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A Comparative Study of the *In Vitro* Dissolution Profiles Of Immediate Release Solid Oral Dosage Forms

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Preface

The work presented in this thesis was developed at the company Labatec Farmacêutica S.A. (Sintra, Portugal), during the period March 2021 to October 2021, under the supervision of MSc. Iva Vinhas. The thesis was cosupervised by Prof. Dr. José Cardoso Menezes.

I declare that this document is an original work of my own authorship and that it fulfills all the requirements of the Code of Conduct and Good Practices of the Universidade de Lisboa.

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To my sisters and brother who were always there for me when I needed them. Thank you for your help, support, and love. I am the luckiest person in the world to have you in my life.

And as the last are the first, to the most important and to whom I owe a lot, my parents, a giant thank you for everything they have done for me. For their permanent support, strength and encouragement, indispensable and fundamental in the motivation, achievement and conclusion of this work.

This Thesis is lovingly dedicated to my mother. Without her endless love and encouragement, I would never have been able to complete my graduate studies. I love you and I appreciate everything that you have done for me.

Abstract

The Dissolution Test is an essential tool throughout the entire life cycle of a drug product preparation, from the initial development stages, through the marketing authorization acquisition, during the quality control of the finished product prior releasing for market distribution, and to proof equivalency in case of changes or regulatory variations. It exploits an *in vitro* physical solubility rate & extend test within a pre-set medium at a certain time point, that is used to proof product performance, consequently, to be absorbed.

Initially, it was designed for oral solid pharmaceutical forms, as these have more variables to consider related with physical characteristics that have direct impact on their bioavailability profile. Currently, the test is widely used for various pharmaceutical forms (including modified release) such as: tablets, capsules, suspensions, transdermal patches, ointments, gels, implants and others.

The current study is a part of product technology transfer project, the product has been developed by the pharmaceutical company Labatec Pharma S.A., then to be transferred to Labatec Farmacêutica S.A., one of the routine health authorities' requirements to proof performance equivalency, is submitting dissolution profiles comparison, between the Portuguese site manufactured product batches, against the current commercialized product produced by the Swiss site.

Dissolution profiles is also a requirement for other markets, such as the Middle East and North Africa region (MENA region) to provide a dissolution profile comparison between product batches produced at different production sites. In this Technology Transfer activity, the goal is to register the new Portuguese site as the future manufacturing site in the targeted market destinations, such as Jordan.

For this purpose, multiple dissolution profile tests were carried out at several pH 1.2, 4.5 and 6.8 to mimic gastric and intestinal conditions, running for different time points (5,10,15,25 and 30 minutes) enabling similarity evidence between test (Portugal site) against reference (Swiss site) batches, therefore, acquiring site approval for future market supply.

Keywords: Baclofen, Dissolution Profiles, Ultra Performance Liquid Chromatography, Immediate Release Solid Oral Dosage Forms, RSD

Resumo

O Teste de Dissolução é uma ferramenta essencial durante todo o ciclo de vida da preparação de um medicamento, desde as fases iniciais de desenvolvimento, passando pela aquisição da autorização de comercialização, durante o controlo de qualidade do produto acabado antes da sua libertação para a distribuição no mercado, e até à prova de equivalência em caso de alterações ou variações regulamentares. Explora-se uma taxa de solubilidade física *in vitro* e estende-se o teste dentro de um meio pré-estabelecido num determinado momento, que é usado para provar o desempenho do produto, consequentemente, para ser absorvido.

Inicialmente, foi concebido para formas farmacêuticas sólidas orais, uma vez que estas são mais problemáticas em termos de libertação de fármacos para alcançar a biodisponibilidade. Atualmente, o teste é amplamente utilizado para várias formas farmacêuticas (incluindo libertação modificada) tais como: comprimidos, cápsulas, suspensões, pomadas, géis, implantes e outros.

O estudo atual faz parte do projeto de transferência de tecnologia do produto, o produto foi desenvolvido pela empresa farmacêutica Labatec Pharma S.A., para depois ser transferido para a Labatec Farmacêutica S.A., um dos requisitos de rotina das autoridades de saúde para comprovar a equivalência de desempenho, é submeter a comparação de perfis de dissolução, entre os lotes de produtos fabricados no *site* português, contra o produto comercializado atualmente produzido pelo *site* suíço.

Os perfis de dissolução são também um requisito para outros mercados, tais como o Médio Oriente e a região do Norte de África (região MENA) para fornecer uma comparação de perfis de dissolução entre os lotes de produtos produzidos em diferentes locais de produção. Nesta atividade de Transferência de Tecnologia, o objetivo é obter e manter autorização de fabrico para a nova unidade industrial em Portugal, segundo os requisitos em vigor para os mercados da MEMA, como por exemplo a Jordânia.

Por este motivo, foram efetuados testes para caracterizar o perfil de dissolução sob diversas condições de pH 1.2, 4.5 e 6.8 para mimetizar as condições digestivas gástricas e intestinais. Foram efetuados doseamentos em diferentes tempos (5,10,15, 25 e 30 minutos), permitindo a verificação de similaridade de perfis entre as formulações de teste e de referência, sendo informação relevante para integrar o processo de registo da instalação fabril.

Palavras-chave: Baclofeno, Perfis de Dissolução, Cromatografia líquida de ultra eficiência, Formas Orais de Libertação Imediata, RSD

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List of Abbreviations

API	Active Pharmaceutical Ingredient
BCS	Biopharmaceutics Classification System
BP	British Pharmacopoeia
EMA	European Medicines Agency
HPLC	High Performance Liquid Chromatography
GMP	Good Manufacturing Practices
LC	Liquid Chromatography
N	Number of theoretical plates
QC	Quality Control
Rpm	Rotations per minute
RRT	Relative Retention Time
RT	Retention Time
Rx	the drug product is a prescription drug
SST	System Suitability Test
SUPAC	Scale-up and post-approval changes
US FDA	United State Food and Drug Administration
USP	United States Pharmacopeia
UV	Ultra-violet
UV/VIS	Ultraviolet-Visible Spectroscopy
WHO	World Health Organization
OTC	Over The Counter
MENA	Middle East and North Africa
BE	Bioequivalence
MA	Market Authorization
GIT	Gastrointestinal Tract
GI	Gastro Intestinal
CNS	Central Nervous System

BBB	Blood-Brain Barrier
GABA	Gamma-aminobutyric acid
AUC	Area Under Curve
IVIVC	In Vitro-In Vivo Correlation
CR	Controlled Release
SR	Sustained Release
IR	Immediate Release
SOP	Standard operating procedure
CMA	Critical Material Attributes
CQA	Critical Quality Attributes
CPP	Critical Process Parameters
QTPP	Quality Target Product Profile
WS	Working Stand
RT	Room temperature

Chapter 1 Framework and goals

The main objective of this MSc project was to compare in vitro dissolution profiles of baclofen containing tablets that best discriminate between batches, i.e., the comparison must be able to discriminate between acceptable and non-acceptable batches of the same formulation.

The dissolution method used was done as stipulated within the marketing authorization dossier and analytically transferred from Geneva site, Labatec Pharma S.A. to the Portuguese site, Labatec Farmacêutica S.A. with the objective of establishing a comparative dissolution study for future site to evaluate batches from the new site.

Dissolution testing was executed because of the need to ascertain bioavailability, bioequivalence for future batches prior market release, and because is assumed an *in vivo-in vitro* correlation (IVIVC) can be proven, that is, to predict the in vivo release behavior of a drug, consequently its absorption rate and extent, or even the similarity between different pharmaceutical forms, when applicable, all with the aid of some mathematical models by comparing dissolution profiles. [1]

In the pharmaceutical industry and in the various stages of research, development and optimization of new pharmaceutical forms, dissolution tests have shown to be relevant in the selection of an ideal pharmaceutical composition and in the biopharmaceutical characterization of the drug.[2]

Baclofen is present in the formulation of the generic drug under study Baclocalm®, This is a drug intended for the treatment of antinociceptive effect in neurological diseases associated with spasm of skeletal muscles.[3]

A generic is an identical, or bioequivalent, drug product preparation to an innovator drug with respect to pharmaceutical form (tablet, capsules, oral suspensions, among others), safety, dosage, route of administration, quality, efficacy and purpose [4]. Nowadays about half of the drugs on the market can be replaced by a generic product [5]. Its main benefit is the health costs for the patients at the price being marketed. This is feasible due to the fact that generics do not entail such high research and development costs, mainly clinical studies, as they are developed on the basis of innovative products formulation, whose patents might have expired and do not need to demonstrate clinical efficacy [6].

Oral absorbed tablets exert their action through the systemic route; therefore, Bioavailability/bioequivalence (BD/BE) studies are the centerpiece of their evaluation, letting know if the generic drug has the same amount of the active substance(s) in the same pharmaceutical form when compared to the reference drug. [6] And if the rate of absorption into the bloodstream after becoming available at the action side is equivalent, complying with an international requisite. [7]

Comparison of the dissolution profiles of three test batches against three commercial batches for each one of the strengths 10 mg and 25 mg were carried out in different dissolution media to evaluate the consistency and homogeneity of the produced batches to proof the in vitro similarity between them. The batches were produced within the scope of technological transfer between Labatec Pharma and Labatec Farmacêutica to register the new Portuguese site as the future commercial manufacturing site for the corresponding health authorities, mainly the swiss and some MENA countries.

Chapter 2 Labatec Pharma SA and Labatec Farmacêutica SA

Figure 2.1 Labatec logo.[8]

Labatec (figure 2.1) is a privately owned Swiss based pharmaceutical company with more than 50 years of manufacturing experience in providing high quality products to the Swiss and Middle Eastern markets. It was founded and established in Switzerland in 1957 acquired by the founder of hikma pharmaceuticals, in 2008 through which it has witnessed remarkable expansion in an emerging market, entering 16 new countries in the last eight years, with presence in both retail and hospital segments leading to the establishment of hospital injectables portfolio of over 65 products from 2010 to 2013, enabling the launch of its products. [8]

Labatec has successfully leveraged the two cornerstones of its business model:

- Its strong portfolio of hospital injectables with a unique safety packaging concept
- Its reputation as a Swiss quality brand to introduce a range of OTC and Rx products in MENA markets.

According to its noticeable growth in 2018, Labatec started the construction of a factory in Mem Martins, Sintra. More than three years and 15 million euros later, the company now has officially started the production of its drug in Portugal. With about 4000 square meters of total area, the factory includes offices, production, packaging, laboratories and warehouse. This unit has an initial production capacity of 250 million tablets, it will be possible to expand its production to two billion tablets per year.[8]

The company's new factory, Labatec Farmacêutica SA, was projected to produce oral dosage forms, as Baclofen, designed for the treatment of diseases related to the musculoskeletal system, the category that represents around 47% of its total revenue as shown in the figure 2.2 chart below:

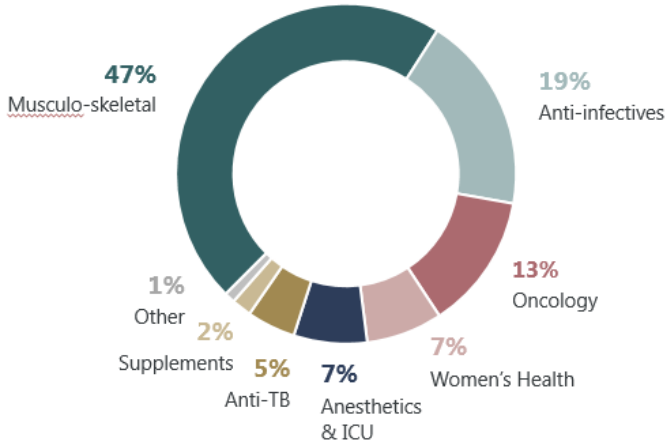

Figure 2.2 Labatec revenues by therapeutic category.[8]

All medicines produced in this factory will be destined for the international market, namely Switzerland and several countries in the MENA (Middle East and North Africa) region. Portugal is geographically well positioned for this purpose.

The factory organization has an extremely specialized structure, as it has a high number of graduates and experts in the different key functions of the company. This framework is supported by an integrated management and information system.[8]

The design of the installations, infrastructures, and the organization of the integrated management system all ensure pharmaceutical production in compliance with the Guide to Good Manufacturing of Pharmaceuticals and other applicable legislation. [9]

In addition, it is an aim of the company, through listing potential pollution sources, to avoid any adverse effects in suitable time and to carry out the necessary corrective measures. For this reason, periodical inspections are carried out by INFARMED which certifies compliance with the Good Manufacturing Practices and authorizes the manufacturing of medicines for human use. [10]

Chapter 3 Theoretical general overview

3.1 Generic Drugs

According to the US FDA (United State Food and Drug Administration) and EMA (European Medicines Agency), all substances designated for the diagnosis, alleviation, prevention, or treatment of illness usage should be considered a medicine. Any patent protected drug has a trade name and is not allowed to be manufactured or resold by a third party is called a "branded drug" while any drug that is a surrogate or a copy and equivalent (in terms of safety, efficacy, dosage and use) to a branded drug is called a "generic drug" which is more affordable and accessible. [11]

In this context, it becomes important to note there are different requisites that generic drugs must meet. However, one of the main assumptions that underpins the safe and effective use of generic drugs is the concept of bioequivalence [4].[12]

The FDA evaluates generic drugs in science reviews based on interchangeability or therapeutic equivalence means that the drug must be pharmaceutically equivalent (if they contain the same quantity of the active substance(s) in the same pharmaceutical form according to the same or similar standards as the reference medicinal product).[13]

Drug product performance comparison for oral generic drug products may be measured either by in vivo bioequivalence studies in normal healthy adult subjects under fasted and fed conditions, or in vitro dissolution profiles comparisons.[13]

The WHO estimates that 30% of the world's population lacks access to essential medicines owing to the cost of prohibited pharmaceuticals. Governments in less advanced countries are unfortunately failing to address these problems within the short term, which leads to rising government expenditure on healthcare. [14][15] Therefore, Generic drugs are promoted in developed and developing countries as they play an essential role in lowering health care costs for the population because they do not incorporate research and development costs into the price, and hence, 20% or 35% cheaper than the reference drugs.

The steps involved in the preparation of a generic drug include the characterization of the reference drug, development of the generic drug and finally the performance of a BE study with the reference drug [16].

In addition, there is the chance that generic companies may have an interest in uncovering the procedures behind the production of an original drug. To make this alternative possible, there are patent royalties. These are payments made by the licensee to the licensor for the use of the patent. In this way, it is ensured that the inventor is fairly compensated for the use of his property. By setting royalty rates as a percentage of the revenue generated from the patent, both sides benefit from the arrangement. Royalty rates can range from 0.1% to 25% and can vary across products and industries. In this way, the licensee can use the invention to boost its business activities and avoid the time wasted in discovering a new product or technology. [12]

In Europe, the body responsible for the approval of pharmaceutical products is the European Medicines Agency. Besides EMA, each member state has a competent authority for such approval [4].

In case of Portugal, the regulatory authority supervising the human medicines sector is INFARMED. Whenever a new drug is to be placed in the market or changes are made to a drug, it is mandatory to submit a support documentation that will be assessed by the NRA (National regulatory authority) and if approved then MA (Market Authorization) or post-approval variation is granted.

As for MENA (Middle East and North Africa) countries generic producers dominate most of the local pharmaceutical drug production. [17][18]

3.2 Baclofen

Spasticity is a common outcome in CNS (Central Nervous System) injuries, affecting the life quality of the affected individuals.[19]

The spinal cord injuries, traumatic or other, are included in the set of CNS pathology. Baclofen is one of the most used antispastic drugs, being marketed orally since 1974 as an effective therapy in about 30% of individuals with severe spasticity. It was approved by the FDA for the treatment of spasticity of medullary origin in 1992 and cerebral in 1996. [19]

3.2.1 Physical and chemical properties

Baclofen is designated chemically as 4-amino-3-(4-chlorophenyl) butanoic acid, with the molecular formula $C_{10}H_{12}ClNO_2$ and a corresponding molecular mass of 213.7 g/mol. Its chemical structure is shown in the figure 3.1 below [20]

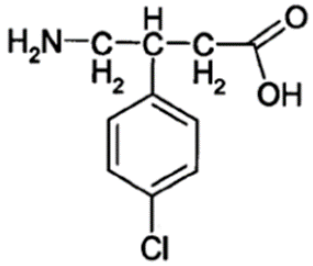

Figure 3.1 Chemical structure of Baclofen. [21]

Described by the following chemical names: [21][22]

I. 4-Amino-3(p-chlorophenyl) butyric acid
II. β-(Aminomethyl)-4-chlorobenzenepropionic acid
III. β-(Aminomethyl)-p-chlorohydrocinnamic acid
IV. y-Amino-$-(p-chloropheny1) butyric acid
V. β -(4-Chlorophenyl) GABA (Gamma-aminobutyric acid)

Baclofen is a white or off-white mostly odorless crystalline powder with slightly bitter taste., its real density is 1.2069 g/cm^3 with a melting point ranging from 206°C to 208°C. Regarding its solubility, it is slightly soluble in water (4 mg/ml at pH 7.6), very slightly soluble in methanol, and insoluble in chloroform. [22]

Based on the characterization of the reference drug, the QTPP (Quality Target Product Profile) was elaborated, and the criteria for the product quality, safety, and efficacy of the targeted formulation were designed. (Table 3.1)

Table 3.1 QTPP designed criteria for the Baclofen product.

QTPP	Target Parameters
Dosage form	Tablet
Dosage design	Immediate release tablet
Route of administration	Oral
Dosage Strength	10 mg and 25 mg
Pharmacokinetics	Immediate release enabling T_{max} in 1.5 hours or less. Bioequivalent to reference drug
Stability	At least 12-months shelf life at room temperature
Dissolution %	Not less than 75% (Q) of the labelled claim released within 30 minutes

3.2.2 Pharmacokinetics

Absorption:

Baclofen is rapidly and completely absorbed from the gastro-intestinal tract. Maximum concentrations of unchanged drug are attained in plasma in 2 to 4 hours after an oral dose. The bioavailability of oral baclofen is 70 to 80%.

Following oral administration of a single dose of 40 mg baclofen, peak serum concentrations of 500 to 600 nanogram/mL are reached. The serum concentration remains above 200 nanogram/mL for 8 hours. The onset of action is highly variable and may range from hours to weeks. [22] [23] [24]

Distribution:

The distribution volume of baclofen amounts to 0.7 l/kg. In cerebrospinal fluid, the active substance attains concentrations approx. 8.5 times lower than in the plasma.

Baclofen is bound to plasma proteins to the extent of about 30%.[22] [23] [24]

Metabolism:

About 15% of a dose of baclofen is metabolized in the liver. Deamination yields the main metabolite, β-chlorophenyl-γ-hydroxybutyric acid, which is pharmacologically inactive. [22] [23] [24]

Elimination:

Approximately 70% of baclofen is eliminated in the urine in unchanged form. The plasma elimination half-life of baclofen averages 3 to 4 hours. Within 72 hours, approximately 75% of the dose is excreted via the kidneys, approximately 5% of this quantity being in the form of metabolites. The remainder of the dose, including 5% as metabolites, is excreted in the faeces. [22] [23] [24]

3.2.3 Pharmacodynamic

Baclofen is a highly effective spinal antispasmodic. Its mechanism of action and pharmacological properties differentiate it from other antispastic agents. it inhibits both Mono sympathetic and polysynaptic reflex transmission by stimulating GABA receptors. This stimulation, in turn, inhibits the release of B excitatory amino acids, glutamate and aspartate. Neuromuscular drive is not affected by baclofen. Further, it has an antinociceptive action. In neurological disorders associated with skeletal muscle spasms, the clinical treatment effects of Baclofen are beneficial on reflex muscle contractions and give improved relief on painful spasms, automaticity, and clonus. Baclofen tablets (figure 3.2) stimulates the secretion of gastric acid and improves the patient's mobility, allowing them to move without assistance, facilitating passively and actively physiotherapy, leading to a better quality of life for the patient.[21][23]

Figure 3.2 Baclofen tablets captured during the analysis.

3.2.4 Process Manufacturing

Baclofen tablets should be manufactured by direct blending approach, where simply all raw materials are combined into a uniform, free-flowing, squeezable final blend followed by the compression process.

Figure 3.3 shows the flowchart of the manufacturing process of Baclofen 10mg and 25mg.

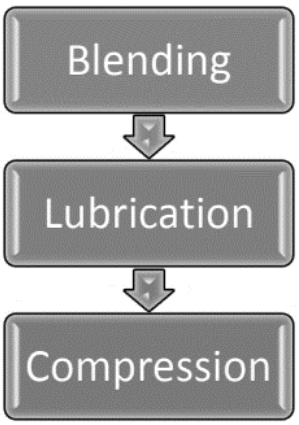

Figure 3.3 Flowchart of manufacturing process.

A risk analysis, in accordance with ICH Q9, was used to establish which variables and unit operations were likely to have the greatest impact on product quality. This initial risk assessment is shown in Table 3.2.

Table 3.2 Manufacturing Risk Assessment. [25][26]

Drug Product CQAs	Process steps									
	Room Conditions		Dry Mixing Excipients		Pre-Blend			Final Blending/Lubrification		Compression
CPP	Temperature	Humidity	Time of mixing	Mixing Speed	Geometry Of Blender	Time Of Mixing	Rotation Speed	Time Of Mixing	Rotation Speed	Compression Force
Dissolution	Low	Low	Low	Low	Low	Low	Low	High	Low	Medium

Following the definition of the process critical steps, they are studied to mitigate risk affecting the final product quality, as shown in table 3.3.

