the quantitation limit that is 0.006 ppm. Which indicates that the suggested cleaning procedure is sufficient and effective for Olanzapine residues. In addition, the results observed in Table 5. 33 insure that no cleaning agent residue was found on Bin Mixer surface which indicates that the cleaning agent is completely removed from the machine surface.

5.3.2 Tablet Press

5.3.2.1 Execution During Swab and Soak Sampling Tests

Olanzapine standard curve for swab and soak technique

Different concentrations of standard solution were prepared using the stock standard solution (2.273 ppm) to prepare (0.02273, 0.09092, 0.18184, 0.2273, 0.4546 ppm that are equivalent to 10%, 40%, 80%, 100% and 200% of the MACO concentration during swab test of Olanzapine = 0.2273 ppm). The data and results are in Table 5. 34.

Table 5. 34 Olanzapine standard curve for Tablet Press swab sampling technique data and results.

Conc. (ppm)	***% of MACO conc. (0.2273 ppm)***	***Area (AU)***	***Avg. Area***	***SD***	***RSD (%)***
0.02273	10	1.442, 1.405, 1.418	1.4217	0.0188	1.3203
0.09092	40	5.542, 5.513, 5.481	5.5120	0.0305	0.5536
0.18184	80	11.229, 11.323, 11.218	11.2567	0.0577	0.5127
0.2273	100	13.993, 14.054, 14.023	14.0233	0.0305	0.2175

0.4546	200	28.457, 28.517, 28.316	28.4300	0.1032	0.3629

After drawing Area VS. STD concentration plot Figure 5. 13, the equation was: y = 62.663x - 0.1206, where R^2 = 0.9999 that more than 0.980, slope = 62.663 and Y-intercept = - 0.1206.

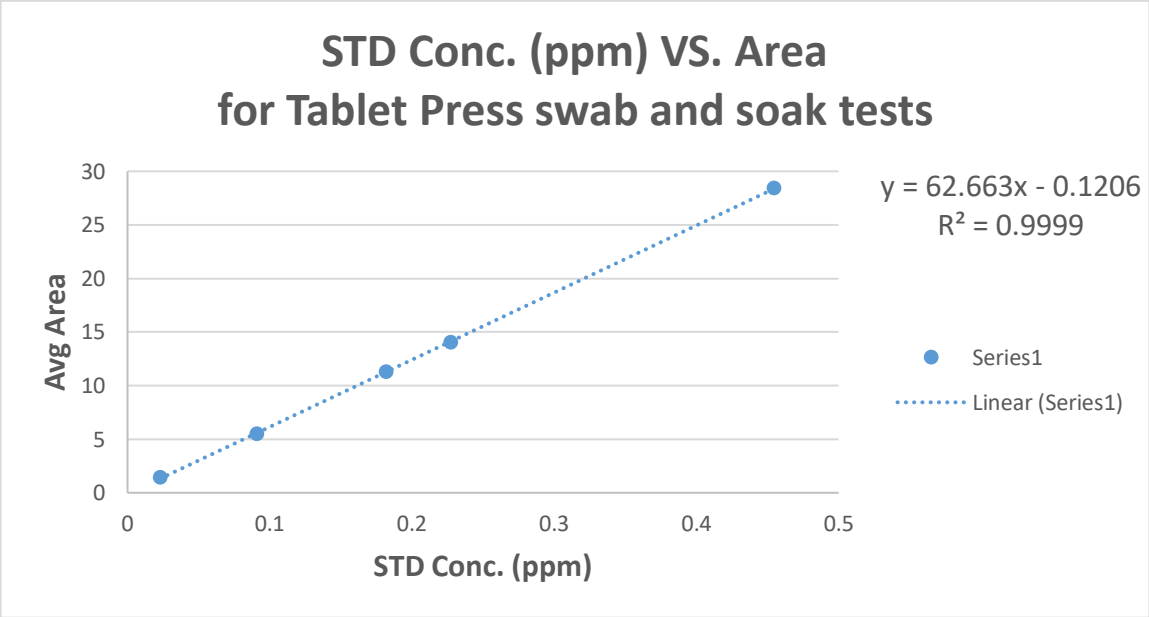


Figure 5. 13 Olanzapine standard curve for Tablet Press swab and soak sampling tests data plot.

Swab samples applied over 5 cm * 5 cm of Tablet Press surface

Swab technique was applied on the worst-case Tablet Press sampling locations as clarified in section 3.7 . The data and results are in Table 5. 35.

Table 5. 35 Swab samples over 5 cm * 5 cm of worst-case Tablet Press sampling locations data and results.

Swab #	***Area (AU)***	***Avg. Area***	***RSD (%)***	***Olanzapine Conc.* (ppm)***	***Actual Olan. Conc.** (ppm)***	***Limit (NMT 0.2273 ppm)***
S1	1.123, 1.128, 1.104	1.1183	1.1322	0.0198	0.0258	Pass
S2	1.277, 1.226, 1.328	1.2770	3.9937	0.0223	0.0291	Pass
S3	1.287, 1.229, 1.308	1.2747	3.2101	0.0223	0.0291	Pass
S4	0.987, 0.916, 0.969	0.9573	3.8555	0.0172	0.0224	Pass
S5	0.975, 0.958, 1.029	0.9873	3.7548	0.0177	0.0231	Pass
S6	1.008, 0.982, 0.990	0.9933	1.3406	0.0178	0.0232	Pass
S7	1.320, 1.338, 1.360	1.3393	1.4958	0.0233	0.0304	Pass
S8	0.926, 0.863, 0.851	0.8800	4.5781	0.0160	0.0209	Pass
S9	1.321, 1.318, 1.303	1.3140	0.7339	0.0229	0.0298	Pass

*: Concentration of Olanzapine in swab sample that are calculated from Olanzapine standard curve equation for Tablet Press swab and soak sampling tests.

**: The actual quantity for Olanzapine residue found on the machine surface was obtained by dividing Olanzapine concentration by the swab recovery factor for the swab sampling technique that equal 76.73%.

From the results in Table 5. 35, it was observed that Olanzapine concentrations in all swab samples over 5 cm * 5 cm of worst-case Tablet Press sampling locations were below the MACO concentration which is 0.2273 ppm. Which indicate that the suggested cleaning procedure is sufficient and effective for Olanzapine residue.

Soak technique applied on Tablet Press Punches and Dies surface to detect Olanzapine residue

Punches and Dies of Tablet Press machine was soaked in 10 ml and 100 ml Diluent respectively as clarified in section 3.7. The data and results are in Table 5. 36.

Table 5. 36 Soak samples for Tablet Press Punches and Dies data and results.

Soak #	***Area (AU)***	***Avg. Area***	***RSD (%)***	***Olanzapine Conc.* (ppm)***	***Actual Olan. Conc.** (ppm)***	***Limit (NMT 0.02 ppm & 0.03 ppm respectively)***
SK1	0.676, 0.634, 0.645	0.6517	3.3421	0.0123	0.0138	Pass
SK2	0.743, 0.758, 0.759	0.7533	1.1898	0.0139	0.0156	Pass

*: Concentration of Olanzapine in soak sample that are calculated from Olanzapine standard curve equation for Tablet Press swab and soak sampling tests.

**: The actual quantity for Olanzapine residues found on the machine surface was obtained by dividing Olanzapine concentration by the soak recovery factor for the soak sampling technique, that equal 89.03% for soak Tablet Press Punches and 89.19% for soak Tablet Press Dies.

From the results in Table 5. 36, it was observed that Olanzapine concentration in soak samples of Tablet Press Punches and Dies were below the MACO concentration which are 0.020980 ppm and 0.031817 ppm respectively, which indicates that the suggested cleaning procedure is sufficient and effective for Olanzapine residue.

Soak sampling technique applied on Tablet Press Punches and Dies surface to detect SLS residues

To detect the cleaning agent (SLS) residues, the soaking technique was repeated by soak separately the tablet press Punches and Dies in 100 ml purified water for 10 minutes. The data and results are shown in Table 5. 37.

Table 5. 37 Determination of cleaning agent residue for Tablet Press Punches and Dies using Conductivity test for water.

Soak #	***Conductivity of purified water as standard reference***	***Conductivity of the soak water***	***Limit (should be NMT ± 0.2 µs/cm)***
SK1	1.06 about 1.1	1.08 about 1.1	Pass
SK2	1.06 about 1.1	1.07 about 1.1	Pass

From the results in Table 5. 37, it was observed that no cleaning agent residues were found on Tablet Press Punches and Dies surfaces after performing the cleaning procedure as written and approved.

5.3.3 Coating Pan

5.3.3.1 Execution During Swab Test

Different concentrations of standard solution were prepared using the stock standard solution (2.273 ppm) to prepare (0.02273, 0.09092, 0.18184, 0.2273, 0.4546 ppm that are equivalent to 10%, 40%, 80%, 100% and 200% of the MACO concentration during swab test of Olanzapine = 0.2273 ppm). The data and results are in Table 5. 38.

Table 5. 38 Olanzapine standard curve for Coating Pan swab sampling technique data and results.

Conc. (ppm)	***% of MACO conc. (0.2273 ppm)***	***Area (AU)***	***Avg. Area***	***SD***	***RSD (%)***
0.02273	10	1.403, 1.420, 1.364	1.3957	0.0287	2.0572
0.09092	40	5.433, 5.432, 5.396	5.4203	0.0211	0.3889
0.18184	80	11.546, 11.592, 11.566	11.5680	0.0231	0.1994
0.2273	100	14.216, 14.337, 14.348	14.3003	0.0732	0.5122
0.4546	200	28.777, 28.781, 28.790	28.7827	0.0067	0.0231

After drawing Area VS. STD concentration plot Figure 5. 14, the equation was: $y = 63.672x - 0.1531$, where $R^2 = 0.9998$ that more than 0.980, slope = 63.672 and Y-intercept = - 0.1531.

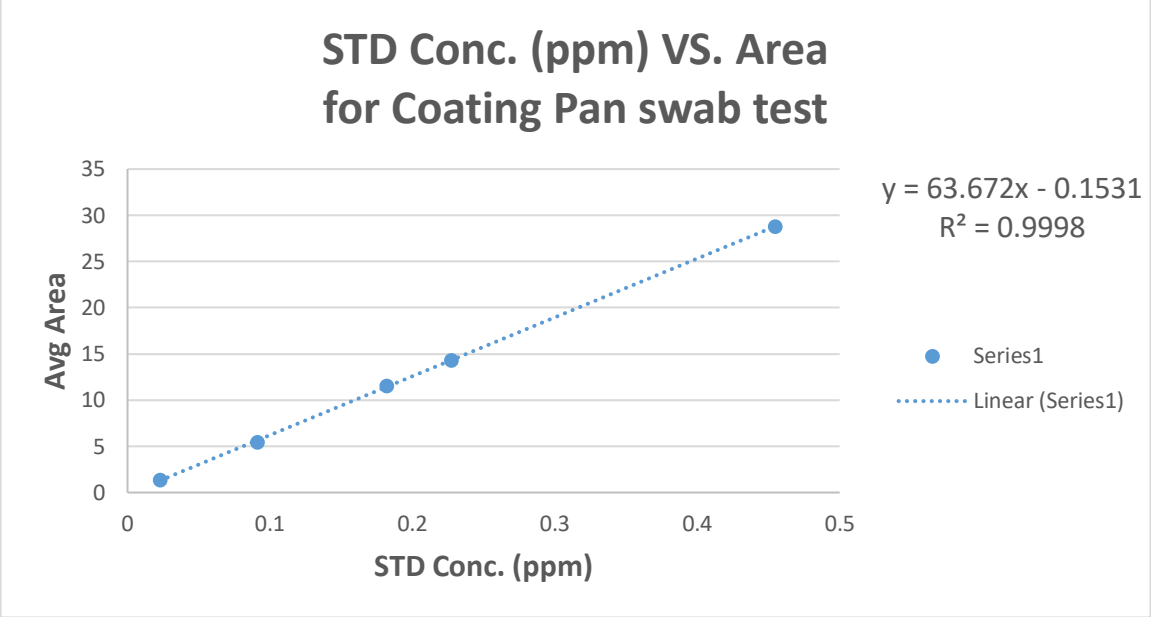


Figure 5. 14 Olanzapine standard curve for Coating Pan swab sampling test data plot.

Swab samples applied over 5 cm * 5 cm of Coating Pan surface

Swab sampling technique was applied on the worst-case Coating Pan sampling locations as clarified in section 3.7 . The data and results are in Table 5. 39.

Table 5. 39 Swab samples over 5 cm * 5 cm of worst-case Coating Pan sampling locations data and results.

Swab #	***Area (AU)***	***Avg. Area***	***RSD (%)***	***Olanzapine Conc.* (ppm)***	***Actual Olan. Conc.** (ppm)***	***Limit (NMT 0.2273 ppm)***
S1	1.032, 0.995, 0.995	1.0073	2.1206	0.0182	0.0237	Pass
S2	1.017, 1.092, 1.072	1.0603	3.6627	0.0191	0.0249	Pass
S3	1.465, 1.439, 1.445	1.4497	0.9391	0.0252	0.0328	Pass
S4	1.241, 1.240, 1.259	1.2467	0.8577	0.0220	0.0287	Pass

S5	1.208, 1.229, 1.194	1.2103	1.4555	0.0214	0.0279	Pass
S6	0.926, 0.928, 0.955	0.9363	1.7298	0.0171	0.0223	Pass
S7	1.164, 1.147, 1.164	1.1583	0.8473	0.0206	0.0268	Pass
S8	0.959, 0.943, 0.976	0.9593	1.7202	0.0175	0.0228	Pass

*: Concentrations of Olanzapine in swab samples that are calculated from Olanzapine standard curve equation for Coating Pan swab sampling test.

**: The actual quantity for Olanzapine residue found on the machine surface was obtained by dividing Olanzapine concentration by the swab recovery factor for the swab sampling technique that equal 76.73%.

From the results in Table 5. 39, it was observed that Olanzapine concentrations in all swab samples over 5 cm * 5 cm of worst-case Coating Pan sampling locations were below the MACO concentration which is 0.2273 ppm. Which indicate that the suggested cleaning procedure is sufficient and effective for Olanzapine residue.

5.3.3.2 Execution During Rinse Sampling Test

Different concentrations of standard solution were prepared using the stock standard solution (0.62489 ppm) to prepare (0.062489, 0.249956, 0.499912, 0.62489, 1.24978 ppm that are equivalent to 10%, 40%, 80%, 100% and 200% of the MACO concentration of Olanzapine during rinse sampling test for Coating Pan = 0.62489 ppm). The data and results are in Table 5. 40.

Table 5. 40 Olanzapine standard curve for Coating Pan rinse sampling technique data and results.

Conc. (ppm)	*% of MACO conc. (0.62489 ppm)*	*Area (AU)*	*Avg. Area*	*SD*	*RSD (%)*
0.062489	10	3.758, 3.682, 3.752	3.7307	0.0423	1.1326
0.249956	40	15.054, 15.058, 15.030	15.0473	0.0151	0.1006
0.499912	80	30.656, 30.626, 30.555	30.6123	0.0519	0.1694
0.62489	100	38.316, 38.297, 38.289	38.3007	0.0139	0.0362
1.24978	200	76.568, 76.502, 76.464	76.5113	0.0526	0.0688

After drawing Area VS. STD concentration plot Figure 5. 15, the equation was: $y = 61.368x - 0.1389$, where $R^2 = 1$ that more than 0.980, slope = 61.368 and Y-intercept = - 0.1389.

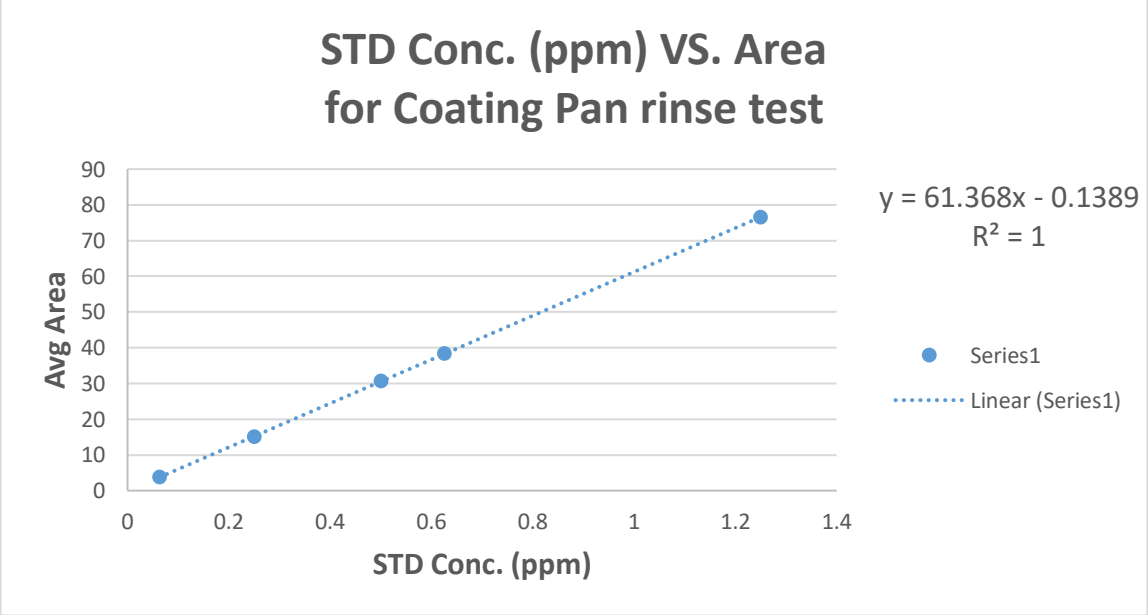


Figure 5. 15 Olanzapine standard curve for Coating Pan rinse sampling test data plot.