Table 3.3 Justification of manufacturing risk assessment. [25][26]

Manufacturing Step	Parameter	Risk	Justification
Drug Product CQA	**Dissolution**		
Room Conditions	Temperature and Humidity	Low	If left unchecked, the facility's variations in temperature and RH can impact the drug's CQAs. the routine room temperature and RH set point in the GMP plant are set at 25°C ± 5% and 40% - 60% respectively and will be tracked during manufacture.
Dry Mixing Excipients	Time of Mixing	Low	Process of simple mixing intended to promote initial homogenization This parameter is not critical for dissolution
	Mixer Speed	Low	The mixer speed is fixed in lab equipment. No different speed is expected
Pre-Blend	Geometry of blende	Low	The geometry of the impeller blade in the laboratory is the same as in production. This parameter is not critical.
	Time of Mixing	Low	The time of blending for homogenization on this step does not affects dissolution.
	Speed of Mixer	Low	Rotation speed is fixed
Final Blending/Lubrification	Time of Lubrication	High	Too much lubrication due to an excessive number of rotations can affect the disintegration and dissolution of the tablets
	Rotation Speed	low	Rotation speed is fixed by equipment
Compression	Compression force	Medium	Machine error, human error. higher compressions forces lead to lower drug releases via higher hardness tablet values

From the study it can be concluded that using the following manufacturing process conditions to the prepared dosage forms of Baclofen (Table 3.4), we have a controlled process and a product with the desired quality.

Table 3.4 Manufacturing process parameters selected for Baclofen.

Process Step	Equipment	Process Parameters
Blending	IBC Blender (780 Liters full capacity)	25 minutes at 12 rpm
Lubrification	IBC Blender (780 Liters full capacity)	5 minutes at 12 rpm.
Compression	Rotary Press Machine	70 N, 50000 Tablets/Hour

3.3 BCS Classifications of Baclofen

The effectiveness of drugs relies on the provision of proper active concentrations at the site of intended pharmacodynamic effect. The drug uptake by oral dosage forms is reliant on the liberation of baclofen API (Active Pharmaceutical Ingredient) from the drug, the API becoming dissolved under physiological conditions, and its permeability through the membranes of the gastrointestinal tract. [27]

Drugs are categorized based on their water solubility and the permeability of the intestinal membrane using a scientific framework, the biopharmaceutical classification system. BCS (Biopharmaceutic Classification System) of drug absorption. these drivers are dissolution, solubility and intestinal permeability.[28][29]

BCS has grouped drug substances into four classes regarding the represented table 3.5.

Table 3.5 Biopharmaceutic Classification System of Drugs.[29]

Class	Aqueous Solubility	Membrane Permeability
I	High	High
II	Low	High
III	High	Low
IV	Low	Low

The BCS solubility rating of a drug is based on the highest strength in an IR (Immediate Release) product. A drug substance is deemed to be highly soluble when the highest concentration is soluble in 250 mL or less of aqueous media in the pH range of 1.0-7.5; otherwise, the drug is considered poorly soluble.

The volume estimate of 250 mL is as derived from typical bioequivalence studies protocols that prescribe administration of a drug to fasting human volunteers with a glass (about 8 ounces) of water.[30]

On the other hand, permeability ratings rely either directly on the extent of gut absorption of a drug substance in humans or indirectly on measuring the rate of mass transfer across the human intestinal membrane. A drug substance is deemed to be highly permeable if the extent of intestinal absorption is 90% or more.[30]

An IR drug is categorized as a fast-dissolving product when not <85% of the labelled amount of the drug substance dissolves within 30 minutes using the USP apparatus.

Studies carried out by different authors indicate that baclofen can be classified as **class III**. [24]

3.4 Dissolution Tests

3.4.1 Evolution and fundamentals of dissolution testing

Solid dosage forms administered orally (tablets and capsules) comprise a large fraction of pharmaceutical products. Pills and capsules are designed to liberate the API through the GI (Gastrointestinal) tract of the patient in a prescribed mode. Our comprehension of the *in vivo* delivery and absorption mechanism of API is a fundamental aim to fast-track and optimize the development of orally administered drugs. Dissolution testing is an *in vitro* laboratory based performance test that assesses the effectiveness with which a drug is released from its pharmaceutical form.[31]

Dissolution tests are designed to measure the amount of API dissolved over time across different media, simulating the entry of the tablet into the body. As seen in figure 3.4, dissolution of a dosage form involves at least two consecutive steps: the release of the solute or matrix substance from the formulation matrix (Disintegration/Deaggregation), followed by dissolution of the drug (solubilization of the drug particles) in dissolution medium. [31]

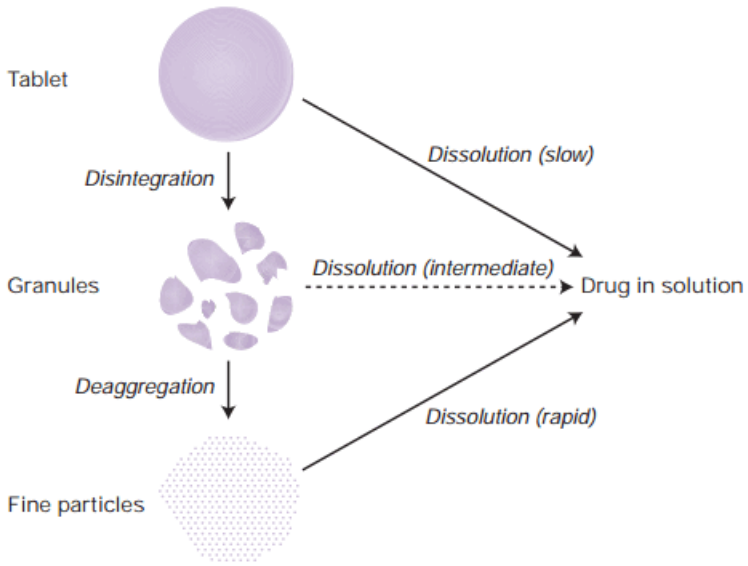

Figure 3.4 Dissolution of a solid oral dosage form.[32]

Dissolution process is based on the interaction of the solute with the solvent molecules and the diffusion of the outermost solute molecules into the surrounding medium, through the diffusion layer.

A dissolution test is an *in vitro* tool that provides important insights into the similarity of drug release between different batches and brands. These assays were created in response to the demand to ascertain the bioavailability, bioequivalence, and *in vivo* performance of drugs to ensure their quality. [33]34]

The reasons why dissolution studies are carried out emerge from the need of:[33][34]

- Formulation effects evaluation and process variables on the bioavailability of a drug
- Ensuring that the prepared batches comply with the specifications listed

- Indicating the *in vivo* conditions of the preparations or in other words, the correlation between *in vitro* dissolution and the uptake of the drug *in vivo*.

In 1897 the first mention of drugs dissolution appeared when Noyes and Whitney conducted the first dissolution experiments and published the article "The Rate of Solution of Solid Substances in Their Own Solution". Both Arthur A. Noyes and Willis R. Whitney investigated the dissolution of two poorly soluble compounds: benzoic acid and lead chloride.[35][36]

As verified by the authors, the speed of dissolution (dC/dt) was proportionately related to the difference between the saturation concentration of the drug in the medium (Cs) and that of the drug at time t (Ct). This ratio can be voiced through the mathematical formula evolved by both, Equation 3.1:[37]

$$\frac{dC}{dt} = k\,(C_s - C_t) \qquad \textbf{Equation 3.1}$$

Where K is a constant. The authors have, throughout the experiment, made sure that the outer surface of the test substances was constantly exposed with the dissolution media as well as an excess of the necessary amount of substance to reach the saturation concentration of the medium itself. They ascribe the phenomenon of molecules dissolution to a thin layer forming around the particles, by which they diffuse into the surrounding medium. This model is called the "Diffusion Layer Model" figure 3.5 [37].

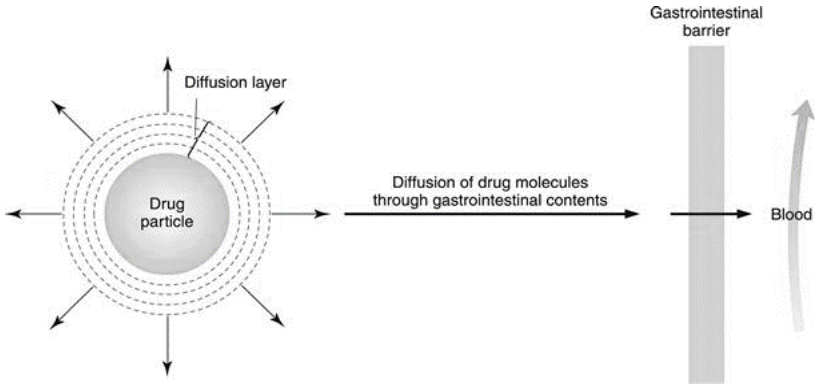

Figure 3.5 schematic representation of drug particle dissolution in the GI fluids.[36]

In 1900 Brunner and Tolloczko introduced new boundary conditions for which the equation is valid, demonstrating that the velocity of dissolution varies as a function of the surface area of the exposed particle, the agitation rate, temperature, surface structure and the arrangement of the equipment [37]. This can be observed from Equation 3.2:

$$\frac{dC}{dt} = K_1 S\,(C_s - C\,) \qquad \textbf{Equation 3.2}$$

Where S is the surface area and K is a constant. After further advances and studies in the area, Equation 3.2 was changed in 1904 and based on the diffusion layer concept of Fick's second law, with new parameters such as the diffusion coefficient (D), the surface area (S), the thickness of the diffusion layer (h) and the volume of the dissolution medium (V) being introduced, expressed in Equation 3.3 .[37][38]

$$\frac{dC}{dt} = \frac{D\,S}{V\,h}\,(C_s - C) \qquad \textit{Equation 3.3}$$

If the volume of the dissolution medium is enough to verify that the concentration at a given time is lower than the saturation concentration, then the solute in the solvent does not affect the speed of dissolution. It is a condition that all dissolution tests must fulfil, known as sink conditions.

The dissolution rate theory, stated in Equation 3.3, is widely accepted in science. Yet, the speed of dissolution can be correlated with the initial mass of the substance and the mass left undissolved as a function of time. Hixon and Crowell developed the equation 3.4, which equates the solute surface area by mass and assumes that part of the initial substance mass dissolves into the surrounding medium and the size of the particle decreases over time.[38]

$$dw = \frac{D\,S}{V\,h}\,C_s\,dt \qquad \textit{Equation 3.4}$$

Finally, Hixson and Crowell have in 1931 designed a mathematical model of the dissolution process, known as the "law of the cube root", whereby the initial mass of the substance and the remaining mass to be dissolved are related with time Equation 3.5.

$$\sqrt[3]{w_0} - \sqrt[3]{w} = k_2 * t \qquad \textit{Equation 3.5}$$

In which W_0 is the initial mass of substance, W is the amount of substance that remains undissolved and k_2 a constant [39].

So, in 1970, with growing evidence of the association between dissolution and drug performance, the first official dissolution test was published by USP [40]. Where three types of dissolution tests for immediate release drugs were listed as [41]:

I. Single-point dissolution test:

It serves as a routine test for quality control (for products with high solubility and which dissolve quickly).

II. Two-point dissolution test:

Characterizes the quality of the product and as a routine test for certain products.

III. Comparison of dissolution profiles (multiple points)

to assess the similarity of assorted products, to exempt lower strength of a development product from *in vivo* studies or to exempt some drug classes from *in vivo* study (it is explained in more detail in section (3.4.3).

Dissolution of a drug from its dosage form depends on many factors, which include not only the physicochemical properties of the drug, but also the formulation of the dosage form and the process of manufacturing. Therefore, it is essential to determine the CMAs (Critical Manufacturing Attributes) and CPPs (Critical Process Parameters) that are based upon physical and chemical properties of the API, formulation, and process selections, (figure 3.6), which, in turn, can be linked to the dissolution performance. [42]

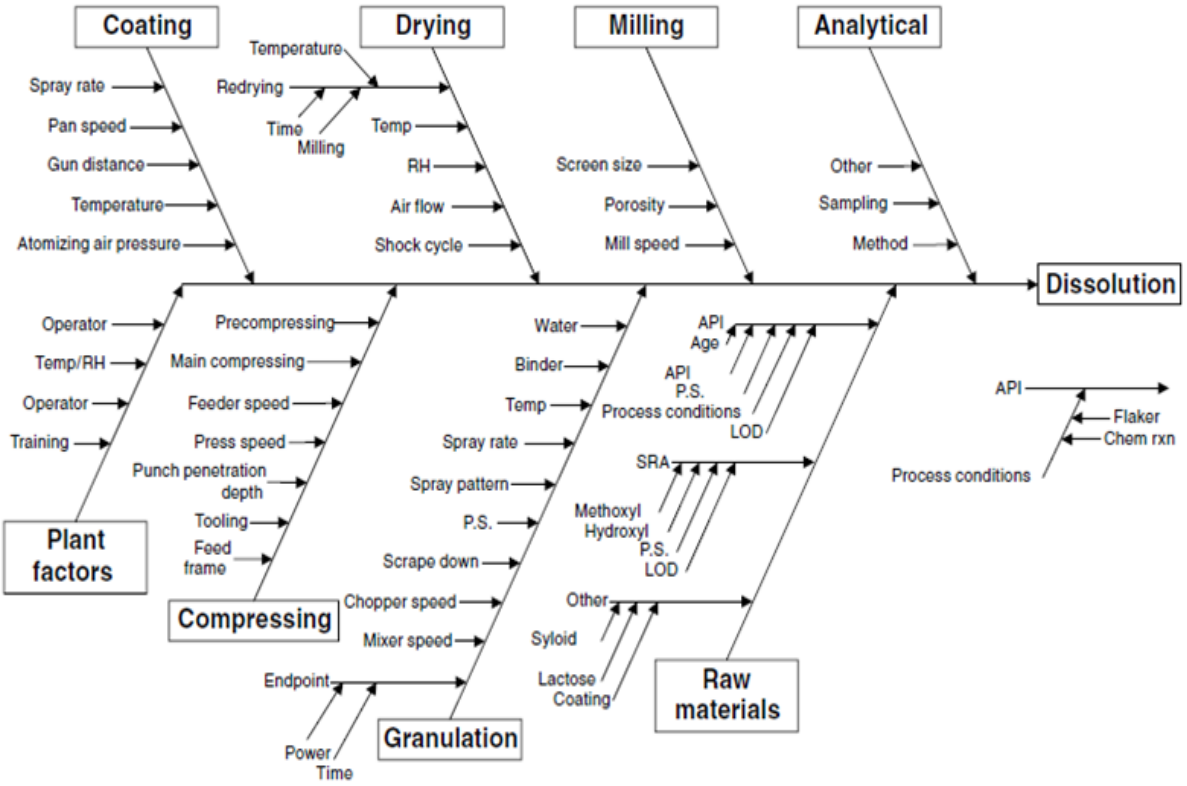

Figure 3.6 Ishikawa diagram for dissolution testing assessment.[43]

3.4.2 Dissolution method development

The dissolution procedure entails several components that are suitably chosen to develop a reproducible method for day-to-day operation and capable of being transferred between laboratories. [33][35] These elements are dissolution medium, dissolution apparatus, type of dissolution test (one, two and multiple points) and test mode.

Conditions According to USP section <1092>, *The Dissolution Procedure: Development and Validation*, that may affect drug dissolution and release are listed as the following:

- Drug Substance;
  - Particle size
  - Polymorph
  - Surface area
  - Chemical stability in dissolution media

- Formulation of drug product
  - Excipients (lubricants, suspending agents)
  - the impact of the compression force

- Media
  - Volume
  - pH
  - Temperature

- Co-solvents, added enzymes/surfactants

- Apparatus
  - Agitation rate
  - Shape of dissolution vessel
  - Placement of tablet in vessel
  - Sinkers (for floating products and products that stick to side of vessel) [34]

The first step in the design of dissolution test is the choice of the appropriate media, considering the physical and chemical properties of the active substance, the most common dissolution media are dilute hydrochloric acid, buffers (phosphate or acetate) in the physiological pH range of 1.2 to 7.5 and water. [34] The variation of biological fluids along GIT is displayed in figure 3.7.

For poorly soluble drugs, surfactants may be added to the aqueous solution (buffer solutions) enhancing their solubility.

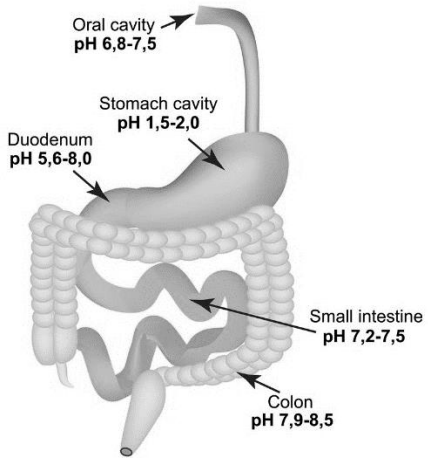

Figure 3.7 pH values in Healthy Humans along the Gastrointestinal Tract. [44]

Sink conditions are defined by the USP as "the volume of dissolution medium 3 times greater than that needed to create a saturated solution of the drug".[38].

As the volume of the medium, usually ,900 mL, relies on substance solubility and sink conditions, these are taken into consideration, to avoid drug saturation and enhance the melting speed of the drug particles.[36]

Regarding temperature, 37± 0.5 ^{0}C must be held to mimic body temperature.

According to the European Pharmacopoeia the dissolution apparatus shall be chosen considering the type of pharmaceutical and physicochemical properties of the drug form. As per describing in the table 3.6, the most common ones used for tablets are apparatus 1 and 2. [30]

Table 3.6 USP-NF and non USP-NF dissolution apparatus [38]

Apparatus	Name	Agitation method	Drug product
Apparatus 1	Rotating Basket	Rotating stirrer	Tablets, capsules
Apparatus 2	Paddle	Rotating stirrer	Tablets, capsules, modified drug products, suspensions
Apparatus 3	Reciprocating cylinder	Reciprocation	Extended-release drug products
Apparatus 4	Flow cell	Fluid movement	Drug products containing low-water-soluble drugs
Apparatus 5	Paddle over disk	Rotating stirrer	Transdermal drug products
Apparatus 6	Cylinder	Rotating stirrer	Transdermal drug products
Apparatus 7	Reciprocating disk	Reciprocation	Extended-release drug products
Rotating bottle	(Non-USP-NF)	-	Extended-release drug products (beads)
Diffusion cell (Franz)	(Non-USP-NF)	-	Ointments, creams, transdermal drug products

The unit with mesh basket operates in the same way as the rotary paddle apparatus [38]. This study will focus only on the rotating paddle equipment, since this is where the dissolution test of the API under study, Baclofen, is conducted.

3.4.2.1 **Equipment with stirring paddle**

According to the European pharmacopeia, to carry out the test in this equipment, the sample is introduced inside allowing the tablet to sink to the bottom of the vessel prior to the rotation of the paddle.

The cylindrical hemispherical-bottomed containers of glass, are inserted into a previously heated water bath at a temperature of (37ºC ± 0.5°C), which should be kept constant during the test with due care to avoid air bubbles forming on the surface of the sample.

As represented in the figure 3.8 below, rods axis must not be more than 2 mm from the vertical axis of the container and its lower part more than 25 ± 2 mm from the bottom of the container.

Rotational speed be set in the range 50-100 rpm. Since below 50 rpm the associated hydrodynamics are unreliable, and the turbulence experienced above 150 rpm compromises the results. [37]. Samples are collected automatically by autosampler at the indicated time. [31]

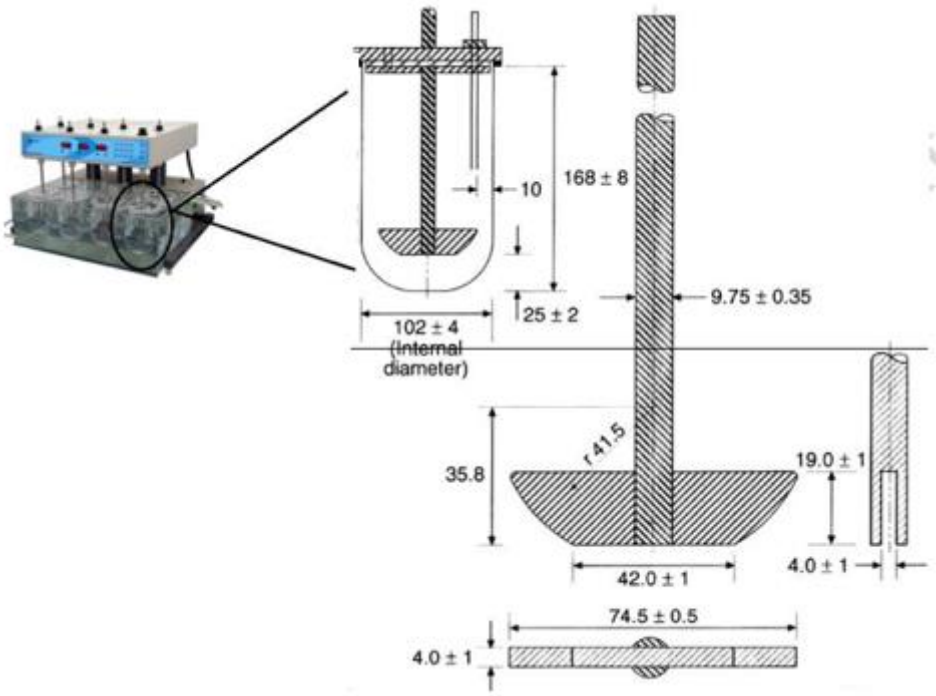

Figure 3.8 Dissolution Apparatus with Paddle (dimensions in millimeters). [31]

For the work under study, the analytical method used, which was transferred from the Swiss site, had already been validated and was being implemented in the laboratory before the start of the present studies.

3.4.3 Dissolution profiles

The current dissertation complied with the "Guideline on the Investigation of Bioequivalence", for which the dissolution profiles obtained from the dissolved percentage of the drug at different sampling times of three test batches were compared with the three reference batches for each strength (10mg and 25 mg), to assure the manufacturing process have not altered the behavior of the product as indicated in its registration dossier and to assess batch-to-batch quality during product quality control. [10]

Dissolution profile testing of drugs is carried out in at least three dissolution media in order to study their stability and release characteristics in different physiological conditions that they may be subjected to *in vivo*. The recommended dissolution media are 0.1N HCl as well as buffer solutions of pH 4.5 and 6.8. Water can be used as an additional medium. [45][46]

Nevertheless, several methods have been proposed for the comparison of dissolution profiles and are grouped into: (**i**) method based on analysis of variance, (**ii**) dependent model methods and (**iii**) independent model methods [47] which is currently the widely recommended method for the comparison of dissolution profiles when three or more dissolution time points are available as suggested by the FDA guidelines.