Rinse sampling technique applied on Coating Pan surface to detect Olanzapine and SLS residues

Rinse sampling technique was applied on Coating Pan surface by washing the internal surface of the Coating Pan with 5000 ml of purified water as clarified in section 3.7. In addition, the conductivity of this rinse was measured to detect cleaning agent (SLS) residues. The data and results for Olanzapine and SLS residue are shown in Table 5. 41 and Table 5. 42 respectively.

Table 5. 41 Rinse sample for Coating Pan data and results.

Rinse #	***Area (AU)***	***Avg. Area***	***RSD (%)***	***Olanzapine Conc.* (ppm)***	***Actual Olan. Conc.** (ppm)***	***Limit (NMT 0.45453 ppm)***
R1	0.244, 0.246, 0.276	0.2553	7.0205	0.006	0.006	Pass

*: Concentrations of Olanzapine in rinse sample that are calculated from Olanzapine standard curve equation for Coating Pan rinse sampling test.

**: The actual quantity for Olanzapine residues found on the machine surface was obtained by dividing Olanzapine concentration by the rinse recovery factor for the rinse sampling technique that is equal to 102.99%.for Coating Pan.

Table 5. 42 Determination of cleaning agent residues in Coating Pan using Conductivity test.

Conductivity of purified water as standard reference	***Conductivity of the final rinse water***	***Limit (should be NMT ± 0.2 µs/cm)***
1.07 about 1.1	1.08 about 1.1	Pass

From the results in Table 5. 41, it was observed that Olanzapine concentration is below the MACO concentration that is 0.62489 ppm. Which indicates that the suggested cleaning procedure is sufficient and effective for Olanzapine residues. In addition, the results observed in Table 5. 42 insures that no cleaning agent residues was found on the Coating Pan surface.

5.4 Microbiological Contamination Tests

In our cleaning validation study we used contact plates or RODAC agar plates (for flat surfaces) to detect and determine microbiological contaminants from Bin Mixer, Tablet Press and Coating Pan worst-case locations as clarified in section 3.7. The data and results are shown in Table 5. 43.

Table 5. 43 Microbiological contaminants for Bin Mixer, Tablet Press and Coating Pan worst-case locations data and results.

	Bin Mixer*			***Tablet Press*****			***Coating Pan*****		
Zero	18/09/2018			20/09/2018			25/09/2018		
Time	**#**	**TAC**	**Y&M**	**#**	**TAC**	**Y&M**	**#**	**TAC**	**Y&M**
		20/09	**24/09**		**22/09**	**25/09**		**27/09**	**01/10**
	S1	Nil	Nil	**S9**	Nil	Nil	**S7**	Nil	Nil
	S2	Nil	Nil						
Two	20/09/2018			22/09/2018			27/09/2018		
Days	**#**	**TAC**	**Y&M**	**#**	**TAC**	**Y&M**	**#**	**TAC**	**Y&M**
		22/09	**25/09**		**24/09**	**27/09**		**29/09**	**02/10**

	S1	S9	S9	**S9**	Nil	Nil	**S7**	Nil	Nil
	S2	Nil	Nil						
Four		22/09/2018			24/09/2018			29/09/2018	
Days	**#**	**TAC**	**Y&M**	**#**	**TAC**	**Y&M**	**#**	**TAC**	**Y&M**
		24/09	**27/09**		**26/09**	**29/09**		**01/10**	**04/10**
	S1	Nil	Nil	**S9**	Nil	Nil	**S7**	Nil	Nil
	S2	Nil	Nil						
Seve		25/09/2018			27/09/2018			02/10/2018	
n	**#**	**TAC**	**Y&M**	**#**	**TAC**	**Y&M**	**#**	**TAC**	**Y&M**
Days		**27/09**	**01/10**		**29/09**	**02/10**		**04/10**	**08/10**
	S1	Nil	Nil	**S9**	Nil	Nil	**S7**	Nil	Nil
	S2	Nil	Nil						
Eleve		29/09/2018			01/10/2018			06/10/2018	
n	**#**	**TAC**	**Y&M**	**#**	**TAC**	**Y&M**	**#**	**TAC**	**Y&M**
Days		**01/10**	**04/10**		**03/10**	**06/10**		**08/10**	**11/10**
	S1	Nil	Nil	**S9**	Nil	Nil	**S7**	Nil	Nil
	S2	Nil	Nil						

*: S1 and S2 worst-case locations in Bin Mixer for microbiological test are for door inlet surface and Outlet door respectively.

**: S9 worst-case location in Tablet Press for microbiological tests are for rotary table.

***: S7 worst-case location in Coating Pan for microbiological tests are for baffles.

The stabilization period for clean hold time for equipment during storage were eleven days as in Table 5. 43. But for risk mitigation we recommend clean hold time to be seven days before equipment reuse or recleaning. If the clean hold time exceeds, equipment should be sprayed again with 70% Ethanol solution prior to be used and verified as clean.

Conclusion

The purpose of cleaning validation is to establish the documented evidence with high degree of assurance that the cleaning process followed as per standard operating procedures for cleaning the equipment used for the processing of different tablets in shared facility, consistently and concurrently yields the results not exceeding predetermined acceptance limits.

During this study an adequate and efficient cleaning process was successfully developed and validated, for removing of the worst-case Olanzapine residues, from tablets manufactured in multi-product facility using shared equipment train, for manufacturing of different solid dosage form products. The developed cleaning process was assured by providing documented evidence that the API residues, cleaning agent residues and microbiological residues from previously manufactured products were removed to acceptable and safe levels, so the subsequently manufactured products did not have any potentially carryover of contaminants from previous products that exceeds the acceptable scientific limits, which may affect the subsequently manufactured product purity, identity, quality and safety and may put the patient under risk as a result of insufficient and ineffective cleaning procedures.

To determine the acceptable quantities of residues after performing the cleaning process, a rapid, specific, accurate and precise RP-HPLC analytical method was developed to detect the residues of the most difficult to clean product (known as the worst-case product) which is Olanzapine tablets. The analytical method was validated

for specificity, LOD and LOQ, linearity, accuracy and precision, ruggedness and robustness as per the [ICH Q2 (R1)] guidelines.

The present study provides documented evidence with a high degree of assurance that the cleaning procedures for the equipment used in production of the three target products (Diclofenac Potassium, Ibuprofen and Olanzapine Tablets) will consistently reduce the residues of the previous product from the equipment contact surface to acceptable limits and leave the equipment safe and ready for manufacturing the subsequent product.

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Appendix I:

- API Certificates of Analysis
- Cleaning SOPs

Appendix II: Supported Data

1. Test Method Validation

1.1. Accuracy

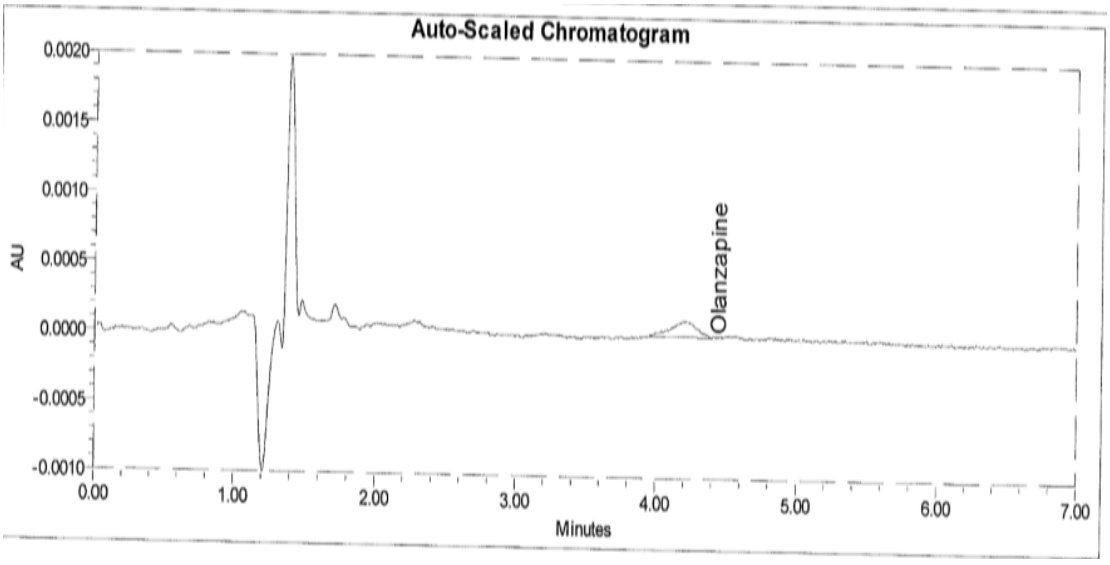

Figure 1 Chromatograph for Olanzapine standard solution (0.02273 ppm).

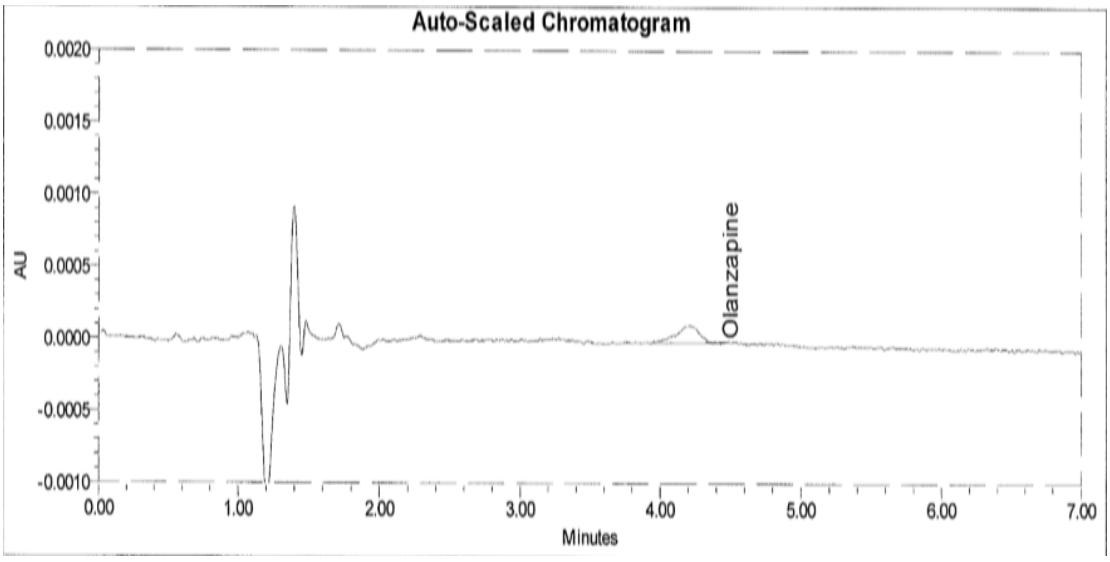

Figure 2 Chromatograph for Olanzapine sample solution (0.02273 ppm).

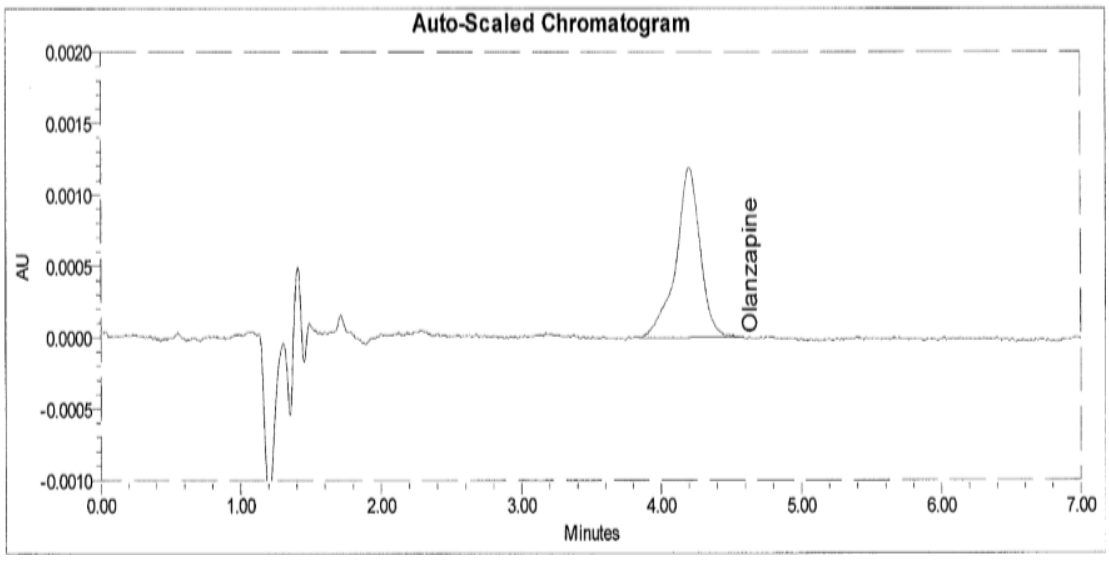

Figure 3 Chromatograph for Olanzapine standard solution (0.2273 ppm).

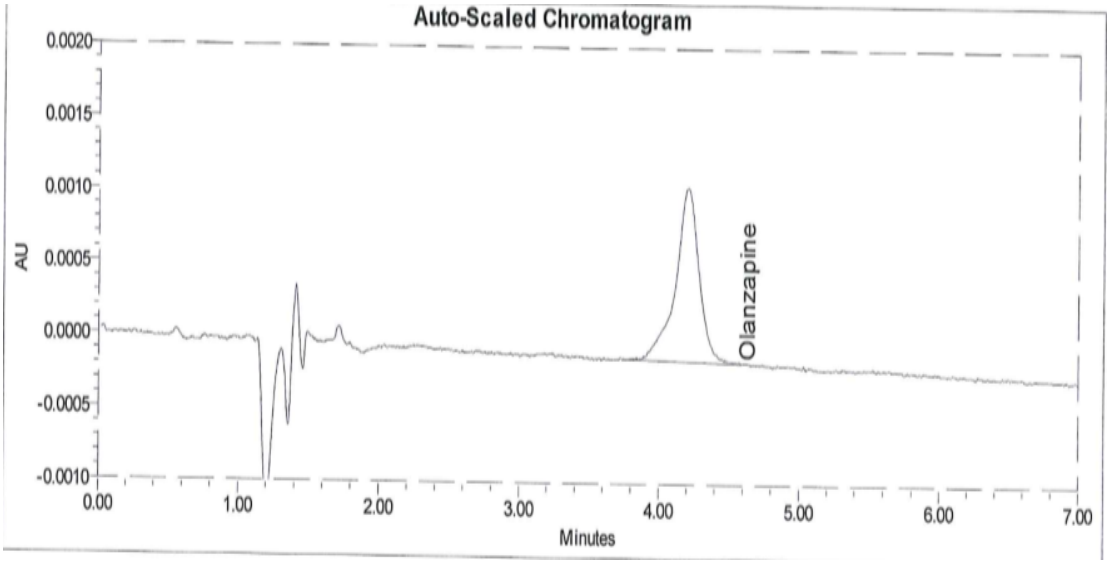

Figure 4 Chromatograph for Olanzapine sample solution (0.2273 ppm).

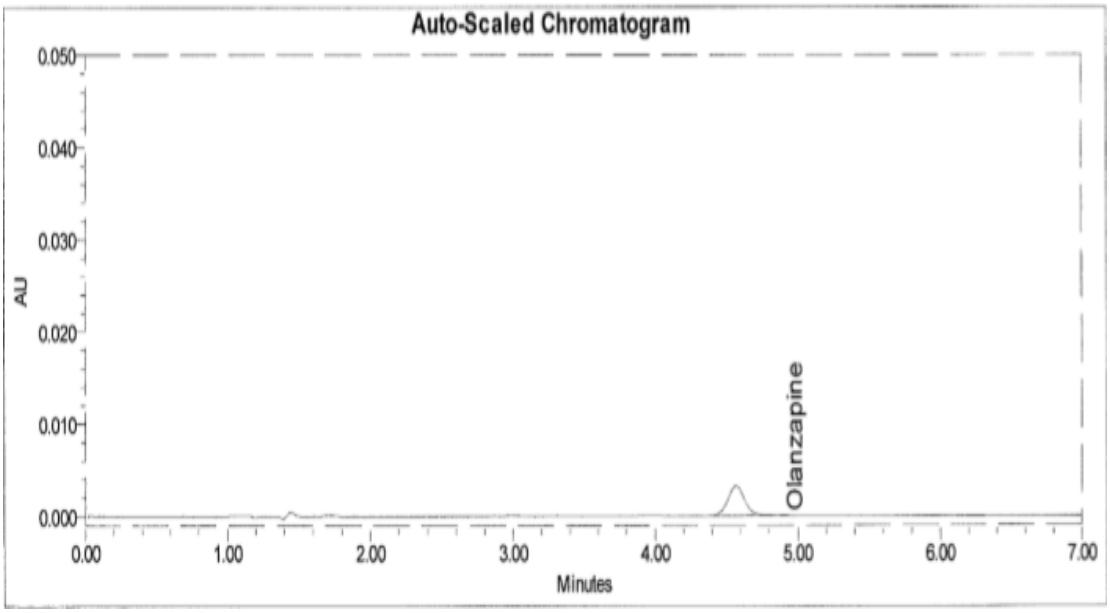

Figure 5 Chromatograph for Olanzapine standard solution (0.4546 ppm).