This model consists of two equations, developed in 1996 by Moore and Flanner. the first equation corresponds to the difference factor (f_1), which is a measurement of the relative error between two curves, that is, in percentage, the difference between two curves represented by the Equation 3.6.[40]

$$f1 = \left[\frac{\sum_{t=1}^{n} |R_t - T_t|}{\sum_{t=1}^{n} R_t} \right] \times 10 \qquad \textit{Equation 3.6}$$

Since:

n = number of sampling points (sampling times);

Rt = % dissolution of the reference drug at time t;

Tt = % dissolution of the test drug at time t.

and the second equation corresponds to the similarity factor (f_2), this one is the logarithmic transformation of the square sum of error. Through this factor it is possible to obtain the percentage similarity of the dissolution profiles between two curves. The similarity factor can be translated by the following equation 3.7 [37].

$$f2 = 50 \times log \left[\frac{1}{\sqrt{1 + \frac{\sum_{t=1}^{t=n} [R(t) - T(t)]^2}{n}}} \right] \qquad \textit{Equation 3.7}$$

According to the "Guidance for Industry – Dissolution Testing of Immediate Release Solid Oral Dosage Forms", Any two dissolution curves are considered similar when f_1 values are inserted in the range 0 to 15 and values of f_2 greater than 50 (50- 100). [48]. Regarding US FDA and EMA, when two dissolution profiles are identical, f_2= 100%. An average dissolution difference of 10% at all measured time points result in an f_2 value of 50%.for this reason, the public standard for similarity of two dissolution profiles had set to 50-100%.

For formulations in which the dissolved percentage is greater than 85% in 15 minutes, no mathematical calculation is necessary, and they are considered similar. The EMA bioequivalence guideline states that the evaluation of the similarity factor is based on the following conditions: the coefficient variation at the first test time must not be more than 20% and for the remaining test times not more than 10%; the test times must be the same for both formulations, with a minimum of 3 times (excluding zero) and no more than one average value above 85% for each formulation. [48]

To perform the evaluation of similarity it is necessary a data of six (6) or twelve (12) units of each product, and three or more dissolution time points, same conditions of testing for reference and test products and same dissolution time points for both profiles.

3.5 Drug factors affecting dissolution rate

Several variables can impact the dissolution profile of active substances and consequently their bioavailability. [48]

To assure the quality of the dissolution process, these parameters were divided into three categories as: (**i**) factors associated with the drug and its formulation, (**ii**) factors related to the dissolution testing and (**iii**) factors related to the analytical method.[48]

3.5.1 Factors associated with physicochemical properties of the drug

Understanding the physical and chemical properties of the drug play an essential role in controlling dissolution testing. The factors of the drug itself can be described as: [49][50]

- **Particle Size**

As per equation 3.3, an increase in the overall surface area of the drug in touch with the GI fluids will lead to an increase in the dissolution rate. If each drug particle is thoroughly wetted by the IG fluids, the actual surface area displayed by the drug will be proportional to the particle size of the drug. Therefore, the smaller the particle size, the greater the effective surface area exhibited by a given mass and the higher the dissolution rate.[39]

Laser diffraction can help to control the micronization of baclofen ingredients to the desired size at a high reproducibility rate. [51] As specified by the baclofen API suppliers, to ensure a controlled release of the ingredients, particle size distribution should be within the interval of:

- $\mathbf{D_{10}}$ NMT 10 µm
- $\mathbf{D_{50}}$ NMT 45 µm
- $\mathbf{D_{90}}$ NMT 150 µm

- **Solubility**

Is the factor that most affects the speed of dissolution. It is a thermodynamic parameter that shows the solution concentration of a drug in equilibrium with the solute. [31]

The Baclofen can be classified according to its solubility, as shown in the Table 3.7.

Table 3.7 Solubility of Baclofen. [21]

Solvent	Temperature	mg/ml
Water (pH 7.6)	23°C	4.3
Methanol	RT	0.045
Ethanol	RT	0.024
Chloroform	RT	0.014
Dimethylformamide	RT	0.008
Acetonitrile	RT	0.004
Phosphate Buffer (PH 7.4)	RT	5.0
0.1N HCl	RT	>20
0.1N NaOH	RT	>20

A variety of strategies can be adopted with the aim of improving drug solubility at the human fluid level, such as the reduction of particle size and the preparation of formulations containing microemulsions, liposomes, cyclodextrins and surfactants.[31]

- **Chemical Nature**

Many drugs are likely to exist in more than one crystalline form, a property referred to as polymorphism; every polymorph has its own different energy and consequently differs in physicochemical terms, such as solubility, melting point, heat of fusion, density and refractive index.

The polymorphic forms have an impact on the physicochemical properties of the drug influencing its bioavailability. [31][52] [53]

According to the monograph in the European Pharmacopoeia, baclofen shows polymorphism. Baclofen, a widely used antispastic agent, has been found to exist in two crystalline forms, the anhydrate and monohydrate as showed in the figure 3.9 [54]

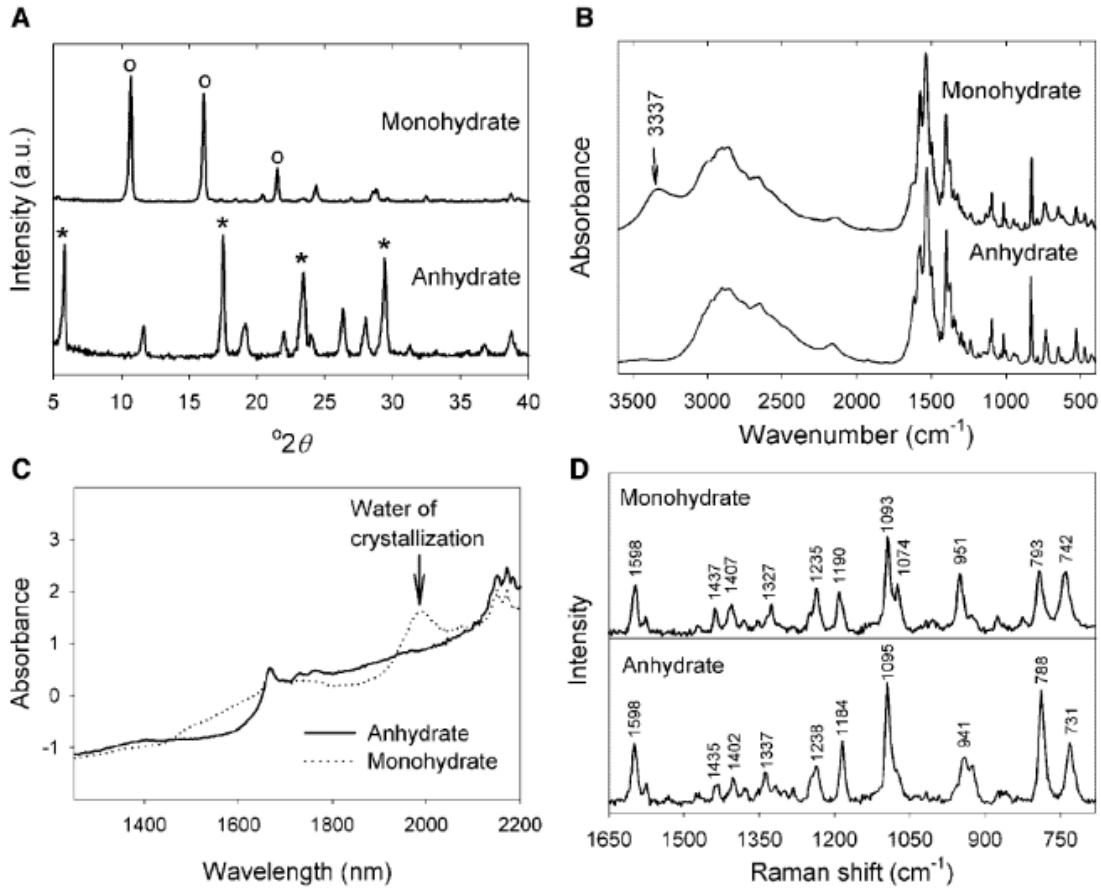

Figure 3.9 Solid-state properties of anhydrate and monohydrate of baclofen:(A) powder X-ray diffraction patterns with the characteristic diffraction peaks of the anhydrate and monohydrate indicated by (0) and (*), respectively; (B) FT-IR, (C) NIR, and (D) Raman spec. [55]

The anhydrous is the form being used in this project.

3.5.2 Factors related to dissolution testing

- **Deaeration**

Several methods are available for medium deaeration. Also automated methods are available. Bubbles are common and will cause problems in the non-deaerated medium. USP General Chapter on Dissolution <711> states that bubbles can interfere with dissolution test results and should be avoided. The dissolved air may retard dissolution by creating a barrier that may adhere to the surface of the tablet or basket screens, therefore, this test should be performed immediately after deaeration. It is best not to rotate the paddle before adding the tablet, as the movement of the paddle aerates the medium. To eliminate the gases, present in the dissolution medium, the Portuguese Pharmacopoeia 9.0 recommends a degassing method, in which the medium is heated to a temperature of 41ºC, gently shaken and filtered at reduced pressure through a filter of 0.45 µm or smaller pore size.[31]

- **Stirring speed**

Stirring decreases the diffusion layer, increasing the contact of the particle with the dissolution medium. This phenomenon also increases the dissolution speed, since it avoids the increase of solute concentration in the diffusion layer, besides favoring the homogenization of the medium.[31]

3.5.3 Factors related to the analytical method

The analytical method for the quantification/dosing of the active substance shall correspond to the analytical method referenced in the respective monograph described in the Pharmacopoeias.

Concerning the filters used during the dissolution tests, these shall be inert, that is, they shall not absorb drug nor release particles into solution.[56]

3.6 Analytical Method Selection for Drug Product Dissolution Testing

3.6.1 High Performance Liquid Chromatography (HPLC)

In general, the intended target of the dissolution test varies over the lifetime of a pharmaceutical form [57]. The primary objective of dissolution testing in Phases 0 and I is to configure the dissolution mechanism. During Phases II and III, the objective changes to further develop insight into the impact of key formulation/process parameters on dissolution and an *IVIVC*. At the time of product registration and thereafter, our objective is to identify a QC dissolution test method to verify process and product consistency. The continuous progression of objectives during the drug life cycle may require different detection methods to have an effective and efficient dissolution test. [58]

The separation and quantifications abilities that an HPLC presents, have made it one of the essential tools used in the laboratory within the pharmaceutical industry.

Although, UV is simpler and requires less solvent in comparison with HPLC method However, the HPLC presents benefits particularly when there is significant interference from excipients or among active substances of the formulation. Its main goal is then to separate the several constituents of a mixture by means of an interaction between the sample molecules and two phases, one stationary and the other mobile. [57] Its main disadvantage is the fact of being expensive, due to the high purity of the reagents used and the high cost of the associated equipment and periodic maintenance and calibration.

Analytes from the sample flow through a stationary phase, with which they have varying affinities, and are dragged with different speeds by a mobile phase, causing the separation of the molecules. The stronger molecules on the stationary phase move more slowly through the column and are therefore trapped longer, unlike molecules weakly trapped by the stationary phase, which are eluted first. [59][60][61]

Normal phase or reversed phase separation can be performed according to the polarity of the separation phases in the system. During normal phase separation, the stationary phase is polar (usually the filler is composed of silica) and the mobile phase is apolar. Here the polar compounds are kept longer in the column whereas the less polar compounds have more affinity for the mobile phase and are more quickly ejected from the chromatographic column.

In the reverse phase, the mobile phase is polar, and the stationary phase is apolar (usually chemically modified silica with hydrocarbon chains is used, commonly called C8 and C18 columns, classified according to the number of hydrocarbons added to the silica base structure), In this case, the less polar compounds are retained in the column.

This last type of separation is the one approached during this project. A typically uses as polar mobile phase herein, is a mixture of methanol or acetonitrile with water.[61]

Its main features as shown in the (figure 3.10) are a pump system, a sample injection system, the chromatographic column, the detector and a system for recording and processing the analytical response of the detector - software and computer.

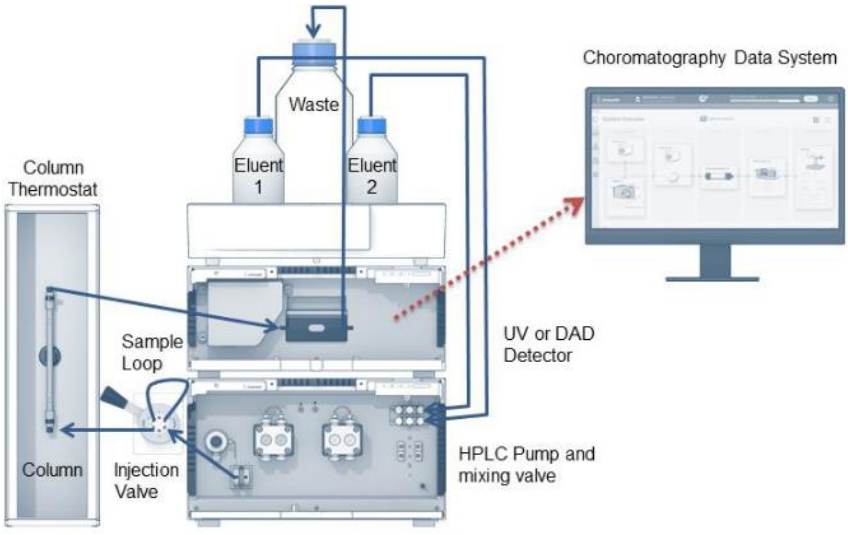

Figure 3.10 HPLC components representation. [60]

Regarding these main components, firstly, there is the mobile phase container, which is pushed to circulate in the equipment through a pump system designed to promote the circulation of the mobile phase at a predetermined flow rate. The pump may be set in constant or variable flow mode over time as well as the introduction of mobile phase components in variable proportions or in a constant proportion, this is referred to as gradient elution and isocratic elution, respectively.

Subsequently, an injector has the role of introducing the sample into the column. As far as the chromatographic column is concerned, its purpose is to detach the sample's constituents, so it is described as the stationary phase.

Next is the detector, which has the function of analyzing and identifying the separation bands of the analytes as they flow out of the column. A sensitivity to capture the signal variations in the concentrations of the analytes to be separated as well as the chemical and physical properties of the analyte must be considered when selecting the detector type.

Lastly, the data gathered by the detector is sent to a data acquiring system which, through a specific software, *empower* in this case, processes the analytical response in the form of a chromatogram, which is a graphical representation of signal intensity as a function of time.[61]

The ideal detector must present features that include the following:

- Ability to detect insignificant amounts of sample.
- The signal needs to be linearly related to the sample concentration.
- It must be stable, that is, resistant to temperature and flow variations in the case of gradient eluting.

UV-visible detectors are the most widely used detectors in HPLC. It consists of a photometer that measures the absorption of light by compounds, within a certain wavelength, between the visible and ultraviolet regions.

3.6.1.1 Ultra-Performance High Liquid Chromatography (UHPLC)

Once, the real separator in the chromatographic column is the material that makes up its filling. The UHPLC is preferable since it has 1.7 µm packing material in comparison of 3-5 µm that HPLC holds.

Furthermore, its system can operate into a higher-pressure range of 6000-15.000 psi and for smaller particles diameter < 2 µm making it overly sensitive and with better resolution.[62]

3.6.2 Procedure

The following describe how to proceed for a chromatographic analysis

3.6.2.1 Column Installation

As a reversed phase column is used here, both the capillaries and the whole mobile phase process between pump and detector should be rinsed with aqueous solvent (ideally in the same proportion as the method's mobile phase buffer). This will prevent any salt or capping residues from the equipment meeting the mobile phase packing of the column and causing precipitation on the column.

In case of normal phase columns, after passing the equipment circuits through an aqueous solution, an either absolute ethanol or 100% isopropanol avoiding immiscibility among mobile phase packing solvents (N-heptane or N-hexane) and the water.

3.6.2.2 Column Stabilization

Ensuring that all packing has been removed from the column. the mobile phase of the analytical method being tested is loaded into the column system and the entire system is equilibrated by moving the mobile phase through. Monitoring should be carried out during this settling using suitable baseline visibility conditions to assess the stability of the system.

3.6.2.3 Chromatographic Conditions

The chromatographic conditions should be defined previously in compliance with the analytical monograph.

The chromatographic working conditions for quantifying the Baclofen substance are done using an UHPLC equipped with variable wavelength detector, which in this method was fixed at 220 nm, and supporting software following the represented specification. (Table 3.8)

Table 3.8 UPLC equipment and chromatographic Conditions.

Chromatographic Conditions	
Mobile phase	Buffer pH 5.0/ Methanol (80/20) (V/V)
Detector	UV, 220 nm
Column	Waters BEH C18 1.7µm, 2.1x 50 mm (C-014, C-016)
Column temperature	30ºC
Autosampler temperature	20ºC
Flow rate	0.3 ml/min
Injection volume	0.5 µL
Retention time	Baclofen= 1.3 min
Run Time	2 min
System Suitability	For Baclofen reference solution: ● RSD for 6 injections ≤ 2.0% ● Symmetry factor ≤ 1.5 ● Theorical plate count ≥ 5000 ● Recovery= [98.0%-102.0%]
Purge, wash and seal wash system solvents	● 20% MeOH/H2O for Purge ● 10% Acetonitrile solution in ultrapure water for seal wash

3.6.3 System Suitability

It is the capacity of the analytical system, to report the system conformity before, during and at the end of the sequential injections regarding the chromatographic quality assessment parameters (retention time, number of theoretical plates, and symmetry or tailing factor).

The determination of these parameters is based on the following calculation:

- **Retention time**: Corresponds to the time that elapses between the injection point and half elution (peak maximum). [63]

- **Symmetry factor or Tailing factor (*T*)**: Corresponds to the peak symmetry measure estimated based on equation 3.8 [63]. (Figure 3.11)

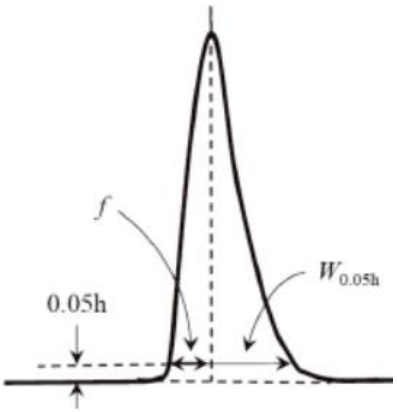

Figure 3.11 calculation of symmetry factor. [63]

$$T = \frac{W_{0.05}}{2f} \qquad \textit{Equation 3.8}$$

Its parameters mean (table 3.9):

Table 3.9 Symmetry Factor Parameters [62]

Parameters	
$\boldsymbol{W_{0.05}}$	Peak width at 5% of height
$\boldsymbol{2f}$	Distance from the maximum of the peak to the leading edge of the peak

A *T* value of 1.0 means symmetry. When *T* > 1.0, the peak is tailing. When *T* < 1.0 the peak is fronting.

- **Number of theoretical plates**: Refers to the efficiency measurement of the chromatographic column. Considering that the chromatographic peak represents a Gaussian distribution, the number of theoretical plates is determined according to the equation 3.9.

$$N = 16\left(\frac{t_R}{W^2}\right) = 5.54\left(\frac{t_R}{W_{\frac{1}{2}}}\right)^2 \qquad \textit{Equation 3.9}$$

Where W and W1/2 are the peak width at the baseline and t_R is the retention time.

- **Recovery**: is a parameter used to ensure accuracy of the reference solution, by preparing a second one and comparing their analytical response using the equation 3.10 below:

$$Recovery\ \% = \frac{Weight_{\ 1^a ref^a Subst} \times Mean_{area\ 2\ injections\ of\ 2^a\ Ref^a\ Subst}}{Weight_{2^a\ Ref^a\ Subst} \times Mean_{Initial\ Injections\ of\ 1^a\ Ref^a\ Subst}} \times 100$$

Equation 3.10

The recovery percent must be between 98.0% - 102.0%.

After verifying the fulfillment of the criteria specified, samples injection can be started. Taking into consideration that the system suitability is valid for 24 hours with unchanged conditions, including flow rate and mobile phase.

- **Repeatability of analytical response:** The repeatability of the analytical response, in areas or in heights as described in the method, must be verified by the relative standard deviation (RSD) calculated on the successive injections of the reference solution described in the method.

When omitted by the analytical method the calculation of repeatability on 6 successive injections of the reference solution is considered for assay tests and the relative standard deviation should be ≤ 2%. For cleaning verifications, the calculation of repeatability on 3 successive injections of the reference solution is considered, and the relative standard deviation should be ≤ 10.0%.

3.6.4 System Control

To ensure system repeatability during the analysis, 2 injections of the reference solution must be done after each 6 successive injections of sample solutions. The response factor between the control reference solution and the initial sequence is expressed by the % recovery that must be kept among 98%-102%. Equation 3.11.

$$Recovery\ \% = \frac{Mean_{Control\ areas}}{Mean_{Initial\ areas}} \times 100$$

Equation 3.11

If the criteria have felt to meet the specifications, an evaluation of the prepared reference solution must be made.

Chapter 4 Experimental Methodology

Six batches were approached to quantify the active substance, baclofen, by UHPLC through dissolution profiles allowing a comparative study among them. Its respective strengths are shown in Table 4.1.

- Reference batches will be highlighted in blue for easier distinction during this chapter.

Table 4.1 Batches Representation.

Test	Dosage
200347 **200348** **200531**	Baclofen 10mg
200349 **200350** **200532**	Baclofen 25mg
Reference	**Dosage**
3827 **3828** **3830**	Baclofen 10mg
3843 **3751** **3861**	Baclofen 25mg

4.1 Reagents and Standard

The reagents and the Standard used are listed in the following table 4.2

Table 4.2 Reagents and Standard used during the dissolution profile study.