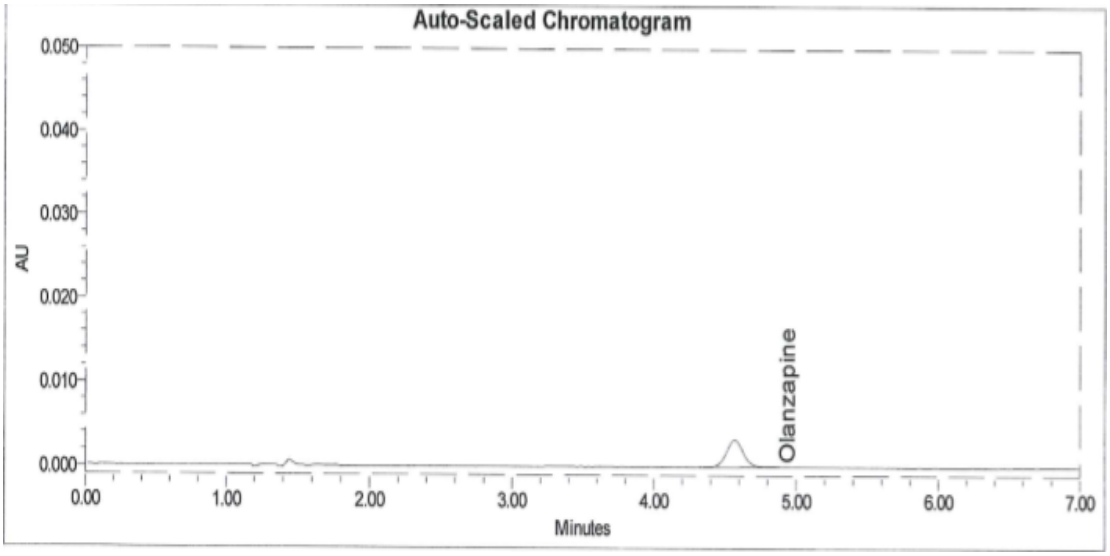

Figure 6 Chromatograph for Olanzapine sample solution (0.4546 ppm).

1 Recovery Test from Coupons

2.1 Recovery from Swab Sampling Test

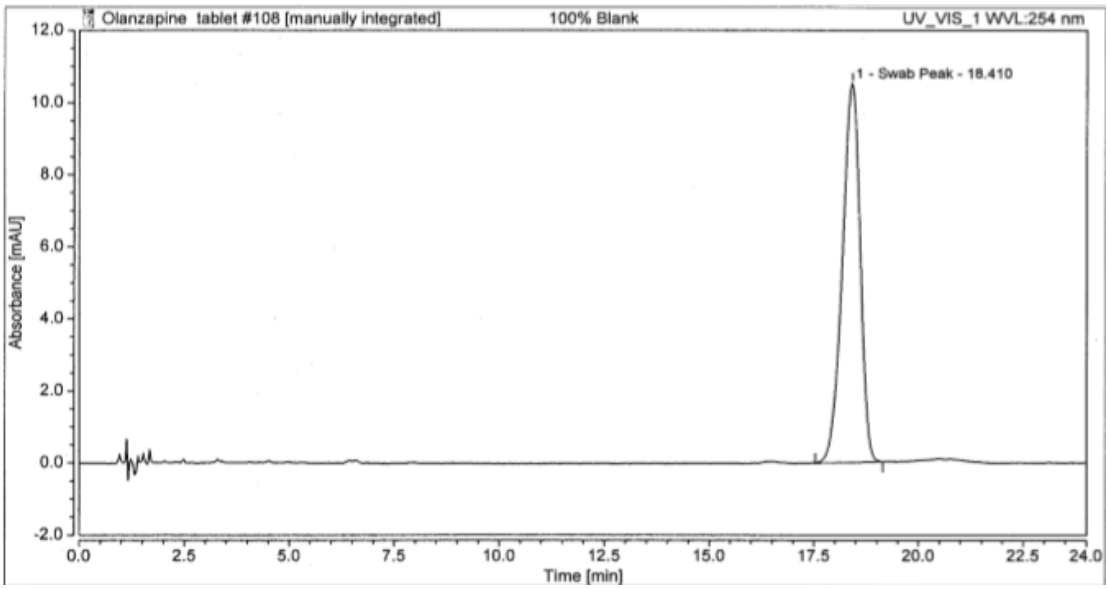

Figure 7 Chromatograph for diluent, a mixture of water and Acetonitrile (55:45, v/v), for swab test.

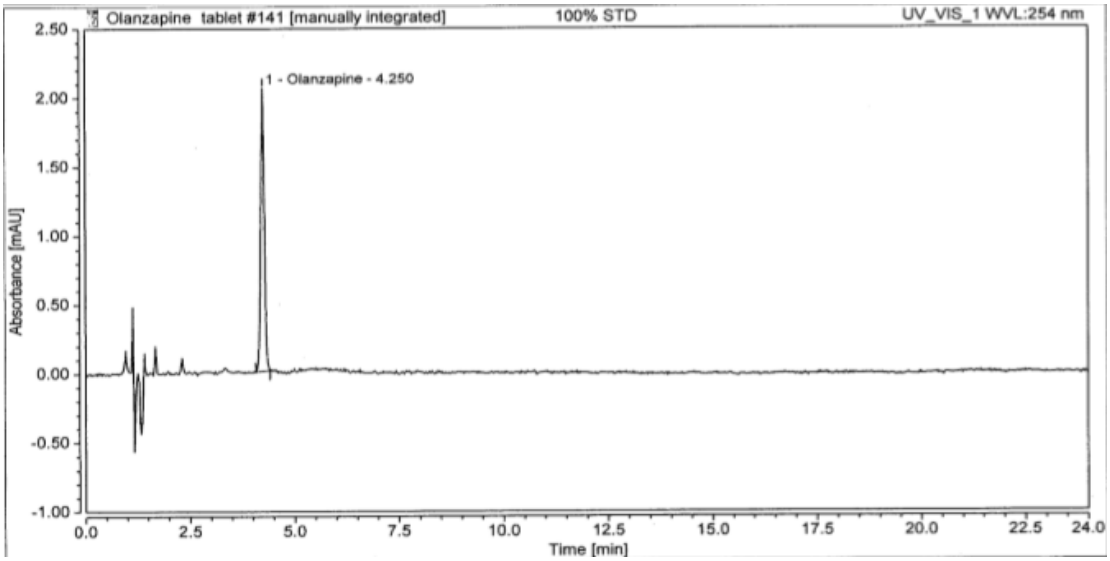

Figure 8 Chromatograph for Olanzapine standard solution (0.2273 ppm), for swab test.

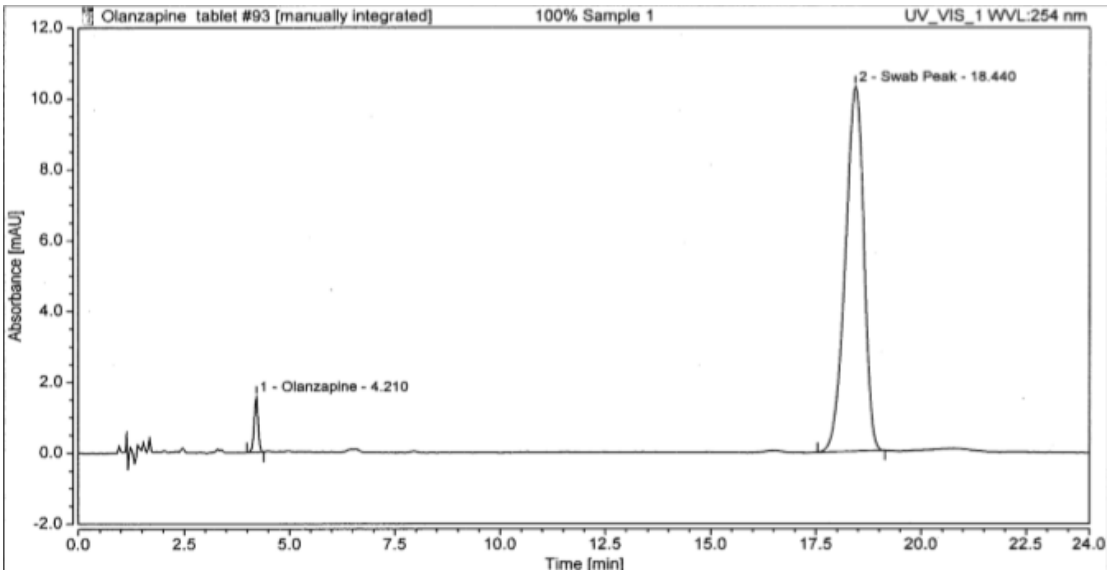

Figure 9 Chromatograph for Olanzapine sample solution (0.2273 ppm), for swab test.

2.2 Execution During Rinse Test for Bin Mixer

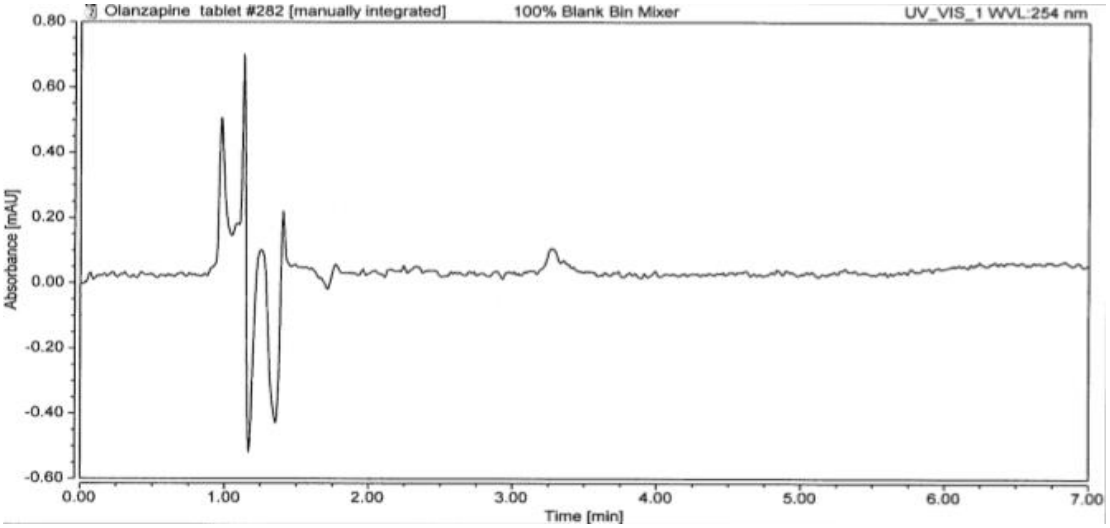

Figure 10 Chromatograph for diluent, a mixture of water and Acetonitrile (55:45, v/v), for Bin Mixer rinse test.

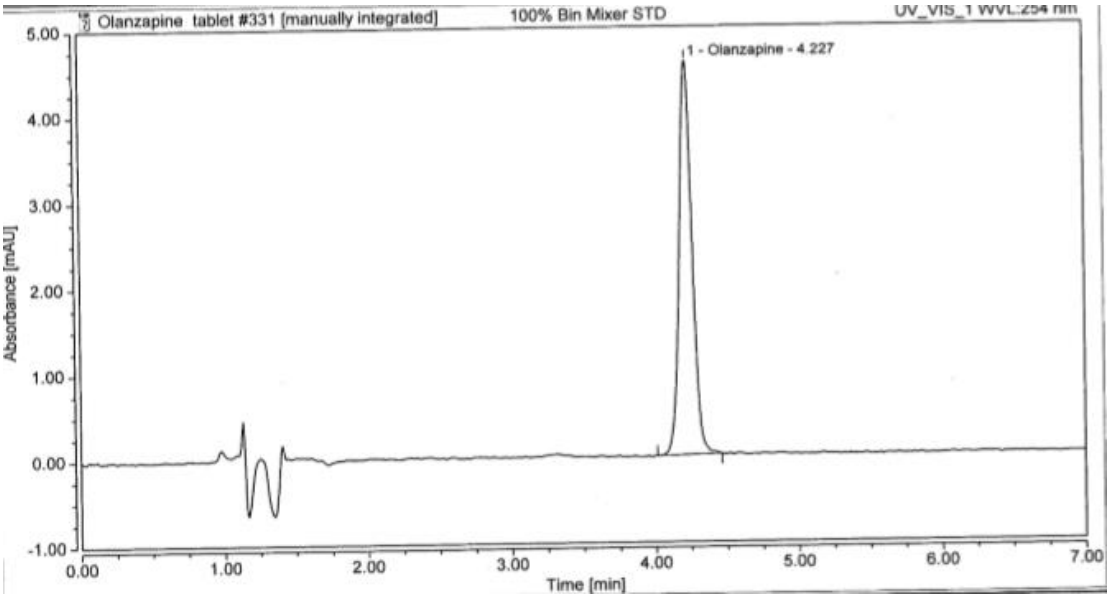

Figure 11 Chromatograph for Olanzapine standard solution (0.45453 ppm) for Bin Mixer rinse test.

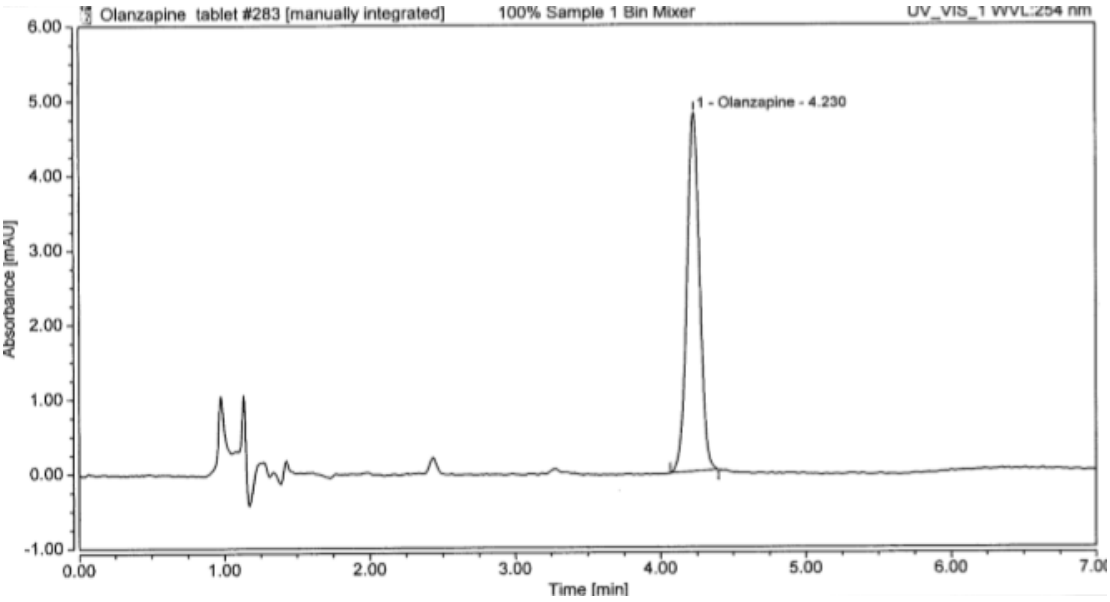

Figure 12 Chromatograph for Olanzapine sample solution (0.45453 ppm), for Bin Mixer rinse test.

2.3 Execution During Soak Test for Tablet Press Punches

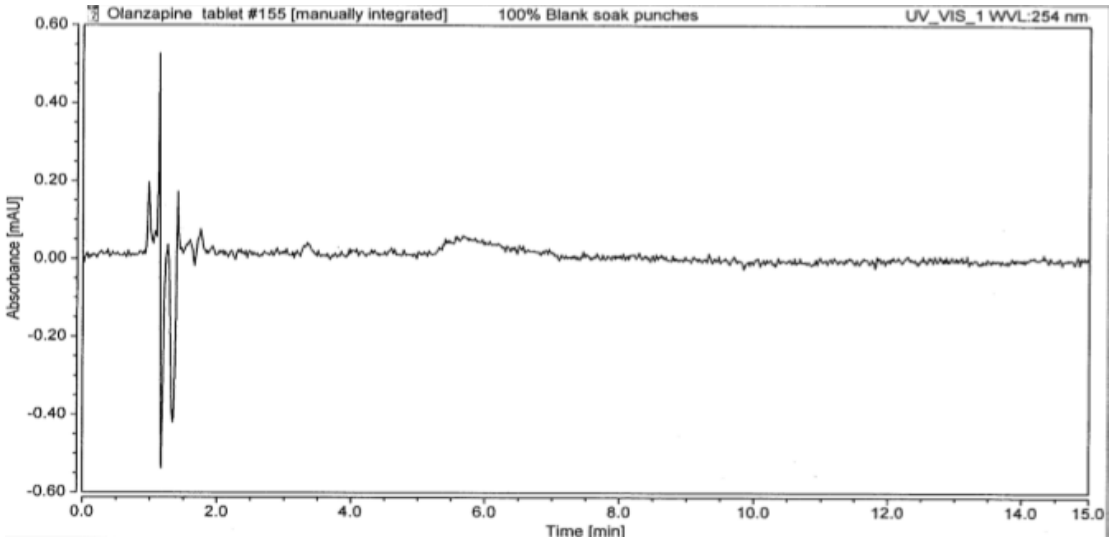

Figure 13 Chromatograph for diluent, a mixture of water and Acetonitrile (55:45, v/v), for Tablet Press soaked test.