Reagent & Standard	Brand	Potency
Baclofen WS	-	99.6%
Baclofen USP	-	99.4%
Ammonium Acetate	VMR	-
Potassium dihydrogen phosphate	VMR	-
Sodium Hydroxide	VMR	-
Sodium Acetate trihydrate	Merck	
Acetonitrile	Carlo Erba	≥99.9
Glacial Acetic Acid	Carlo Erba	99.8%
Hydrochloric Acid	Carlo Erba	37%
Methanol	Carlo Erba	≥99.9
Milli-Q water (purified water)	Millipore	-

4.2 Equipment and Materials

The following laboratory materials were used throughout the project implementation:

- Sotax® dissolving apparatus;
- VWR ultrasound apparatus ;
- Analytical balance accurate to ±0.20 mg, Mettler Toledo;
- Chromatographic column, Waters BEH C18 1.7µm, 2.1x 50 mm;
- High Performance Liquid Chromatography equipment, Agilent Technologies model 1290 series and Waters Acquity UPLC, UV detector, automatic injection, refrigerated and data acquisition system.
- Magnetic agitation plate;
- Mettler Toledo pH meter;
- VWR paper filters;

4.3 Dissolution Method

Regarding the Pharmacopeia's method specification, the dissolution test is performed at 50 rpm using the paddle method with one of the three, HCl 0.1N pH=1.2, acetate buffer pH=4.5 and phosphate buffer pH=6.8 as a dissolution media which should be maintained at 37 $\pm$ 0.5 ^{o}C.

4.3.1 Solutions preparation

- **Acetic Acid 2M**

  Dilute 232 mL of glacial acetic acid into a 2000 mL volumetric flask with purified water. Allow the solution to stabilize at room temperature. Then, complete the volume with purified water and shake it manually

- **Sodium Hydroxide Solution 0.2M**

  Accurately weigh approximately 40 g of sodium hydroxide into a 5000 ml volumetric flask of purified water and shake well until complete dissolution. Make up to volume with the same solvent and shake well by hand.

- **Monobasic Potassium Phosphate 0.2M**

  Accurately dissolve 136.1 g of Potassium dihydrogen phosphate into 5000 mL volumetric flask of purified water, shake well until complete dissolution. Make up to volume with the same solvent.

- **Standard Stock Solution**

  Weigh accurately 27.5 mg of Baclofen reference substances into 100 mL volumetric flask and dissolve with HCl 0.1M solvent. Complete the solution with the same solvent and shake it well until totally dissolved (ultrasonic vibration might be used for further dissolution if needed)
  The solution's stability is valid at room temperature storage for 5 days.

- **Standard Solution/Reference Solution**

1. **Dosage 10mg Baclofen**
   Dilute 1 mL of the standard stock solution into a 25 mL flask. Make up the solution with the same dissolution media solvent.

2. **Dosage 25mg Bacolfen**
   Dilute 2 mL of the reference solution into a 20 mL flask. Make up the solution with the same dissolution media solvent.

The solution must be properly filtered on the day of testing through hydrophilic 0.22 µm membrane, discarding the first 2 mL for further accuracy.

- **Mobile phase Buffer pH 5.0 /Methanol (80/20) (V/V)**

Mix the amount of 800 mL of Buffer plus 200 mL of Methanol. Shake the solution manually, homogenize it and degas before use.

4.3.2 Dissolution Conditions

For the dissolution test, three different solutions were used to simulate the gastro-intestinal tract. Dissolution media were prepared according to USP. The mode of preparation can be seen in Table 4.3

Table 4.3 Dissolution media preparation.

Dissolution Media	Preparation mode
pH=1.2 **0.1N Hydrochloric Acid**	For 6 L volumetric flask of purified water, 50 mL of (HCl 37%) must be added.
pH=4.5 **Acetate Buffer**	In a 10 L flask, dissolve 17.94 g of sodium acetate in distilled water and add 84 ml of 2M acetic acid. Adjust the pH and make up to volume with purified water.
pH=6.8 **Potassium Phosphate Buffer**	1500 mL of 0.2 M potassium phosphate monobasic and 672 mL of 0.2 M sodium hydroxide are placed in a 6 L volumetric flask and made up to volume with purified water.

The conditions used during assessing dissolution profiles are listed in the table 4.4 below:

Table 4.4 Dissolution Conditions for Baclofen tablets 10 mg and 25 mg

Apparatus	**Apparatus Type II (Paddle elements)**		
Equipment	Sotax AT Xtend		
Dissolution Media	HCL 0.1N	Acetate buffer pH 4.5	Phosphate buffer pH 6.8
Dissolution medium volume	900 mL	900 mL	900 mL
Temperature	37± 0.5°C	37± 0.5°C	37± 0.5°C
Rotational speed (rpm)	50 rpm	50 rpm	50 rpm
Sample volume (mL)	1.2 mL	1.2 mL	1.2 mL
Sampling time-points (min)	5, 10, 15, 20, 25 and 30		

Specifying the following criteria for it: table 4.5.

Table 4.5 Product Specification

Physical and Chemical Tests	**Release specification**
Dissolution	≥ 80% (Q) the nominal dosage of the tablets (15 min)

Equation 4.1 allows determining the dissolved percentage of active substance released at each time:

$$\%\ Dissolved = \frac{A \times Wref \times Cref \times Dilref \times Wm}{Aref \times Diltest \times LC \times W} \qquad \textit{Equation 4.1}$$

Where *A* is the Area of baclofen in sample solution, A_{ref} the average area of baclofen in reference solution, W_{ref} weigh of reference substance (mg), Wm average tablet weigh (mg), W weigh of sample (mg), C potency of the reference substance (%), Dil_{ref} dilution factor of the reference solution (0.01) (mL^{-1}) ,Dil_{test} dilution factor of the sample solution (mL^{-1}) and LC is the label claim (mg).

In the dissolution profile case, the volume of the medium is corrected at each time point since the volume removed is not compensated for and a new factor related to the concentration of the solution at the previous point is also introduced.

Chapter 5 Results and Discussion

5.1 System Suitability

In order to ensure the proper management of the qualitative and quantitative analysis during chromatographic evaluation of the released API, six consecutive injections of the standard solution were made initially in the UHPLC.

Only after having read the results of the standards in the *Empower* system and confirmed that they are in conformity with the specified limits and that the functioning of the chromatographic column in relation to pressure and temperature is stable, the samples are injected.

The specified limits were checked according to the samples shown below for each strength.

- **<u>Dosage 10 mg</u>**

Representing batch 3830 in Acetate buffer pH 4.5 media. Figure 5.1

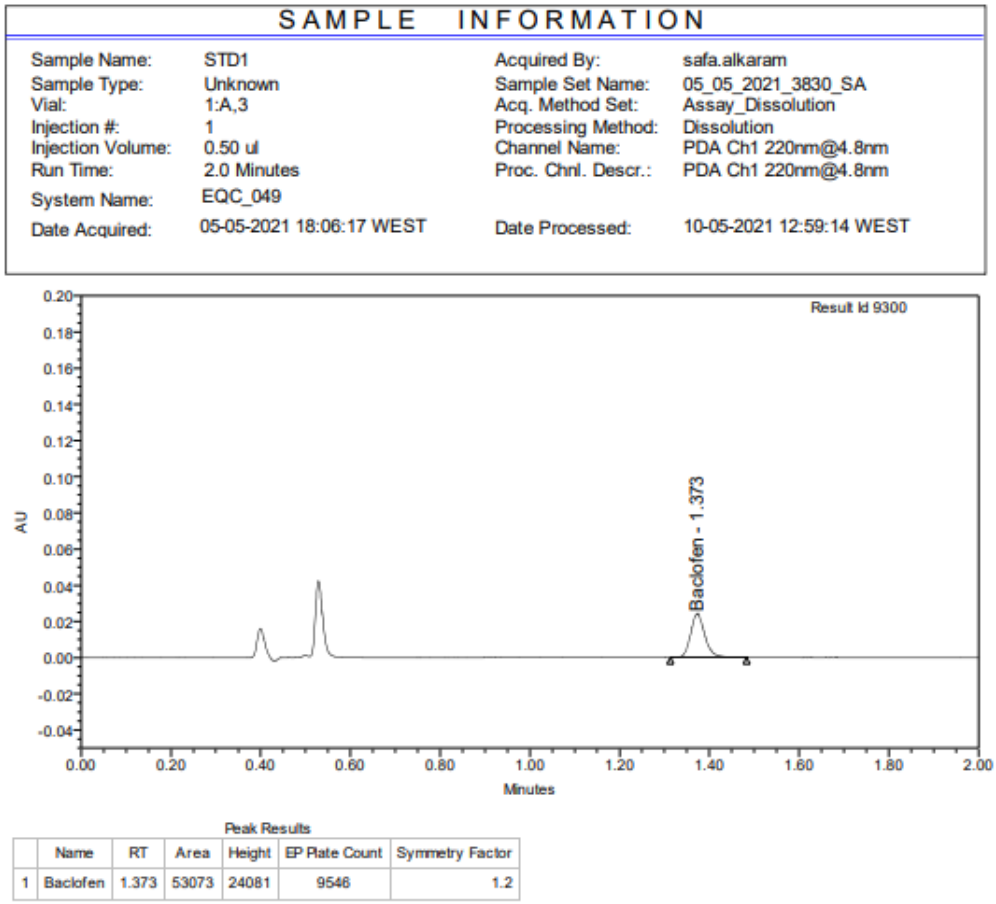

Figure 5.1 Representative chromatogram of batch 3830, 10 mg in Acetate buffer pH 4.5 media.

Obtaining the tableted calculated values:(table 5.1)

Table 5.1 System suitability verification for 10 mg strength.

Standards	Area	Mean	RSD (%)	Recovery (%)	Symmetry Factor	Plate Count	Acceptance Criteria
Standard 1	53073	53081	0.5	N/A	1.2	9546	RSD ≤ 2.0 %
	53150						
	53101						Plate Count ≥5000
	52978						
	52715						Symmetry Factor ≤1.5
	53466						
Standard 2	52688	52442	0.7	101.1	N/A	N/A	Recovery 98-102 %
	52195						

- **Dosage 25 mg**

Figure 5.2 shows a representative chromatogram for batch 200350, in Acetate buffer pH 4.5.

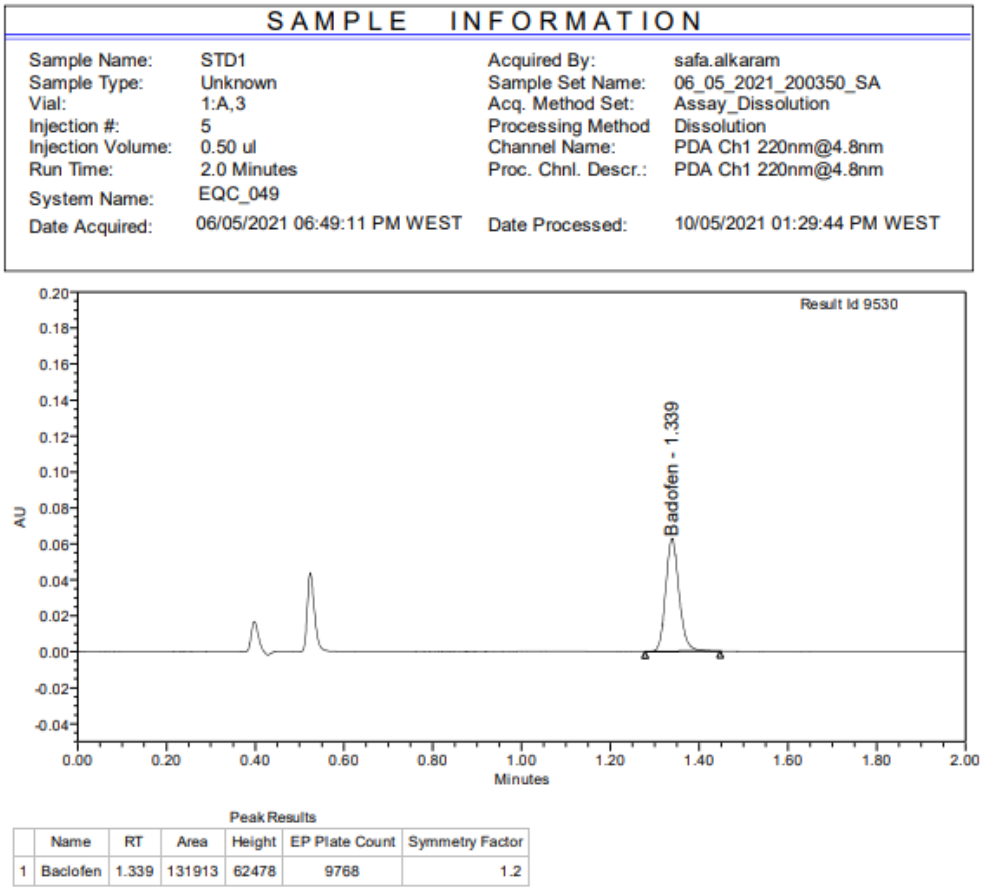

Figure 5.2 Representative chromatogram of Batch 200350, 25 mg in Acetate buffer media.

Validating the system suitability through the calculated listed outcomes. (table 5.2).

Table 5.2 System Suitability of 25 mg strength in Acetate buffer media.

Standards	Area	Mean	RSD (%)	Recovery (%)	Symmetry Factor	Plate Count	Acceptance Criteria
Standard 1	131224	131911	0.4	N/A	1.2	9735	RSD ≤ 2.0 %
	131480						
	132163						Plate Count ≥5000
	132291						
	131913						Symmetry Factor ≤1.5
	132397						
Standard 2	131892	131897	0.0	99.9	N/A	N/A	Recovery 98-102 %
	131901						

Note here that the above-mentioned process was followed for the rest of the samples, evaluating the 12 cups at the different time points in all media. Annexes C and D represent the areas imported from the system for 10 mg and 25 mg dosage forms.

5.2 Dissolution Profiles Comparison

The dissolution profiles were performed according to company's procedure, which is in line with the guidelines. Throughout this subchapter the results obtained for the studied batches, presented in annex A and annex B, will be evaluated in several medias, namely pH 1.2, pH 4.5 and pH 6.8.

The results obtained for the different dissolution media are shown in the graphs/ tables and are expressed in terms of the average percentage of active substance dissolved over time for 12 units.

5.2.1 HCl 0.1N pH 1.2

5.2.1.1 Formulation of Baclofen 10 mg

The dissolution profiles for baclofen dosage 10 mg in 0.1 N HCl pH 1.2 depicted in Figure 5.3 show dissolution values greater than 85% at 15 minutes and present RSD values within the specified limits for the study. According to the "Guideline on the Investigation of Bioequivalence", when this occurs the dissolution profiles are deemed to be similar and there is no further requirement to calculate the similarity factor (f2).

Table 5.3 shows the comparison of the 3 test batches 200347,200348 and 200531 produced in the Portuguese site against the swiss reference batches.

Table 5.3 Average values of dissolved % API of Baclofen 10 mg per pH 1.2

HCl 0.1 N Medium						
Batches	**% API Dissolved for each sampling time point (min)**					
	5	10	15	20	25	30
3827	97.3	100.2	101.0	101.4	102.0	102.4
3828	97.4	99.0	100.0	101.8	101.0	101.3
3830	98.5	100.4	100.6	101.5	101.5	102.0
200347	94.3	95.7	96.5	96.7	97.8	98.0
200348	92.9	94.8	96.5	96.5	97.3	97.4
200531	95.4	97.7	98.3	98.9	99.4	99.6
Mean CH	97.7	99.8	100.6	101.6	101.5	101.9
% RSD CH	0.7	0.8	0.5	0.2	0.5	0.5
Mean PT	94.2	96.1	97.1	97.4	98.2	98.3
% RSD PT	1.4	1.5	1.1	1.4	1.1	1.2

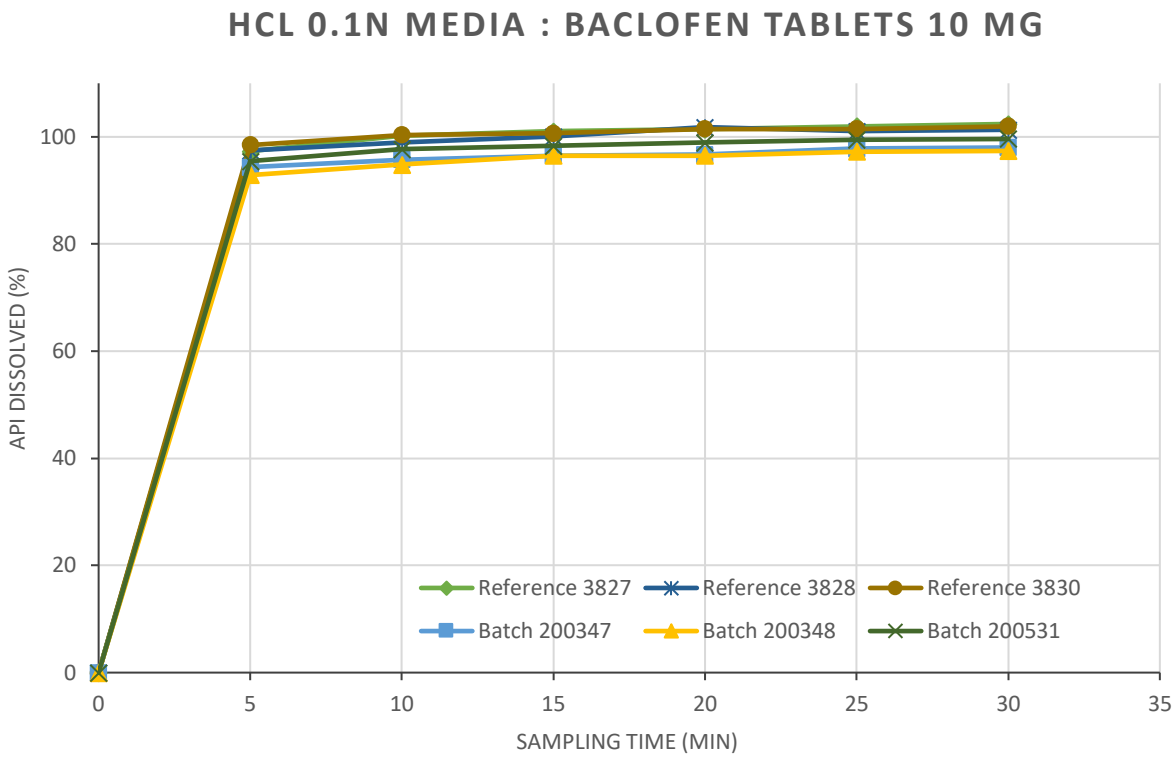

Figure 5.3 Comparison release profiles of test and reference batches in HCl 0.1 N media for baclofen 10 mg dosage form.

Another way of conveying the variability of data, is by means of the variation coefficient, defined by CV, which is interpreted as the variability of data with respect to the mean. The lower the CV, the more homogeneous the data set are.

It represents the dispersion of results obtained under defined conditions, by measuring the degree of repeatability or reproducibility of the analytical method and reflecting the degree of resemblance between values, for a given number of analyses using the same analytical method.

Figure 5.4 states the coefficient variation for each time point in HCl 0.1N media, where the obtained values comply with the acceptance criteria specified in section 3.4.3, since during all the time the CV appears to have lower than 5% of variation when comparing the both means ,test and reference.

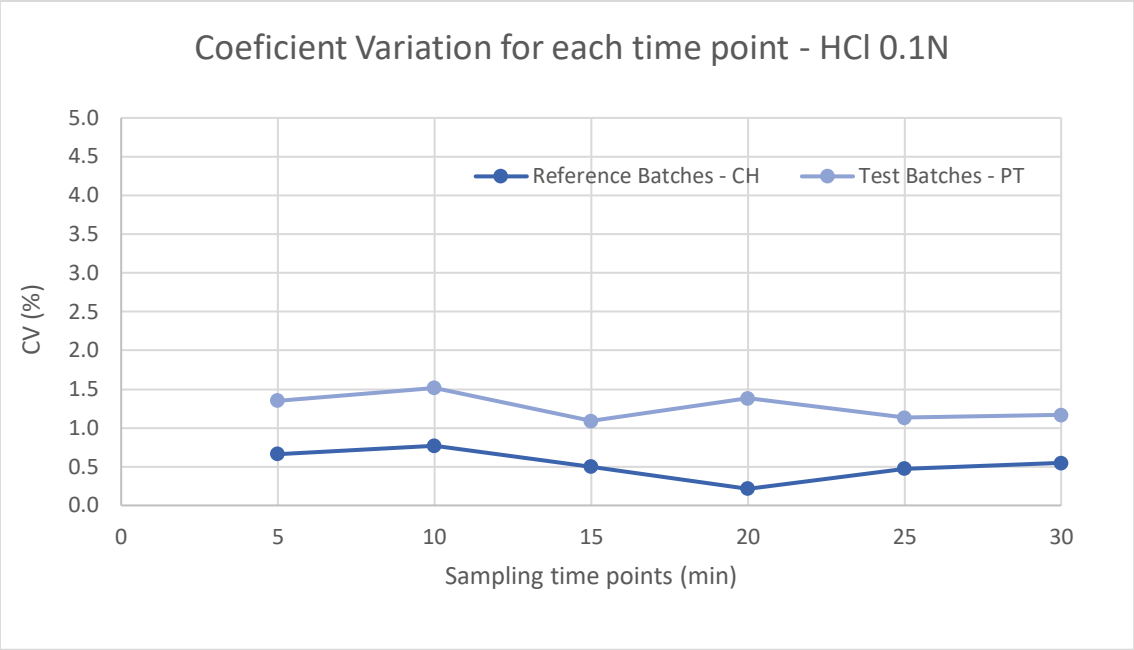

Figure 5.4 Coefficient Variation at each time point for 10 mg dosage form in HCl 0.1N media.

Furthermore, it's important to point out to calculation of similarity and difference factors in order to take conclusions of the behaviors represented above.

The calculation in tables 5.4, 5.5 and 5.6 was done regarding the formulas previously mentioned in section 3.4.3

Table 5.4 f_1 and f_2 calculation against the reference batch 3827.

Reference batch 3827	Test batches		
	200347	200348	200531
Difference Factor f1	4	5	2
Similarity Factor f2	68	65	78

Table 5.5 f_1 and f_2 calculation against the reference batch 3828.

Reference batch 3828	Test batches		
	200347	200348	200531
Difference Factor f1	4	4	2
Similarity Factor f2	71	68	83

Table 5.6 f_1 and f_2 calculation against the reference batch 3830.

Reference batch 3830	Test batches		
	200347	200348	200531
Difference Factor f1	4	5	2
Similarity Factor f2	68	65	78

Once the recommended figures for $f_1 \leq 15$ (0-15) and $f_2 \geq 50$ (50-100) are met, therefore, we conclude the similarity among reference and test batches.

5.2.1.2 **Formulation of Baclofen 25 mg**

In order to confirm the previous observations with a higher strength, a comparison was made between the test performed at the experimental referenced conditions (50 rpm) and the reference performed at the same conditions

Table 5.7 compares the % of API dissolved over time for both commercial and test products.

Table 5.7 Average values of dissolved % API of Baclofen 25 mg per pH 1.2.

HCl 0.1 N Media						
Batches	**% API Dissolved for each sampling time point (min)**					
	5	10	15	20	25	30
3751	87.8	92.0	93.6	95.0	95.8	96.7
3843	89.7	92.8	94.5	95.7	96.7	97.2
3861	90.1	93.7	95.6	97.1	98.3	98.8
200349	92.6	95.3	96.8	97.6	98.1	98.3
200350	87.6	90.3	91.7	92.7	93.4	93.9
200532	87.6	91.5	92.9	94.1	95.1	95.6
Mean CH	89.2	92.8	94.6	95.9	96.9	97.6
% RSD CH	1.4	0.9	1.0	1.1	1.3	1.1
Mean PT	89.3	92.3	93.8	94.8	95.5	96.0
% RSD PT	3.2	2.8	2.8	2.7	2.5	2.3

The dissolution performance for Baclofen 25 mg in 0.1 N HCl pH 1.2 depicted in Figure 5.5 show dissolution values greater than 85% after 15 minutes, for both test and reference products.

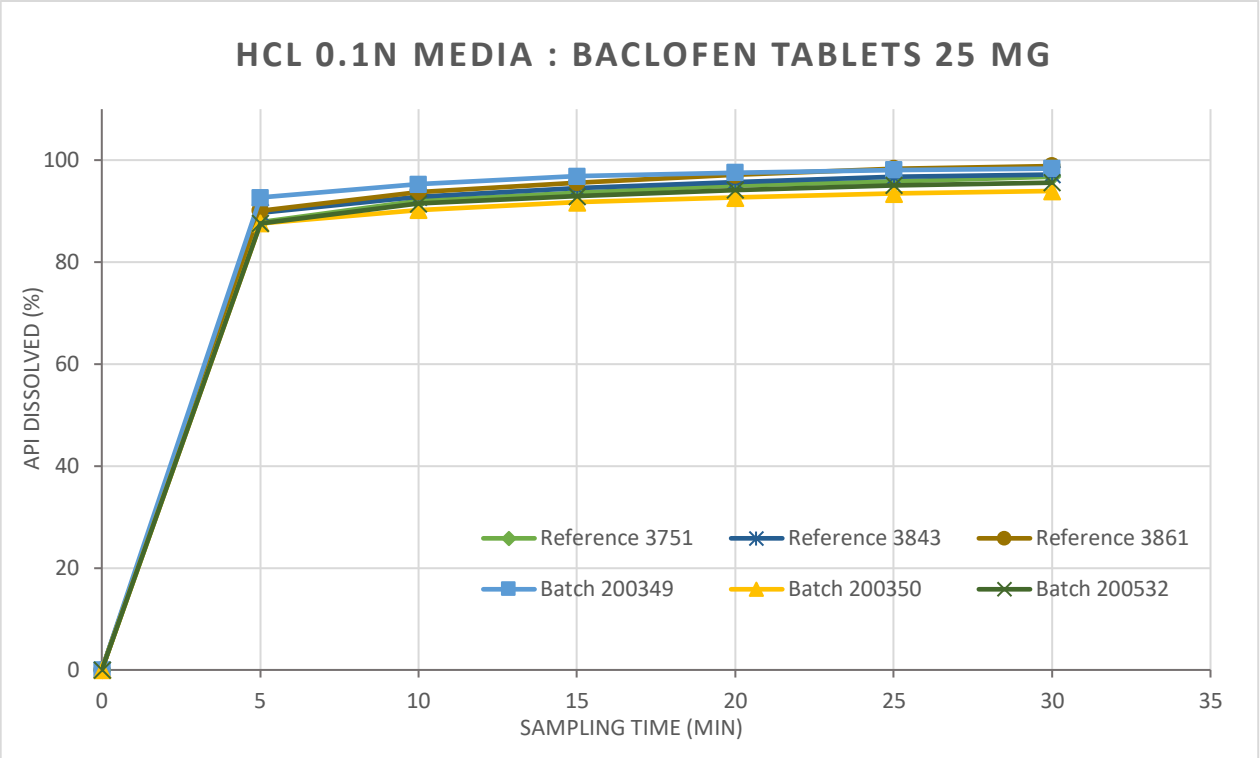

Figure 5.5 Profile release comparison of baclofen 25 mg in HCl 0.1N media.

The RSD obtained (figure 5.6) values complies with the criteria specified ensuring consistency between batches within the selected media.

The CV among reference mean and test mean has lower than 5% of variation complying the criteria specified.

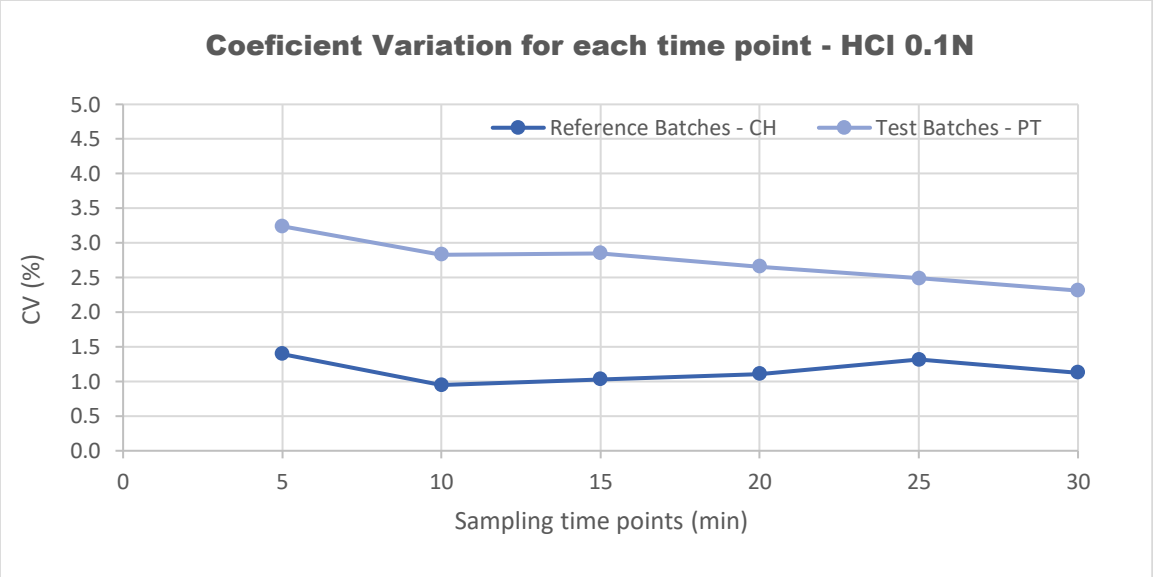

Figure 5.6 Coefficient Variation at each time point for 25 mg dosage form in HCL 0.1N media.

The similarity was shown through tables 5.8, 5.9 and 5.10 and by the calculations of both factors.

Table 5.8 f_1 and f_2 calculation against the reference batch 3751

Reference batch 3751	Test batches		
	200349	200350	200532
Difference Factor f1	3	2	1
Similarity Factor f2	74	82	95

Table 5.9 f_1 and f_2 calculation against the reference batch 3843

Reference batch 3843	Test batches		
	200349	200350	200532
Difference Factor f1	2	3	2
Similarity Factor f2	81	76	86

Table 5.10 f_1 and f_2 calculation against the reference batch 3861

Reference batch 3861	Test batches		
	200349	200350	200532
Difference Factor f1	1	4	3
Similarity Factor f2	89	69	76

Once the recommended figures for $f_1 \leq 15$ (0-15) and $f_2 \geq 50$ (50-100) are met, therefore, we conclude the similarity among reference and test batches of 25 mg in HCl 0.1N media.

5.2.2 Acetate Buffer pH 4.5

5.2.2.1 Formulation of Baclofen 10 mg

The dissolution profiles for Baclofen of 10 mg strength in acetate buffer pH 4.5 are depicted in figure 5.7. As can be seen from table 5.11, both products (test and reference) show dissolution values greater than 85% after 15 minutes as well as complete dissolution by the end of the test time. In this environment the similarity of the products is maintained as can be verified through the results obtained.

Table 5.11 Average values of %API dissolved of baclofen 10 mg per pH 4.5.

Acetate Buffer pH 4.5 Media						
Batches	**% API Dissolved for each sampling time point (min)**					
	5	10	15	20	25	30
3827	91.7	95.8	98.0	98.8	100.2	100.6
3828	89.5	94.2	96.1	97.5	98.4	99.5
3830	88.1	92.7	94.7	96.1	97.2	98.2
200347	87.4	93.1	95.1	96.9	97.6	98.1
200348	87.3	91.3	93.0	94.2	94.9	94.5
200531	88.3	92.5	94.0	95.3	95.9	98.6
Mean CH	89.8	94.2	96.3	97.5	98.6	99.5
% RSD CH	2.0	1.7	1.7	1.4	1.5	1.2
Mean PT	87.7	92.3	94.1	95.5	96.1	97.1
% RSD PT	0.6	1.0	1.1	1.4	1.4	2.3

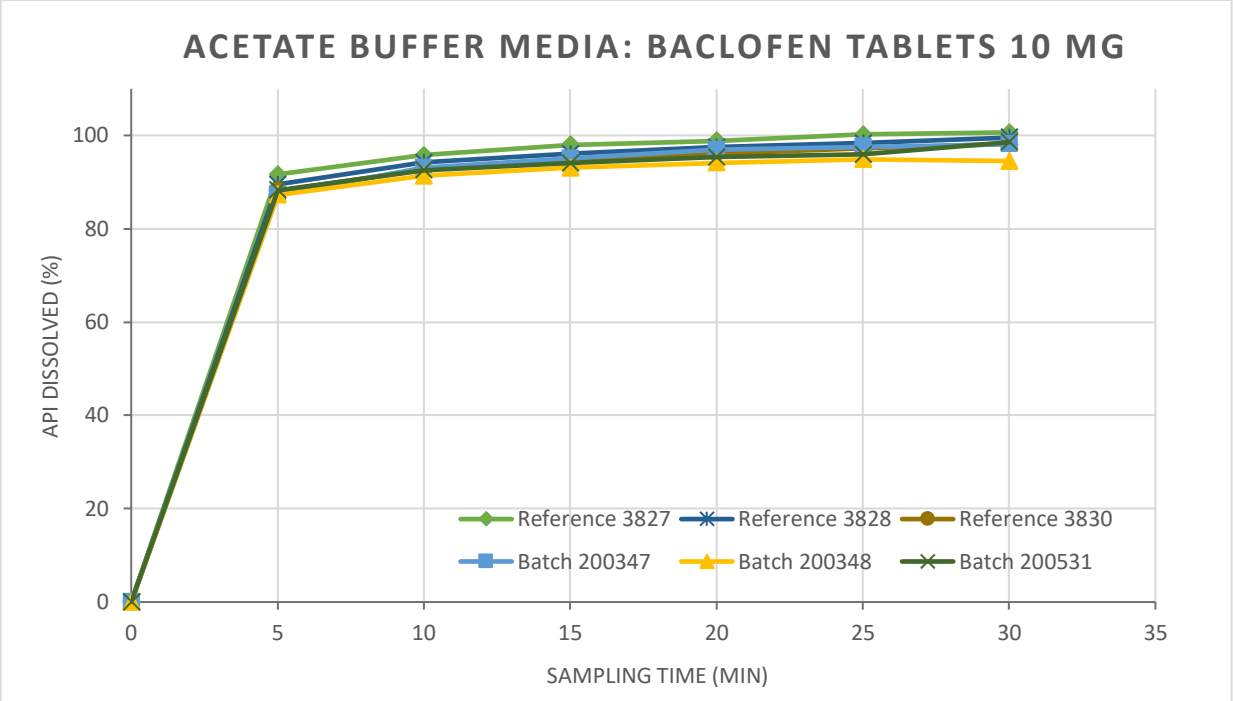

Figure 5.7 Dissolution profiles comparison of Baclofen 10 mg in Acetate Buffer media.

Figure 5.8 shows the RSD value (%) for each sample time in response to the percentage dissolved along the profile.

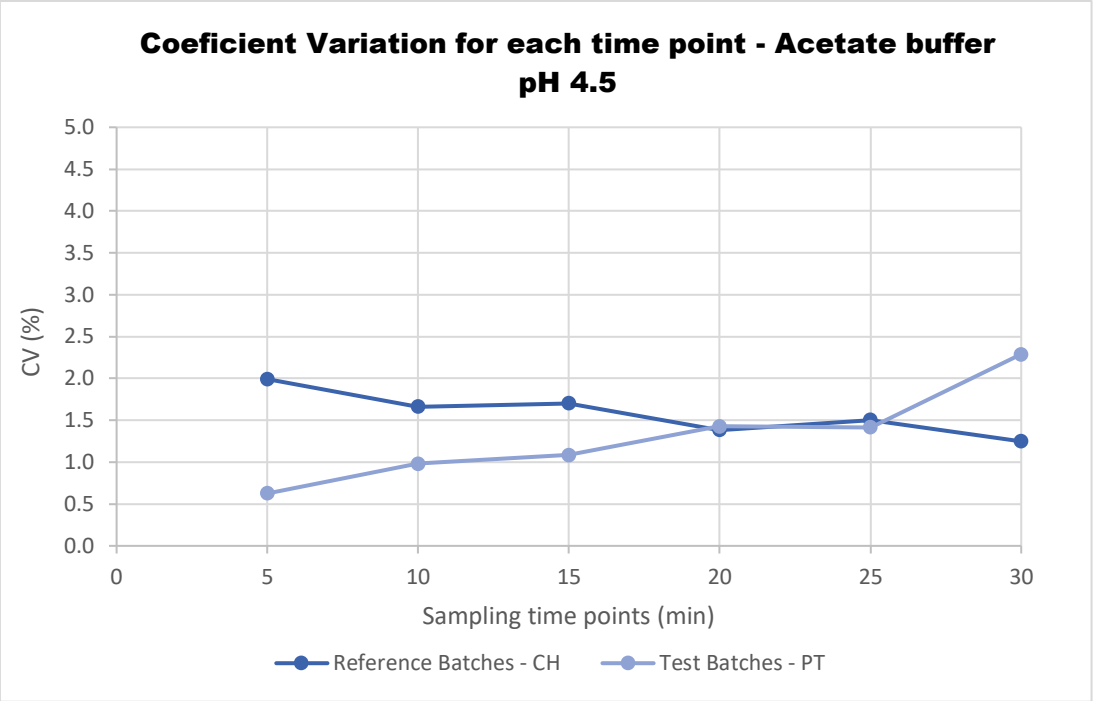

Figure 5.8 Coefficient Variation at each time point of 10 mg dosage form in Acetate Buffer media.

The variation is lower than 5%, hence, RSD values for 10 mg into pH 4.5 media are within specifications defined by the guidelines.

As per the calculation in tables 5.12, 5.13 and 5.14 done regarding the formulas previously mentioned in section 3.4.3.

Table 5.12 f_1 and f_2 calculation against the reference batch 3827

Reference batch 3827	Test batches		
	200347	200348	200531
Difference Factor f1	3	5	3
Similarity Factor f2	76	65	72

Table 5.13 f_1 and f_2 calculation against the reference batch 3828

Reference batch 3828	Test batches		
	200347	200348	200531
Difference Factor f1	1	3	2
Similarity Factor f2	90	72	84

Table 5.14 f_1 and f_2 calculation against the reference batch 3830

Reference batch 3830	Test batches		
	200347	200348	200531
Difference Factor f1	0	2	1
Similarity Factor f2	98	81	96

Once the recommended figures for $f_1 \leq 15$ (0-15) and $f_2 \geq 50$ (50-100) are met, therefore, we conclude the similarity among commercial and test batches of Baclofen10 mg strength in acetate buffer pH 4.5.

5.2.2.2 **Formulation of Baclofen 25 mg**

Analogously analyzed for baclofen 25 strength, through the % of API released showed in the table 5.15 and their profiles releasing behavior in figure 5.9.

Table 5.15 Average values of dissolved %API of baclofen 25 mg at pH 4.5

Acetate Buffer pH 4.5 Media						
Batches	**% API Dissolved for each sampling time point (min)**					
	5	10	15	20	25	30
3751	79.9	85.5	88.5	90.4	92.0	93.7
3843	78.8	84.6	87.0	88.5	90.0	91.1
3861	83.0	87.6	89.9	91.7	93.3	94.4
200349	87.4	91.8	94.0	95.4	96.6	97.7
200350	82.6	86.8	89.1	90.7	91.8	92.9
200532	83.2	86.9	88.9	90.5	91.6	92.6
Mean CH	80.6	85.9	88.5	90.2	91.8	93.1
% RSD CH	2.7	1.8	1.6	1.8	1.8	1.9
Mean PT	84.4	88.5	90.6	92.2	93.3	94.4
% RSD PT	3.1	3.2	3.2	3.0	3.1	3.0

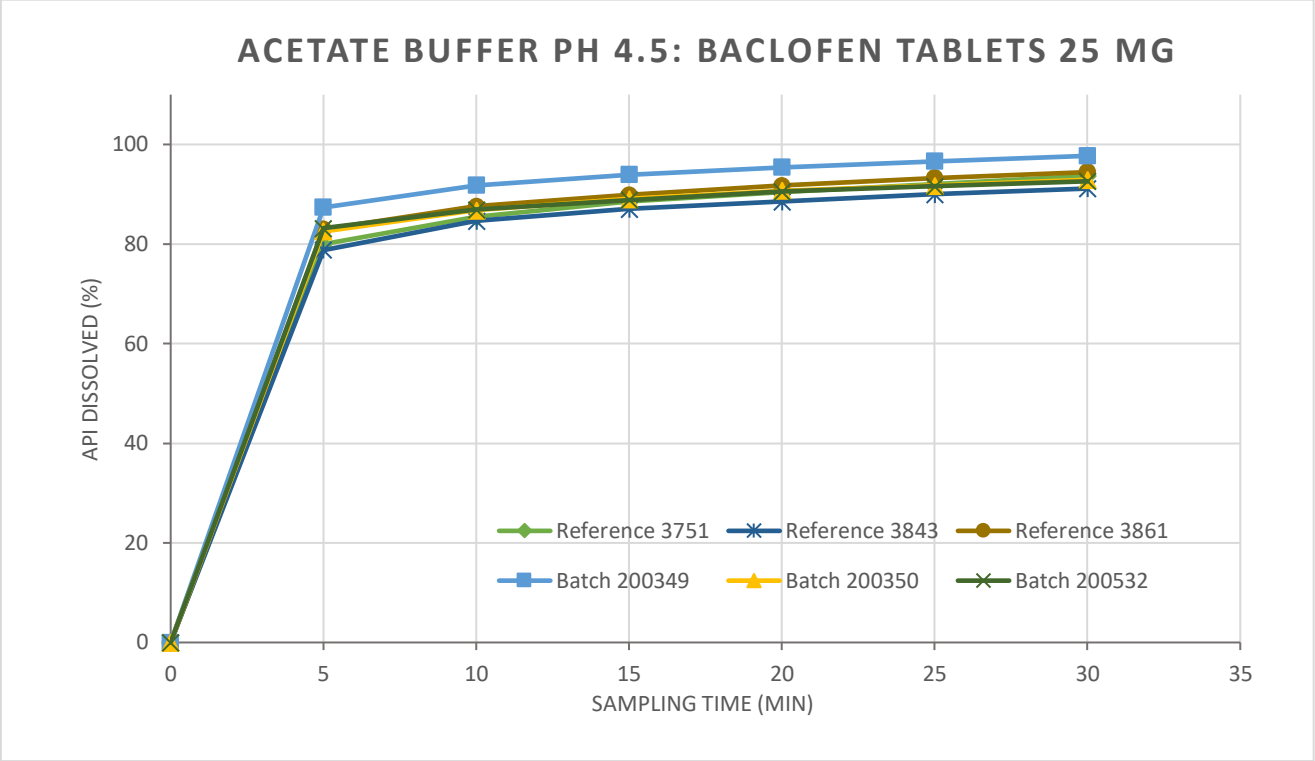

Figure 5.9 Dissolution profiles comparison in Acetate Buffer media for 25 mg dosage form.

Both commercial and test batches have similar dissolution mean patterns representing homogeneity by low variation showed in figure 5.10, ensuring for the first 5 minutes, a variation of coefficient values much lower than 20% and for the remaining times lower than 10%.