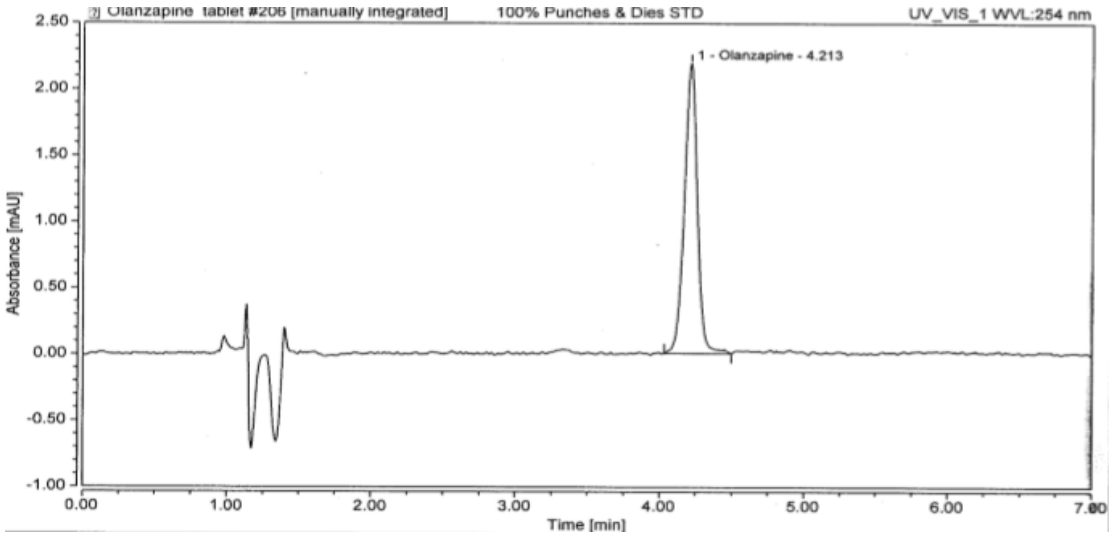

Figure 14 Chromatograph for Olanzapine standard solution (0.2273 ppm) for Tablet Press soaked test.

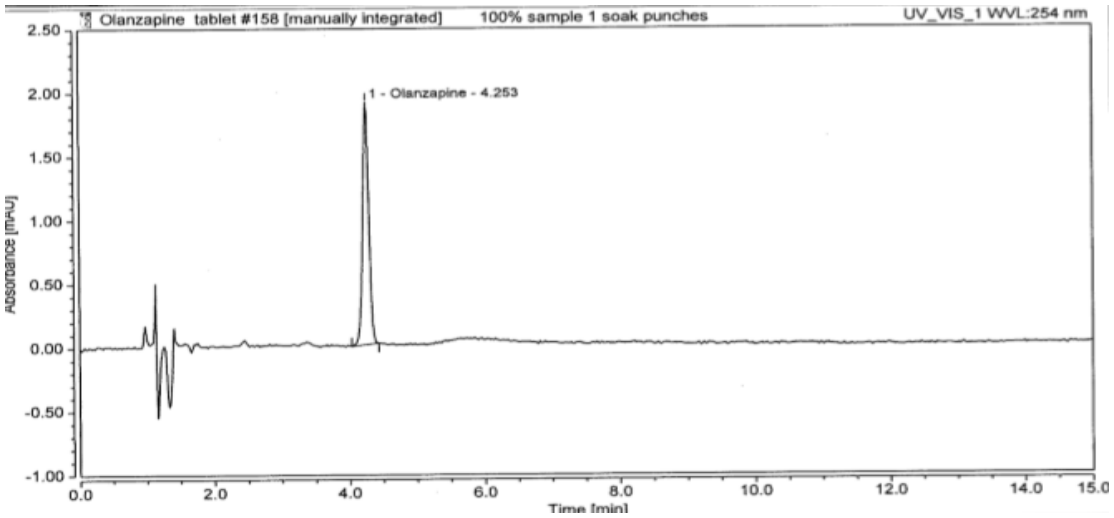

Figure 15 Chromatograph for Olanzapine sample solution (0.2273 ppm), for Tablet Press Punches soaked test.

3. Implementation of Cleaning Procedure Using Pilot Scale Product and Equipment

3.1 Execution During Swab Test for Bin Mixer

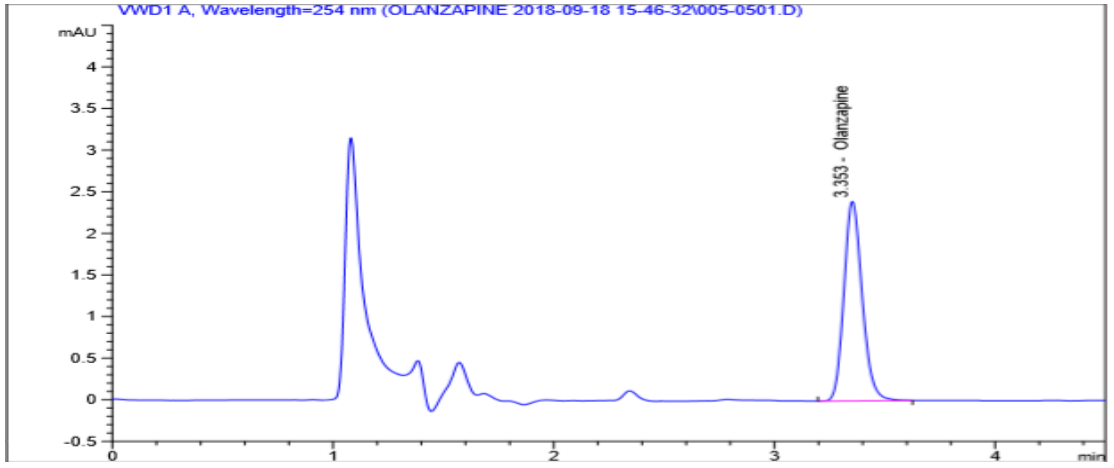

Figure 16 Chromatograph for Olanzapine standard solution (0.2273 ppm) for Bin Mixer swab test.

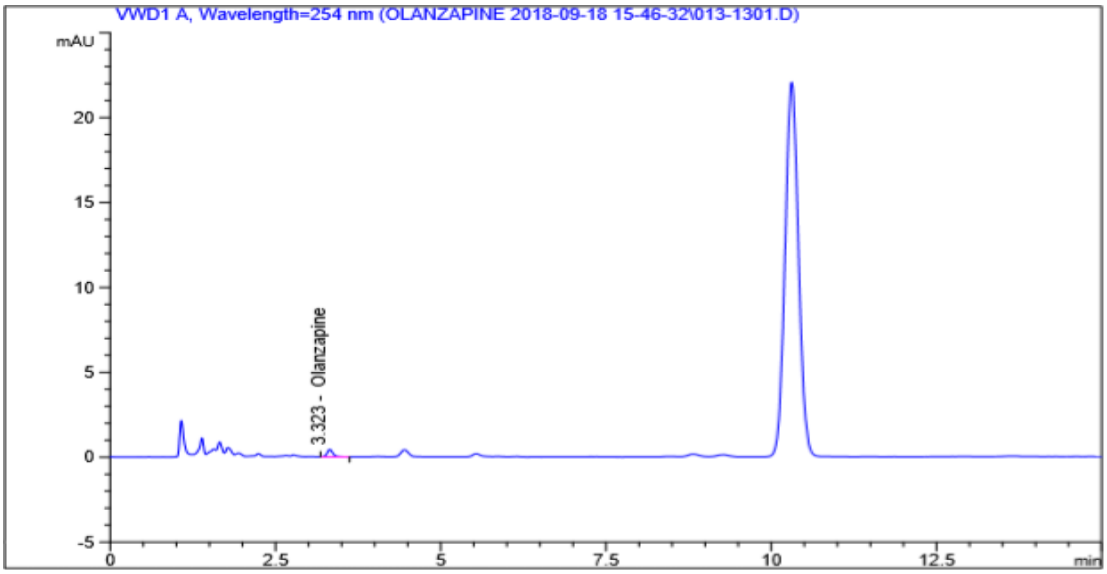

Figure 17 Chromatograph for Olanzapine Sample solution (S1) for Bin Mixer swab test.