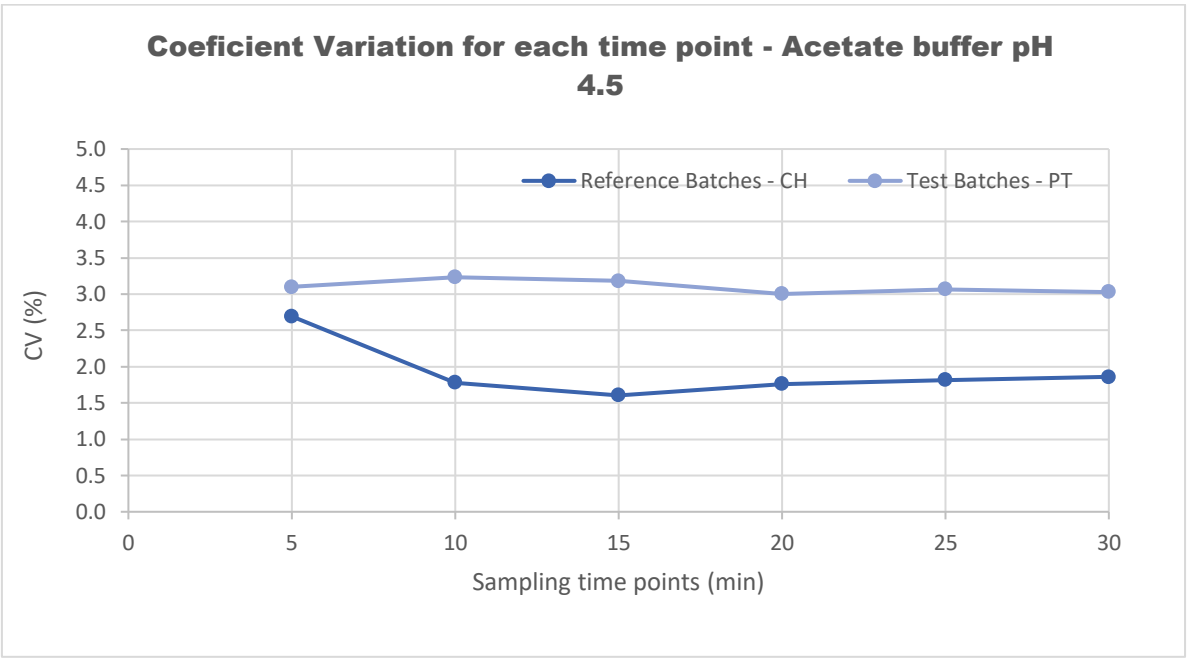

Figure 5.10 Coefficient Variation at each time point for 25 mg strength in Acetate Buffer media.

The similarity was shown through tables 5.16, 5.17 and 5.18 and by the calculations of both factors.

Table 5.16 f_1 and f_2 calculation against the reference batch 3751

Reference batch 3751	Test batches		
	200349	200350	200532
Difference Factor f1	6	1	1
Similarity Factor f2	62	90	87

Table 5.17 f_1 and f_2 calculation against the reference batch 3843

Reference batch 3843	Test batches		
	200349	200350	200532
Difference Factor f1	8	3	3
Similarity Factor f2	57	79	79

Table 5.18 f_1 and f_2 calculation against the reference batch 3861

Reference batch 3861	Test batches		
	200349	200350	200532
Difference Factor f1	4	1	1
Similarity Factor f2	70	92	90

Since the prescribed values for f1 ≤ 15 (0-15) and f2 ≥ 50 (50-100) are fulfilled, we can state the similarity between the reference and test lots of the 25 mg dosage form in acetate buffer medium. Therefore, we conclude identical dissolution profiles in acetate buffer medium.

5.2.3 Phosphate Buffer pH 6.8

Regarding previous demonstration, phosphate buffer with pH 6.8 is not considered to be an ideal environment for a faster dissolution of baclofen in comparison with pH 1.2, thus, a lower API releasing percent is expected herein.

5.2.3.1 Formulation of Baclofen 10 mg

However, a complete dissolution has been achieved by the results shown in table 5.19 and figure 5.11, since baclofen 10 mg was able to free over 85% after 15 minutes.

Table 5.19 Average values of %API dissolved of Baclofen 10 mg per pH 6.8.

Phosphate Buffer pH 6.8 Media						
Batches	**% API Dissolved for each sampling time point (min)**					
	5	**10**	**15**	**20**	**25**	**30**
3827	84.5	91.8	94.6	95.7	96.7	97.5
3828	81.8	91.4	95.2	96.9	97.8	98.7
3830	86.8	94.2	98.5	97.8	98.8	98.5
200347	73.1	87.4	91.6	94.0	95.6	96.2
200348	77.5	86.8	89.6	91.3	92.0	92.8
200531	76.9	86.1	91.6	93.9	95.0	96.0
Mean CH	84.4	92.5	96.1	96.8	97.8	98.2
% RSD CH	3.0	1.6	2.2	1.1	1.1	0.6
Mean PT	75.9	86.8	90.9	93.1	94.2	95.0
% RSD PT	3.1	0.8	1.3	1.7	2.0	2.0

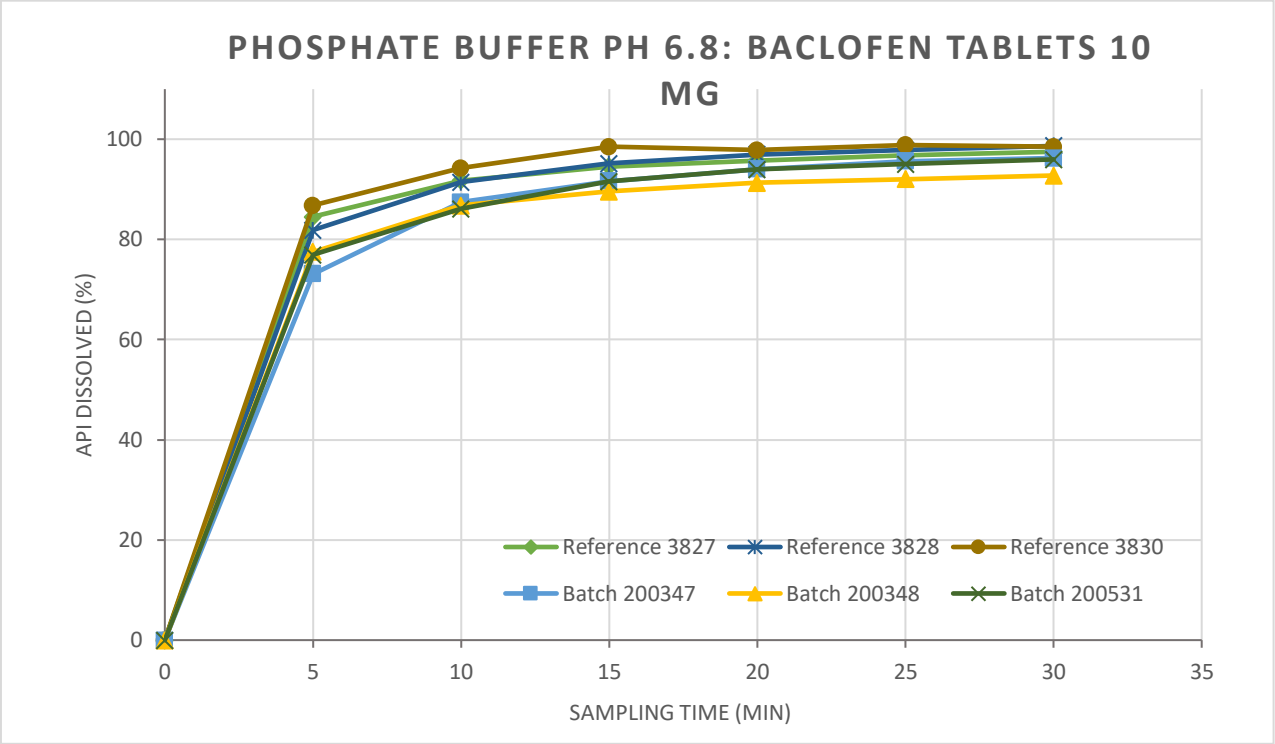

Figure 5.11 Dissolution profiles comparison of Baclofen 10 mg in the Phosphate Buffer pH 6.8 media.

As well as, the CV is lower than 5% for all the samples over time as depicted in the figure 5.12 below.

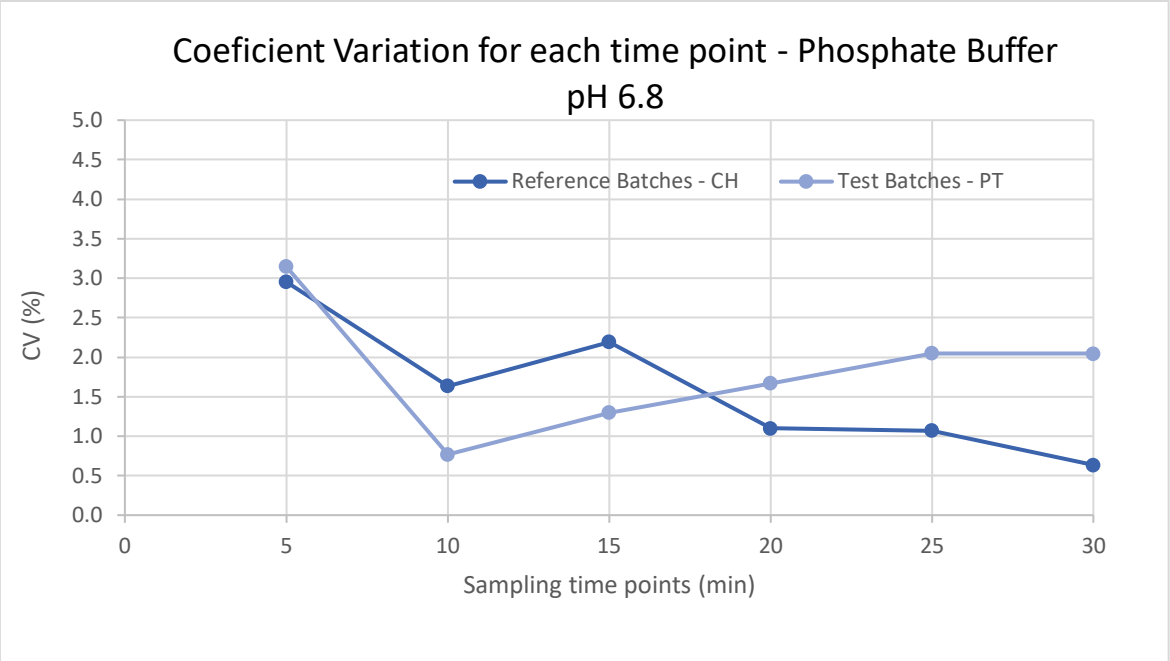

Figure 5.12 Coefficient Variation at each time point for 10mg strength in Phosphate Buffer media.

As per the calculation in tables 5.20, 5.21 and 5.22 done accordingly with the formulas previously mentioned in section 3.4.3.

Table 5.20 f_1 and f_2 calculation regarding the reference batch 3827

Reference batch 3827	Test batches		
	200347	200348	200531
Difference Factor f1	4	6	4
Similarity Factor f2	64	64	68

Table 5.21 f_1 and f_2 calculation regarding the reference batch 3828

Reference batch 3828	Test batches		
	200347	200348	200531
Difference Factor f1	4	6	4
Similarity Factor f2	67	63	70

Table 5.22 f_1 and f_2 calculation regarding the reference batch 3830

Reference batch 3830	Test batches		
	200347	200348	200531
Difference Factor f1	6	8	6
Similarity Factor f2	57	56	59

Given that we have met the recommended values for f1 ≤ 15 (0-15) and f2 ≥ 50 (50-100), we thus conclude the similarity of commercial and test batches for 10 mg strength of Baclofen in phosphate buffer pH 6.8.

5.2.3.2 **Formulation of Baclofen 25 mg**

Finally, and by the same way as done previously, baclofen 25 mg strength was evaluated through the displayed values in table 5.23 and figure 5.13.

Table 5.23 Average values of dissolved %API of 25 mg baclofen per pH 6.8.

Phosphate Buffer pH 6.8 Medium						
Batches	**% API Dissolved for each sampling time point (min)**					
	5	10	15	20	25	30
3751	81.2	86.5	89.0	90.7	91.9	92.8
3843	83.5	88.1	90.2	91.5	92.9	94.5
3861	83.4	88.1	90.1	91.7	92.7	93.6
200349	82.9	86.5	88.7	90.1	91.0	92.1
200350	83.0	87.3	88.6	89.9	91.6	92.6
200532	80.8	84.9	86.8	88.4	89.4	90.5
Mean CH	82.7	87.6	89.8	91.3	92.5	93.6
% RSD CH	1.6	1.1	0.8	0.6	0.6	0.9
Mean PT	82.2	86.2	88.0	89.5	90.7	91.7
% RSD PT	1.5	1.4	1.2	1.1	1.2	1.2

The API for all sampling points accomplished over 85% after 15 minutes.

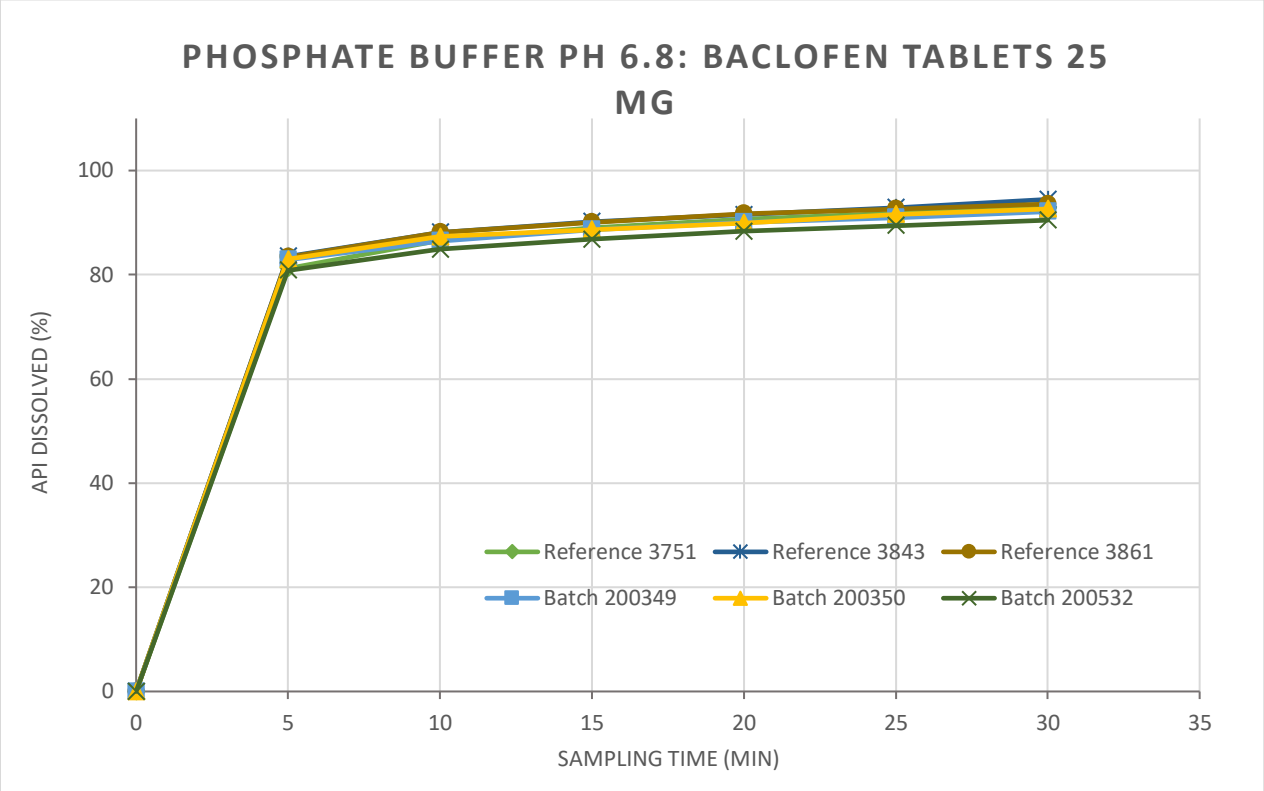

Figure 5.13 Comparison release profiles of Baclofen 25 mg in the Phosphate Buffer pH 6.8 media.

As well as, an RSD patterns lower than 5% ensuring the consistency and reproducibility of the used method. (Figure 5.14)

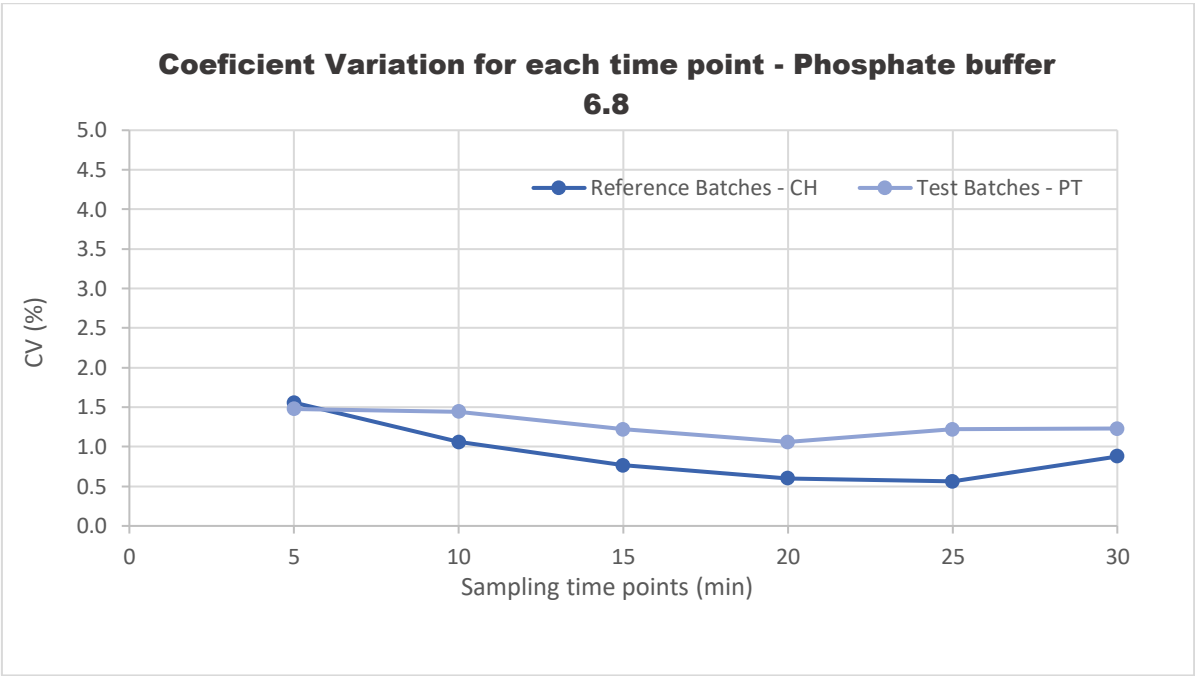

Figure 5.14 Coefficient Variation at each time point of 25 mg dosage form in Phosphate Buffer media.

The similarity was shown through tables 5.24, 5.25 and 5.26 by the calculations of both factors.

Table 5.24 f_1 and f_2 calculation against the reference batch 3751

Reference batch 3751	Test batches		
	200349	200350	200532
Difference Factor f1	1	1	2
Similarity Factor f2	94	94	82

Table 5.25 f_1 and f_2 calculation against the reference batch 3843

Reference batch 3843	Test batches		
	200349	200350	200532
Difference Factor f1	2	1	4
Similarity Factor f2	86	89	73

Table 5.26 f_1 and f_2 calculation against the reference batch 3861

Reference batch 3861	Test batches		
	200349	200350	200532
Difference Factor f1	2	1	3
Similarity Factor f2	88	91	74

As the achieved figures for $f_1 \leq 15$ (0-15) and $f_2 \geq 50$ (50-100) are within the specified values, therefore, we conclude the similarity among reference and test batches of 25 mg in phosphate buffer media. Thus, an identical dissolution profiles at phosphate buffer media.

- **<u>Comparison of formulation 10 mg in different media</u>**

lastly, and by comparing the % of baclofen released over the selected different media, we can conclude that:

1. HCl 0.1N is the dissolution media with highest % of API dissolved over time.
2. Phosphate buffer is the lowest % of API dissolved.

By looking at the curves below in the figure 5.15, we can evidence the similarity of both swiss and portuguese batches.

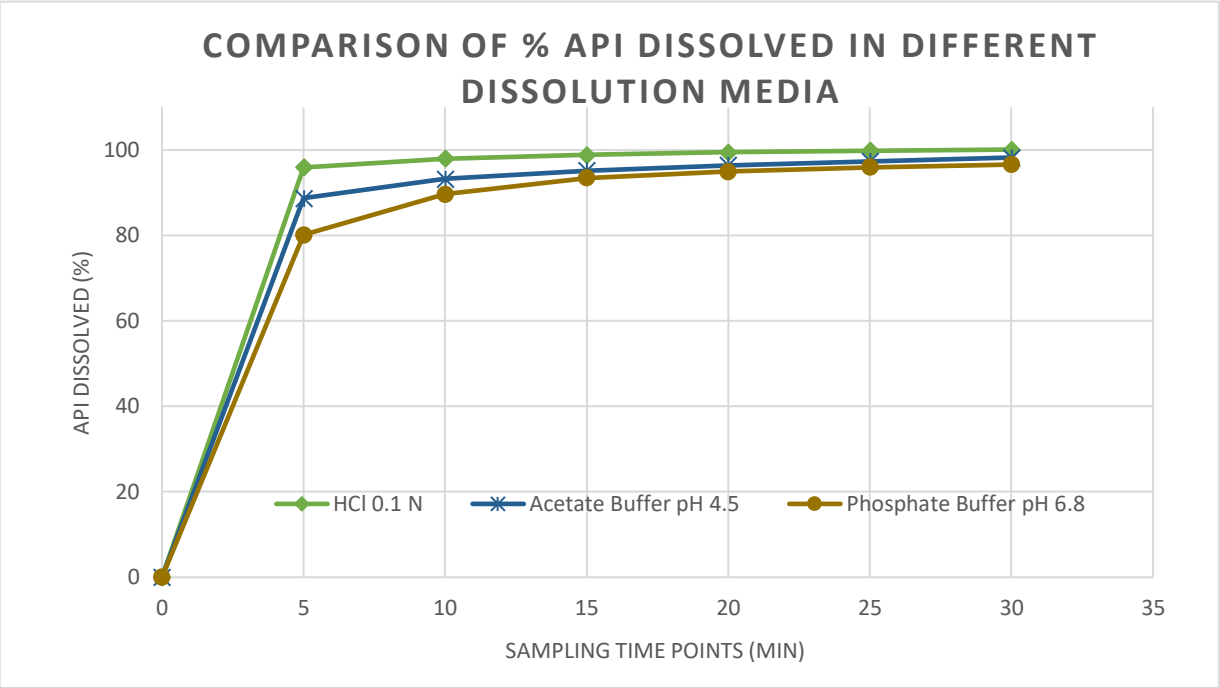

Figure 5.15 Comparative release profile in different media for 10 mg of baclofen solid oral dosage form.

- **<u>Comparison of formulation 25 mg in different media</u>**

By evaluating the indicated strength, a correlation between all batches were done in different media ensuring the acidic media is the most suitable one with highest % of API released over multiple points in comparison with the remaining medias of pH 4.5, pH 6.8 respectively. demonstrated in figure 5.16.

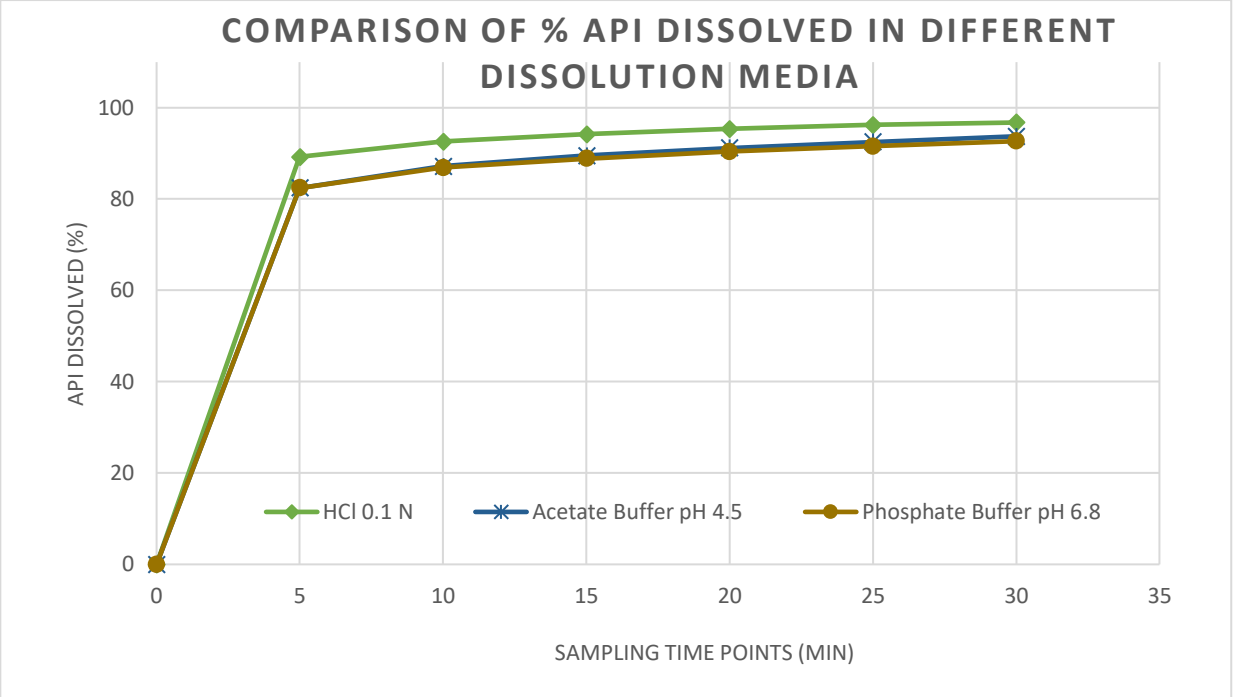

Figure 5.16 Comparative release profile in different media for 25 mg of baclofen solid oral dosage form.

According to the comparison analysis done earlier, for each strength assessment it was concluded the various points listed below:

The dissolution profiles for all the batches listed in (figure 5.16 above) shows almost a perfect dissolution rhythm achieving an average value within the interval of 90%-101% of API dissolved at sampling time 15 minutes, meaning that batches containing the target strength of baclofen tablets have the ability to attain full release in different media, matching the criteria specified (Q≥ 80%) .

The relative standard deviation was shown to be complying with the guidelines, since it showed a variation lower than 5% between test and reference means.

The independent model approached during this work proved to be well implemented.

Realizing therefore, that Labatec Farmacêutica SA. proved the similarity target among both manufactured products in the Portuguese site and the swiss site.

Chapter 6 Conclusions

Dissolution testing is present in all phases of investigation and development on new pharmaceutical forms, assessment/comparison of various formulation compounds, biopharmaceutical characterization of the drug, evaluating changes in composition, manufacturing site and volume of the drug formulation, and conducting comparative investigations of dissolution profiles.

The work carried out took into account essentially a comparison of baclofen dissolution profiles among three batches prepared in Switzerland against three batches prepared in Portugal by employing an analytical method previously validated at the Swiss site for the submission of new Portuguese site as the manufacturing unit in the targeted market destinations.

The effect of various factors on dissolution profile of baclofen was assessed by an independent statistical model method (calculation of similarity and difference factors) carried out in similar gastric fluid dissolution medium containing, 0.1 N HCl pH 1.2, acetate buffer pH 4.5 and phosphate buffer pH 6.8. Accordingly, the findings reached herein enabled us to ascertain similarities between pre-change and post-change batches, hence the presentation of the Portuguese site as a new manufacturing unit.

Although these assays are relatively simple, there are several aspects that should be taken into consideration for the correct performance of the assay. Some parameters that influence the dissolution of active ingredients, such as parameters related not only with the active substance and its formulation but also with the dissolution test itself. Therefore, it is convenient to have prior knowledge of the various characteristics of our pharmaceutical form in order to choose the most appropriate method of dissolution testing.

By assessing the method used, the recovery rate obtained allowed to conclude that there is a good accuracy in the method since the values taken from were within the planned range of 100 ± 2% and the coefficient variation is less than 5%. Hence, the method is robust to all the performed changes (injection volume, wavelength, oven temperature, mobile phase composition and solution stability).

Regarding the chromatography column used for the quantification of dissolved API, it was found to be suitable for the separation of the analyte chromatographic peaks in the dissolution test.

Regardless of whether Baclofen is considered class III (high solubility), permeability will always be the limiting step for this molecule, since upon reaching the stomach (pH 1.2) Baclofen will dissolve rapidly in biological fluids (> 85% after 15 minutes), becoming immediately available for absorption.

Summing up, dissolution tests occupy a central position in the quality control of drugs, not only for their specificity, but also due to the impact of the obtained outcomes and their scope of application.

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Appendices

Appendix A - Average values of the experimental results for % dissolved in different media of 10 mg

Commercial batches are colored in blue for easier distinction.

1. **Dissolution Media: HCl 0.1N**

Table A1- Average dissolved percentage values of the commercial batch **3827**

Unit	Baclofen Tablets 10 mg Batch 3827 Dissolution Profile					
	5 min (%)	10 min (%)	15 min (%)	20 min (%)	25 min (%)	30 min (%)
1	93.7	99.0	99.8	100.5	101.1	102.9
2	99.3	102.1	103.0	103.7	104.3	103.9
3	99.6	101.4	101.8	102.3	102.7	102.8
4	99.9	100.8	101.2	101.3	101.0	102.6
5	95.6	98.4	99.2	100.6	100.8	100.9
6	99.1	101.4	102.3	102.7	103.0	102.1
7	98.2	101.2	101.9	102.5	102.3	103.6
8	99.2	101.3	102.3	101.5	102.7	102.4
9	99.4	101.2	102.0	102.3	103.3	103.1
10	94.4	99.2	100.2	99.1	101.1	102.0
11	92.9	98.1	99.1	100.8	101.1	101.2
12	96.3	98.6	99.6	99.5	100.5	101.4
Mean	**97.3**	**100.2**	**101.0**	**101.4**	**102.0**	**102.4**
RSD	**2.7**	**1.4**	**1.4**	**1.3**	**1.2**	**0.9**
Minimum	**92.9**	**98.1**	**99.1**	**99.1**	**100.5**	**100.9**
Maximum	**99.9**	**102.1**	**103.0**	**103.7**	**104.3**	**103.9**

Table A2- Average dissolved percentage values of the commercial batch **3828**

Unit	Baclofen Tablets 10 mg Batch 3828 Dissolution Profile					
	5 min (%)	10 min(%)	15 min (%)	20 min (%)	25 min (%)	30 min (%)
1	99.2	100.4	101.2	101.4	102.2	102.4
2	96.5	97.9	99.0	98.5	98.7	100.1
3	95.2	96.3	98.5	99.9	100.6	101.4
4	98.5	100.3	100.7	117.8	100.8	101.9
5	94.6	97.3	99.4	100.4	100.9	101.1
6	96.6	97.4	99.4	99.9	100.3	100.4
7	98.9	99.8	100.7	101.5	101.8	101.2
8	96.6	99.4	100.5	100.1	101.9	101.3
9	96.6	99.5	100.0	100.5	100.2	101.3
10	98.9	99.2	100.5	101.3	101.6	101.0
11	98.6	99.8	100.1	100.6	101.6	101.7
12	98.9	100.3	100.6	100.0	101.7	101.8
Mean	**97.4**	**99.0**	**100.0**	**101.8**	**101.0**	**101.3**
RSD	**1.6**	**1.4**	**0.8**	**5.0**	**1.0**	**0.6**
Minimum	**94.6**	**96.3**	**98.5**	**98.5**	**98.7**	**100.1**
Maximum	**99.2**	**100.4**	**101.2**	**117.8**	**102.2**	**102.4**

Table A3- Average dissolved percentage values of the commercial batch **3830**

Unit	Baclofen Tablets 10 mg Batch 3830 Dissolution Profile					
	5 min (%)	10 min (%)	15 min (%)	20 min (%)	25 min (%)	30 min (%)
1	94.8	97.3	98.1	99.3	99.9	100.0
2	96.4	97.8	97.7	99.0	99.6	100.4
3	96.6	98.4	98.6	99.4	101.0	100.9
4	101.3	101.6	102.8	103.1	104.0	103.4
5	97.8	101.7	101.3	103.6	104.3	104.5
6	99.5	99.8	102.3	102.4	102.4	102.9
7	98.7	99.5	99.6	99.9	99.7	99.9
8	103.2	105.0	104.7	104.8	103.7	104.8
9	97.1	99.4	98.6	100.8	99.6	100.8
10	101.4	102.7	102.5	102.3	101.7	102.3
11	97.2	99.4	100.2	100.7	99.2	100.7
12	98.0	101.8	101.4	102.8	102.6	102.8
Mean	**98.5**	**100.4**	**100.6**	**101.5**	**101.5**	**102.0**
RSD	**2.5**	**2.2**	**2.2**	**1.9**	**1.9**	**1.7**
Minimum	**94.8**	**97.3**	**97.7**	**99.0**	**99.2**	**99.9**
Maximum	**103.2**	**105.0**	**104.7**	**104.8**	**104.3**	**104.8**

Table A4- Average dissolved percentage values of the test batch **200347** in HCl 0.1N.

Unit	Baclofen Tablets 10 mg Batch 200347 Dissolution Profile					
	5 min (%)	10 min (%)	15 min (%)	20 min (%)	25 min (%)	30 min (%)
1	87.8	89.5	91.8	92.5	94.1	95.3
2	94.8	97.1	97.6	97.5	98.9	99.2
3	94.5	96.3	97.0	96.2	98.8	99.0
4	96.8	99.2	100.0	99.1	101.2	101.9
5	80.8	81.4	83.3	84.4	87.1	87.8
6	95.8	97.8	97.9	98.0	100.1	100.4
7	94.5	98.2	98.6	98.6	99.8	99.6
8	104.9	97.4	98.2	98.4	98.4	98.5
9	95.5	97.7	98.2	98.2	98.2	98.3
10	100.0	101.9	101.9	102.4	102.2	101.4
11	89.6	93.5	94.6	95.5	95.8	95.8
12	96.8	98.9	98.9	99.4	99.2	98.9
Mean	**94.3**	**95.7**	**96.5**	**96.7**	**97.8**	**98.0**
RSD	**6.5**	**5.7**	**5.0**	**4.7**	**4.1**	**3.8**
Minimum	**80.8**	**81.4**	**83.3**	**84.4**	**87.1**	**87.8**
Maximum	**104.9**	**101.9**	**101.9**	**102.4**	**102.2**	**101.9**

Table A5- Average dissolved percentage values of the test batch **200348** in HCl 0.1N.

Unit	Dissolution Profile, Baclofen 10mg Tablets, Batch 200348					
	5 min (%)	10 min (%)	15 min (%)	20 min (%)	25 min (%)	30 min (%)
1	94.6	97.4	94.2	97.2	97.2	97.6
2	93.4	95.3	96.4	97.3	97.5	97.6
3	93.9	95.1	98.5	96.1	95.8	96.3
4	98.2	99.1	95.7	100.7	100.1	99.8
5	81.4	84.1	94.2	88.9	90.7	91.6
6	94.6	94.5	99.9	95.2	97.0	97.1
7	90.1	92.9	94.2	95.4	96.0	96.4
8	94.6	95.5	96.4	96.9	96.9	96.7
9	96.1	97.9	98.5	99.9	100.6	100.9
10	87.6	93.2	95.7	95.7	98.0	98.7
11	91.9	93.3	94.2	94.5	95.5	94.5
12	98.4	99.2	99.9	100.4	101.9	101.4
Mean	**92.9**	**94.8**	**96.5**	**96.5**	**97.3**	**97.4**
RSD	**5.1**	**4.2**	**2.3**	**3.3**	**3.0**	**2.8**
Minimum	**81.4**	**84.1**	**94.2**	**88.9**	**90.7**	**91.6**
Maximum	**98.4**	**99.2**	**99.9**	**100.7**	**101.9**	**101.4**

Table A6- Average dissolved percentage values of the test batch **200531** in HCl 0.1N.

Unit	Baclofen Tablets 25mg batch 200351 Dissolution Profile					
	5 min (%)	10 min (%)	15 min (%)	20 min (%)	25 min (%)	30 min (%)
1	92.8	95.9	97.1	97.3	98.1	98.1
2	97.9	100.4	100.4	98.1	102.5	102.2
3	94.6	96.1	96.2	98.3	98.1	97.7
4	92.8	96.2	97.0	95.3	98.9	99.3
5	94.3	96.6	97.8	101.2	98.2	96.2
6	89.6	93.5	94.3	97.5	95.8	98.8
7	93.8	95.9	96.4	97.2	97.4	98.2
8	98.6	100.7	101.8	101.5	101.9	102.0
9	93.0	95.3	96.5	97.0	97.2	97.7
10	97.8	99.0	99.4	99.4	99.7	100.4
11	100.2	101.1	101.6	102.1	102.6	102.1
12	100.1	101.1	101.4	102.0	102.5	102.6
Mean	**95.4**	**97.7**	**98.3**	**98.9**	**99.4**	**99.6**
RSD	**3.5**	**2.7**	**2.5**	**2.3**	**2.4**	**2.2**
Minimum	**89.6**	**93.5**	**94.3**	**95.3**	**95.8**	**96.2**
Maximum	**100.2**	**101.1**	**101.8**	**102.1**	**102.6**	**102.6**

2. **Dissolution media: Acetate Buffer pH 4.5**

Table A7- Average dissolved percentage values of the commercial batch **3827**

Unit	Baclofen Tablets 10 mg Batch 3827 Dissolution Profile					
	5 min (%)	10 min (%)	15 min (%)	20 min (%)	25 min (%)	30 min (%)
1	87.4	93.9	97.0	97.8	99.4	100.5
2	92.2	96.1	100.4	102.8	103.3	103.6
3	89.8	95.3	96.8	97.4	99.5	100.7
4	97.6	99.9	101.7	102.4	103.1	103.2
5	90.9	96.0	97.6	98.8	100.4	100.5
6	80.1	85.6	87.2	88.6	90.2	90.4
7	96.0	100.5	101.6	102.7	103.4	103.4
8	92.5	95.9	97.9	98.1	99.6	100.3
9	94.3	98.4	100.5	100.7	101.6	102.3
10	99.0	100.7	102.8	102.8	103.7	103.2
11	93.8	96.1	98.0	98.6	100.6	101.2
12	86.2	91.4	94.3	95.2	97.2	98.4
Mean	**91.7**	**95.8**	**98.0**	**98.8**	**100.2**	**100.6**
RSD	**5.7**	**4.4**	**4.3**	**4.1**	**3.7**	**3.6**
Minimum	**80.1**	**85.6**	**87.2**	**88.6**	**90.2**	**90.4**
Maximum	**99.0**	**100.7**	**102.8**	**102.8**	**103.7**	**103.6**

Table A8- Average dissolved percentage values of the commercial batch **3828**

Unit	Baclofen Tablets 10 mg Batch 3828 Dissolution Profile					
	5 min (%)	**10 min (%)**	**15 min (%)**	**20 min (%)**	**25 min (%)**	**30 min (%)**
1	89.2	92.3	95.0	97.0	98.4	99.9
2	87.8	95.5	96.8	98.3	99.7	100.1
3	90.9	96.2	98.3	98.6	99.6	100.6
4	88.8	92.4	95.1	95.6	96.6	97.2
5	92.6	96.4	97.7	98.3	99.0	99.9
6	86.2	91.5	92.0	94.5	95.3	96.6
7	89.1	92.9	94.8	96.3	97.8	100.0
8	91.7	95.3	97.8	98.1	99.1	99.6
9	93.3	97.2	99.0	101.9	101.2	102.6
10	93.3	97.3	99.3	100,2	100.4	102.1
11	86.3	92.6	94.1	97.0	97.6	98.9
12	84.8	90.5	92.9	94,3	95.7	97.0
Mean	**89.5**	**94.2**	**96.1**	**97.5**	**98.4**	**99.5**
RSD	**3.2**	**2.5**	**2.5**	**2.3**	**1.9**	**1.9**
Minimum	**84.8**	**90.5**	**92.0**	**94.3**	**95.3**	**96.6**
Maximum	**93.3**	**97.3**	**99.3**	**101.9**	**101.2**	**102.6**

Table A9- Average dissolved percentage values of the commercial batch **3830**

Unit	Baclofen Tablets 10 mg batch 3830 Dissolution Profile					
	5 min (%)	10 min (%)	15 min (%)	20 min (%)	25 min (%)	30 min (%)
1	89.0	94.8	95.2	96.3	97.1	98.1
2	92.7	96.3	98.2	99.0	99.9	101.2
3	90.9	93.5	95.4	97.0	98.9	99.3
4	83.6	87.6	89.9	92.1	93.2	93.7
5	83.8	90.0	92.7	95.0	96.8	98.5
6	83.4	88.0	91.0	92.6	93.3	95.5
7	89.2	92.9	95.2	97.1	97.1	99.5
8	88.4	93.3	94.8	96.3	97.3	97.3
9	94.5	98.8	101.0	101.1	102.8	103.0
10	86.9	93.9	94.8	96.5	97.0	97.7
11	88.9	92.5	95.3	96.6	97.6	98.3
12	85.9	90.6	93.1	94.0	95.7	96.0
Mean	**88.1**	**92.7**	**94.7**	**96.1**	**97.2**	**98.2**
RSD	**4.1**	**3.5**	**3.1**	**2.6**	**2.7**	**2.5**
Minimum	**83.4**	**87.6**	**89.9**	**92.1**	**93.2**	**93.7**
Maximum	**94.5**	**98.8**	**101.0**	**101.1**	**102.8**	**103.0**

Table A10- Average dissolved percentage values of the test batch **200347**

Unit	Baclofen Tablets 25mg batch 200347 Dissolution Profile					
	5 min (%)	10 min (%)	15 min (%)	20 min (%)	25 min (%)	30 min (%)
1	86.6	94.1	96.9	99.0	99.6	100.4
2	87.6	93.4	94.8	96.4	96.9	98.1
3	87.4	92.5	93.4	97.0	97.7	97.9
4	87.7	96.6	97.8	98.8	99.8	101.4
5	81.9	87.9	89.6	91.8	92.6	93.4
6	84.7	92.3	94.9	97.3	97.0	98.2
7	90.9	96.0	97.8	98.5	98.6	99.7
8	86.4	91.6	93.2	95.0	94.9	95.0
9	87.1	92.9	96.2	98.7	97.8	99.4
10	89.7	95.4	97.1	97.9	101.8	98.1
11	89.5	91.6	93.7	94.9	96.1	97.9
12	89.0	92.8	95.6	97.1	98.2	97.6
Mean	**87.4**	**93.1**	**95.1**	**96.9**	**97.6**	**98.1**
RSD	**2.8**	**2.5**	**2.5**	**2.2**	**2.5**	**2.2**
Minimum	**81.9**	**87.9**	**89.6**	**91.8**	**92.6**	**93.4**
Maximum	**90.9**	**96.6**	**97.8**	**99.0**	**101.8**	**101.4**

Table A11- Average dissolved percentage values of the test batch **200348**

Unit	Baclofen Tablets 10 mg batch 200348 Dissolution Profile					
	5 min (%)	10 min (%)	15 min (%)	20 min (%)	25 min (%)	30 min (%)
1	86.9	91.5	92.4	94.1	94.1	94.9
2	87.9	92.0	92.8	92.8	94.9	94.4
3	87.2	90.4	91.3	93.3	94.0	94.0
4	87.4	91.0	92.7	93.4	94.5	93.6
5	79.6	85.9	87.9	88.5	89.2	90.4
6	89.8	93.1	96.9	96.1	95.7	97.2
7	85.3	82.8	90.3	91.0	91.6	91.5
8	90.9	98.9	96.7	98.2	99.4	99.1
9	88.6	93.0	94.4	95.6	96.3	96.5
10	90.9	95.5	95.6	97.7	97.3	98.9
11	83.6	87.1	88.2	90.9	92.3	94.0
12	89.4	94.6	97.3	98.5	99.3	89.6
Mean	**87.3**	**91.3**	**93.0**	**94.2**	**94.9**	**94.5**
RSD	**3.7**	**4.8**	**3.5**	**3.4**	**3.2**	**3.2**
Minimum	**79.6**	**82.8**	**87.9**	**88.5**	**89.2**	**89.6**
Maximum	**90.9**	**98.9**	**97.3**	**98.5**	**99.4**	**99.1**