3.2 Execution During Rinse Test for Bin Mixer

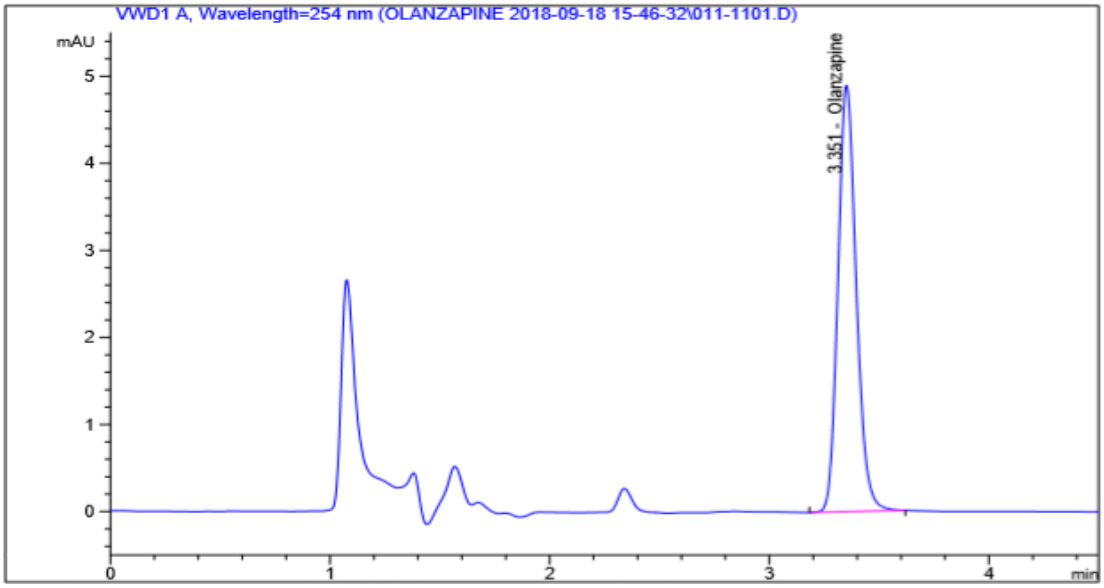

Figure 18 Chromatograph for Olanzapine standard solution (0.45453 ppm) for Bin Mixer rinse test.

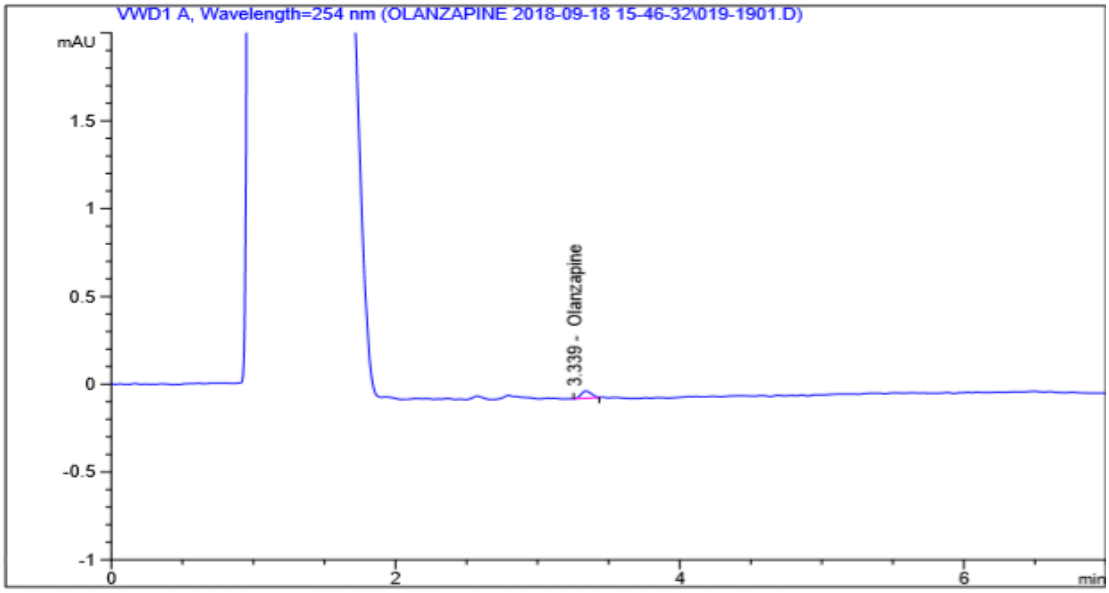

Figure 19 Chromatograph for Olanzapine sample solution (R1) for Bin Mixer rinse test.

3.3 Execution During Soak Test for Tablet Press Punches

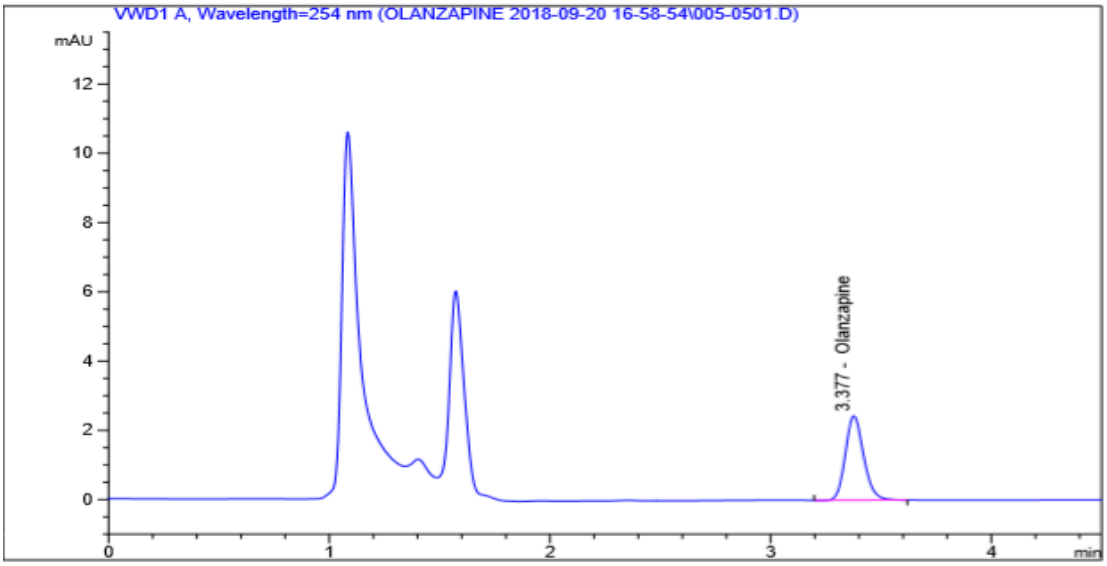

Figure 20 Chromatograph for Olanzapine standard solution (0.2273 ppm) for Tablet Press soaked test.

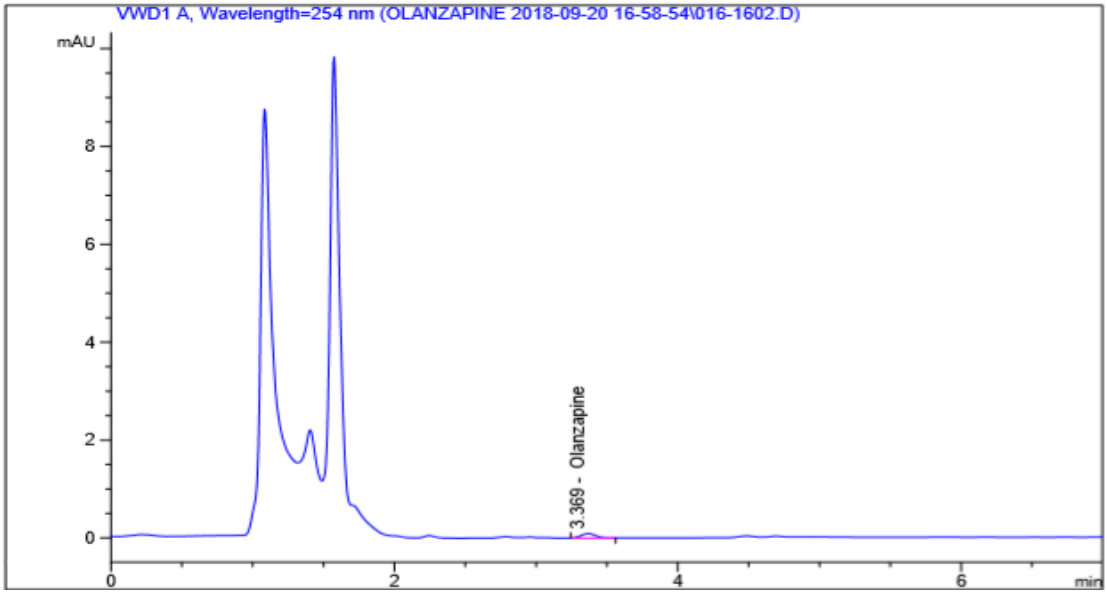

Figure 21 Chromatograph for Olanzapine sample solution (SK1), for Tablet Press Punches soaked test.