Table A12- Average dissolved percentage values of the test batch **200531**

Unit	Baclofen Tablets 10 mg batch 200351 Dissolution Profile					
	5 min (%)	10 min (%)	15 min (%)	20 min (%)	25 min (%)	30 min (%)
1	91.1	93.6	95.5	92.9	96.9	101.5
2	84.8	88.7	91.0	92.9	93.6	93.6
3	89.6	93.1	93.4	95.2	96.3	98.0
4	93.0	101.1	98.1	98.8	100.0	102.2
5	81.2	86.1	91.0	93.5	94.3	95.0
6	87.1	91.6	93.4	94.4	95.4	95.1
7	86.3	89.9	93.1	92.6	94.0	95.1
8	89.7	93.6	94.7	96.3	96.9	98.1
9	94.4	97.7	98.2	99.2	98.4	106.1
10	90.9	95.6	96.1	96.8	96.3	102.1
11	82.9	87.6	89.3	91.6	91.8	92.6
12	88.4	91.7	94.6	99.9	97.2	103.3
Mean	**88.3**	**92.5**	**94.0**	**95.3**	**95.9**	**98.6**
RSD	**4.5**	**4.6**	**2.9**	**3.0**	**2.3**	**4.5**
Minimum	**81.2**	**86.1**	**89.3**	**91.6**	**91.8**	**92.6**
Maximum	**94.4**	**101.1**	**98.2**	**99.9**	**100.0**	**106.1**

3. **Dissolution Media: Phosphate Buffer pH 6.8**

Table A13- Average dissolved percentage values of the commercial batch **3827**

Unit	**Baclofen Tablets 10 mg batch 3827 Dissolution Profile**					
	5 min (%)	10 min (%)	15 min (%)	20 min (%)	25 min (%)	30 min (%)
1	92.1	95.0	97.5	98.1	98.9	100.5
2	88.4	92.5	93.2	94.7	96.2	96.0
3	89.5	95.1	97.1	98.6	98.6	99.6
4	84.1	94.0	99.2	99.6	100.2	101.5
5	85.6	97.2	99.8	101.1	100.0	100.5
6	74.1	82.0	85.0	87.4	89.1	89.8
7	82.3	91.5	96.2	97.0	97.9	97.5
8	84.4	89.5	90.9	92.0	93.4	94.0
9	83.9	90.6	94.1	94.6	97.0	97.7
10	81.8	88.2	89.5	90.8	92.1	93.1
11	78.0	92.4	97.4	98.3	99.4	100.4
12	89.7	93.1	95.0	96.4	98.1	99.2
Mean	**84.5**	**91.8**	**94.6**	**95.7**	**96.7**	**97.5**
RSD	**6.1**	**4.3**	**4.6**	**4.2**	**3.6**	**3.7**
Minimum	**74.1**	**82.0**	**85.0**	**87.4**	**89.1**	**89.8**
Maximum	**92.1**	**97.2**	**99.8**	**101.1**	**100.2**	**101.5**

Table A14- Average dissolved percentage values of the commercial batch **3828**

Unit	Baclofen Tablets 10 mg batch 3828 Dissolution Profile					
	5 min (%)	10 min (%)	15 min (%)	20 min (%)	25 min (%)	30 min (%)
1	87.8	98.7	101.9	101.5	101.8	100.9
2	76.2	88.5	93.1	95.6	96.8	99.4
3	78.8	85.3	87.9	88.7	90.0	91.1
4	64.7	84.3	91.9	94.9	96.9	97.3
5	96.4	101.2	102.7	103.3	103.2	104.0
6	83.9	89.1	91.0	94.3	93.2	95.6
7	70.2	84.2	91.7	95.4	96.7	98.2
8	78.3	87.4	91.6	93.1	94.5	95.4
9	85.1	96.8	99.0	99.8	102.2	101.5
10	84.9	96.6	98.0	99.0	99.0	98.9
11	76.2	99.8	101.0	101.9	101.8	102.4
12	99.0	85.3	92.0	95.6	97.6	99.3
Mean	**81.8**	**91.4**	**95.2**	**96.9**	**97.8**	**98.7**
RSD	**12.1**	**7.2**	**5.3**	**4.4**	**4.1**	**3.6**
Minimum	**64.7**	**84.2**	**87.9**	**88.7**	**90.0**	**91.1**
Maximum	**99.0**	**101.2**	**102.7**	**103.3**	**103.2**	**104.0**

Table A15- Average dissolved percentage values of the commercial batch **3830**

Unit	Baclofen Tablets 10 mg batch 3830 Dissolution Profile					
	5 min (%)	10 min (%)	15 min (%)	20 min (%)	25 min (%)	30 min (%)
1	85.7	97.7	100.8	85.4	102.4	102.2
2	89.4	92.6	93.7	101.8	95.4	96.9
3	87.7	99.1	102.6	94.9	104.7	88.2
4	85.8	97.4	105.4	103.1	103.3	103.8
5	90.9	99.4	101.3	103.0	105.0	106.0
6	59.5	78.0	102.3	104.4	89.3	92.2
7	90.8	94.2	95.8	95.9	98.4	98.6
8	90.7	97.3	98.1	99.8	99.1	100.7
9	89.4	93.1	94.3	95.0	95.6	96.5
10	96.0	98.5	100.2	100.0	101.2	102.4
11	89.8	91.5	93.5	95.8	96.2	97.3
12	85.9	91.6	93.6	94.9	95.5	96.8
Mean	**86.8**	**94.2**	**98.5**	**97.8**	**98.8**	**98.5**
RSD	**10.4**	**6.2**	**4.3**	**5.4**	**4.7**	**5.1**
Minimum	**59.5**	**78.0**	**93.5**	**85.4**	**89.3**	**88.2**
Maximum	**96.0**	**99.4**	**105.4**	**104.4**	**105.0**	**106.0**

Table A16- Average dissolved percentage values of the test batch **200347**

Unit	Baclofen Tablets 10 mg batch 200347 Dissolution Profile					
	5 min (%)	10 min (%)	15 min (%)	20 min (%)	25 min (%)	30 min (%)
1	61.0	78.7	86.7	90.4	94.8	95.3
2	84.4	91.5	95.2	94.8	97.2	95.7
3	81.9	90.4	93.8	96.2	97.0	98.9
4	64.1	81.5	84.9	89.0	90.9	91.9
5	90.1	96.2	97.9	98.4	99.3	99.2
6	75.4	80.5	83.4	84.2	86.2	86.6
7	84.5	93.5	97.3	99.1	97.5	97.2
8	79.4	89.0	92.0	94.7	96.4	97.3
9	57.4	91.7	97.4	98.4	97.9	99.6
10	41.2	77.3	85.4	92.4	96.6	98.0
11	82.4	93.7	96.7	98.9	100.6	100.9
12	75.6	85.0	88.7	91.5	93.0	94.3
Mean	**73.1**	**87.4**	**91.6**	**94.0**	**95.6**	**96.2**
RSD	**19.6**	**7.5**	**6.0**	**4.9**	**4.1**	**4.1**
Minimum	**41.2**	**77.3**	**83.4**	**84.2**	**86.2**	**86.6**
Maximum	**90.1**	**96.2**	**97.9**	**99.1**	**100.6**	**100.9**

Table A17- Average dissolved percentage values of the test batch **200348**

Unit	Baclofen Tablets 10 mg batch 200348 Dissolution Profile					
	5 min (%)	10 min (%)	15 min (%)	20 min (%)	25 min (%)	30 min (%)
1	84.2	88.6	91.2	93.4	93.2	94.3
2	87.2	92.4	94.3	95.6	96.0	96.7
3	84.3	87.7	89.0	91.3	91.6	92.7
4	67.6	87.9	91.4	92.2	92.7	94.2
5	82.9	87.1	92.9	93.5	95.1	94.8
6	85.2	90.5	92.3	94.3	94.8	95.6
7	75.7	80.7	83.6	85.3	86.4	88.5
8	81.7	86.0	88.8	91.2	91.8	91.4
9	86.8	91.3	89.9	92.5	92.6	94.2
10	70.8	79.8	81.5	83.7	85.8	84.6
11	55.2	91.1	98.9	99.6	100.7	100.4
12	68.8	78.0	80.8	82.7	83.5	85.7
Mean	**77.5**	**86.8**	**89.6**	**91.3**	**92.0**	**92.8**
RSD	**12.9**	**5.5**	**6.0**	**5.5**	**5.2**	**4.9**
Minimum	**55.2**	**78.0**	**80.8**	**82.7**	**83.5**	**84.6**
Maximum	**87.2**	**92.4**	**98.9**	**99.6**	**100.7**	**100.4**

Table A18- Average dissolved percentage values of the test batch **200531**

Unit	Baclofen Tablets 10 mg batch 200348 Dissolution Profile					
	5 min (%)	10 min (%)	15 min (%)	20 min (%)	25 min (%)	30 min (%)
1	79.8	88.4	91.3	93.3	93.8	95.6
2	77.4	84.9	88.0	89.3	91.7	92.1
3	60.5	80.3	87.5	89.8	92.1	93.3
4	91.0	95.5	97.1	99.4	99.3	100.1
5	72.2	86.4	90.7	95.8	95.8	95.5
6	79.0	84.4	88.7	90.2	90.6	93.4
7	85.0	89.7	92.4	94.5	95.6	96.0
8	84.7	91.6	94.3	96.6	97.0	96.5
9	90.8	81.7	101.4	101.0	101.2	101.5
10	65.9	83.6	89.8	92.8	94.5	94.3
11	65.2	86.9	95.6	99.0	100.4	102.7
12	71.4	79.7	82.0	85.5	88.0	90.7
Mean	**76.9**	**86.1**	**91.6**	**93.9**	**95.0**	**96.0**
RSD	**13.1**	**5.5**	**5.6**	**5.0**	**4.3**	**3.9**
Minimum	**60.5**	**79.7**	**82.0**	**85.5**	**88.0**	**90.7**
Maximum	**91.0**	**95.5**	**101.4**	**101.0**	**101.2**	**102.7**

Appendix B - Average values of the experimental results for % dissolved in different media of 25 mg

1. **Dissolution Media: HCl 0.1N**

Table B1- Average dissolved percentage values of the commercial batch **3751**

Unit	Baclofen Tablets 25 mg Batch 3751 Dissolution Profile					
	5 min (%)	10 min (%)	15 min (%)	20 min (%)	25 min (%)	30 min (%)
1	89.8	93.7	94.9	95.6	96.3	97.4
2	88.5	92.4	94.2	95.1	96.4	96.7
3	93.0	96.9	98.7	99.0	100.7	99.8
4	68.2	76.9	79.5	82.3	84.1	84.5
5	88.0	91.1	91.8	94.1	94.7	95.5
6	77.6	82.0	84.2	85.9	87.7	88.6
7	82.1	87.4	90.0	90.9	92.2	94.1
8	97.8	100.6	100.6	103.4	103.6	103.9
9	95.0	98.3	99.3	100.2	100.9	101.6
10	92.0	96.3	97.5	98.6	98.0	99.6
11	89.6	94.0	95.9	97.2	97.1	99.0
12	91.9	94.2	96.8	98.1	97.9	100.2
Mean	**87.8**	**92.0**	**93.6**	**95.0**	**95.8**	**96.7**
RSD	**9.3**	**7.4**	**6.8**	**6.4**	**5.8**	**5.7**
Minimum	**68.2**	**76.9**	**79.5**	**82.3**	**84.1**	**84.5**
Maximum	**97.8**	**100.6**	**100.6**	**103.4**	**103.6**	**103.9**

Table B2- Average dissolved percentage values of the commercial batch **3843.**

Unit	Baclofen Tablets 25 mg Batch 3843 Dissolution Profile					
	5 min (%)	10 min (%)	15 min (%)	20 min (%)	25 min (%)	30 min (%)
1	93.3	95.8	97.4	98.9	99.0	99.5
2	90.3	95.7	98.0	100.0	100.0	101.1
3	97.9	96.8	98.0	98.4	99.6	99.6
4	94.1	96.7	98.4	98.6	99.5	100.7
5	83.9	88.3	90.5	91.9	93.7	95.7
6	92.8	95.6	98.2	98.9	101.9	98.6
7	92.0	94.0	95.8	97.0	97.6	97.8
8	86.8	89.5	90.9	92.0	93.0	93.9
9	84.2	87.0	88.3	89.6	91.5	92.6
10	84.6	89.2	91.2	92.4	93.0	93.5
11	84.5	90.1	91.1	93.1	93.9	94.7
12	92.2	94.5	96.1	97.7	97.8	98.3
Mean	**89.7**	**92.8**	**94.5**	**95.7**	**96.7**	**97.2**
RSD	**5.3**	**3.9**	**4.0**	**3.8**	**3.6**	**3.1**
Minimum	**83.9**	**87.0**	**88.3**	**89.6**	**91.5**	**92.6**
Maximum	**97.9**	**96.8**	**98.4**	**100.0**	**101.9**	**101.1**

Table B3- Average dissolved percentage values of the commercial batch **3861.**

Unit	Baclofen Tablets 25 mg Batch 3843 Dissolution Profile					
	5 min (%)	10 min (%)	15 min (%)	20 min (%)	25 min (%)	30 min (%)
1	99.1	100.6	101.8	103.6	104.4	104.9
2	94.6	98.8	100.8	102.4	103.2	103.3
3	81.8	88.6	90.7	93.5	95.8	97.1
4	93.7	96.6	99.1	100.6	102.3	101.8
5	94.7	98.3	100.4	100.8	102.8	103.4
6	95.1	97.7	99.8	101.8	102.6	103.4
7	96.1	97.4	99.4	99.9	100.3	100.4
8	79.6	85.3	87.5	90.5	92.1	93.0
9	94.9	97.1	98.0	98.6	99.9	100.2
10	91.0	97.1	96.9	98.6	98.8	98.7
11	78.7	82.3	86.0	87.8	88.9	89.7
12	82.2	85.0	86.5	87.1	88.7	90.0
Mean	**90.1**	**93.7**	**95.6**	**97.1**	**98.3**	**98.8**
RSD	**8.1**	**6.9**	**6.3**	**6.0**	**5.7**	**5.4**
Minimum	**78.7**	**82.3**	**86.0**	**87.1**	**88.7**	**89.7**
Maximum	**99.1**	**100.6**	**101.8**	**103.6**	**104.4**	**104.9**

Table B4- Average dissolved percentage values of the test batch **200349.**

Unit	Baclofen Tablets 25 mg Batch 200349 Dissolution Profile					
	5 min (%)	10 min(%)	15 min (%)	20 min (%)	25 min (%)	30 min (%)
1	93.5	96.3	98.5	98.7	99.9	99.6
2	89.4	93.1	95.2	96.4	96.7	98.1
3	98.5	100.8	101.4	102.2	102.7	102.7
4	89.4	92.8	94.9	96.5	97.4	97.4
5	92.0	95.5	96.7	97.2	98.5	98.8
6	87.3	90.2	92.5	94.1	95.0	95.6
7	94.2	96.5	97.8	98.0	99.0	98.2
8	87.9	91.7	93.7	94.7	95.2	96.0
9	90.6	94.2	94.6	96.8	96.2	96.4
10	91.8	92.8	94.7	95.5	95.3	95.9
11	99.2	100.4	102.0	101.1	101.4	100.6
12	97.8	98.9	99.9	99.8	99.9	100.8
Mean	**92.6**	**95.3**	**96.8**	**97.6**	**98.1**	**98.3**
RSD	**4.4**	**3.6**	**3.2**	**2.6**	**2.6**	**2.3**
Minimum	**87.3**	**90.2**	**92.5**	**94.1**	**95.0**	**95.6**
Maximum	**99.2**	**100.8**	**102.0**	**102.2**	**102.7**	**102.7**

Table B5- Average dissolved percentage values of the test batch **200350.**

Unit	Baclofen Tablets 25 mg Batch 200350 Dissolution Profile					
	5 min (%)	10 min (%)	15 min (%)	20 min (%)	25 min (%)	30 min (%)
1	90.5	92.6	93.4	93.6	94.3	94.7
2	83.4	86.5	88.6	89.3	90.6	91.5
3	89.2	91.3	92.7	93.5	93.9	94.5
4	92.0	94.6	95.8	96.1	96.2	96.3
5	83.4	86.2	87.7	89.7	90.6	91.5
6	87.5	90.5	92.5	93.5	94.7	95.6
7	83.2	86.7	88.6	89.9	90.9	91.4
8	90.4	92.0	93.5	93.7	94.1	94.1
9	85.8	87.7	89.8	90.6	91.6	92.0
10	91.1	93.6	94.1	95.2	95.7	96.0
11	91.6	94.4	95.9	96.6	97.7	97.6
12	83.2	87.1	88.1	90.3	90.7	92.2
Mean	**87.6**	**90.3**	**91.7**	**92.7**	**93.4**	**93.9**
RSD	**4.1**	**3.6**	**3.3**	**2.8**	**2.7**	**2.3**
Minimum	**83.2**	**86.2**	**87.7**	**89.3**	**90.6**	**91.4**
Maximum	**92.0**	**94.6**	**95.9**	**96.6**	**97.7**	**97.6**

Table B6- Average dissolved percentage values of the test batch **200532.**

Unit	Baclofen Tablets 25 mg Batch 200532 Dissolution Profile					
	5 min (%)	**10 min(%)**	**15 min (%)**	**20 min (%)**	**25 min (%)**	**30 min (%)**
1	80.2	86.4	88.3	90.1	91.4	91.4
2	78.4	82.0	85.3	86.1	88.0	88.0
3	90.4	93.9	94.2	95.6	96.5	96.5
4	90.0	94.6	95.2	96.6	97.9	97.9
5	94.4	96.6	97.5	97.8	99.3	99.3
6	92.4	95.0	95.9	97.4	97.8	97.8
7	92.5	95.9	96.8	98.2	97.7	99.0
8	68.7	77.6	81.0	82.7	84.3	86.1
9	92.1	94.4	95.1	96.3	97.5	97.7
10	91.0	94.7	96.4	97.0	97.4	98.7
11	90.9	93.5	95.2	96.3	97.4	97.7
12	90.6	93.1	94.3	95.5	96.0	97.0
Mean	**87.6**	**91.5**	**92.9**	**94.1**	**95.1**	**95.6**
RSD	**8.8**	**6.7**	**5.6**	**5.4**	**4.9**	**4.7**
Minimum	**68.7**	**77.6**	**81.0**	**82.7**	**84.3**	**86.1**
Maximum	**94.4**	**96.6**	**97.5**	**98.2**	**99.3**	**99.3**

2. **Dissolution Media: Acetate Buffer pH 4.5**

Table B7- Average dissolved percentage values of the commercial batch **3751.**

Unit	Baclofen Tablets 25 mg Batch 3751 Dissolution Profile					
	5 min (%)	**10 min (%)**	**15 min (%)**	**20 min (%)**	**25 min (%)**	**30 min (%)**
1	87.6	90.2	91.9	92.8	94.4	95.7
2	90.1	94.7	96.8	97.4	98.3	100.4
3	80.5	87.8	92.9	96.3	98.2	100.2
4	75.7	81.0	82.0	84.3	84.8	86.9
5	77.3	85.2	89.3	91.4	93.9	95.6
6	74.2	79.6	83.6	86.5	90.0	91.3
7	80.8	85.0	87.7	89.3	90.7	91.9
8	75.9	83.3	85.6	87.1	88.4	89.9
9	75.8	81.8	85.5	87.6	88.8	90.0
10	86.3	90.1	92.5	93.0	94.7	97.0
11	81.6	88.2	92.9	95.7	96.7	98.8
12	73.4	78.7	81.4	83.1	85.5	86.7
Mean	**79.9**	**85.5**	**88.5**	**90.4**	**92.0**	**93.7**
RSD	**7.0**	**5.7**	**5.6**	**5.3**	**5.1**	**5.2**
Minimum	**73.4**	**78.7**	**81.4**	**83.1**	**84.8**	**86.7**
Maximum	**90.1**	**94.7**	**96.8**	**97.4**	**98.3**	**100.4**

Table B8- Average dissolved percentage values of the commercial batch **3843.**

Unit	Baclofen Tablets 25 mg Batch 3843 Dissolution Profile					
	5 min (%)	10 min(%)	15 min (%)	20 min (%)	25 min (%)	30 min (%)
1	86.4	90.0	91.4	93.0	94.1	94.2
2	76.5	83.3	85.9	87.9	89.5	91.0
3	74.2	83.6	86.6	88.5	90.6	90.8
4	84.6	89.1	90.7	91.0	92.8	93.6
5	86.3	90.0	91.2	92.8	93.8	94.8
6	80.3	84.0	86.0	87.8	89.3	90.7
7	92.3	96.2	97.9	99.7	100.1	100.9
8	80.3	86.2	88.1	89.8	90.0	91.6
9	71.1	79.4	82.5	84.4	85.4	87.7
10	70.6	75.9	78.8	80.1	82.2	83.2
11	74.0	82.4	86.7	88.4	90.8	92.5
12	68.9	75.4	78.7	79.2	81.2	82.8
Mean	**78.8**	**84.6**	**87.0**	**88.5**	**90.0**	**91.1**
RSD	**9.4**	**7.2**	**6.3**	**6.4**	**5.8**	**5.4**
Minimum	**68.9**	**75.4**	**78.7**	**79.2**	**81.2**	**82.8**
Maximum	**92.3**	**96.2**	**97.9**	**99.7**	**100.1**	**100.9**

Table B9- Average dissolved percentage values of the commercial batch **3861.**

Unit	Baclofen Tablets 25 mg Batch 3843 Dissolution Profile					
	5 min (%)	10 min(%)	15 min (%)	20 min (%)	25 min (%)	30 min (%)
1	88.2	90.7	93.7	94.6	96.4	96.8
2	76.3	82.2	84.7	87.0	88.3	89.2
3	85.5	91.4	93.5	94.9	96.9	98.2
4	89.0	91.9	94.1	95.7	96.0	97.4
5	73.7	79.2	81.9	85.0	88.6	89.5
6	82.5	85.8	88.4	89.9	91.4	92.8
7	84.1	87.2	89.5	91.9	93.1	95.4
8	83.9	88.3	90.4	91.5	93.0	93.4
9	90.7	94.2	95.7	96.9	98.5	99.0
10	76.9	84.1	85.7	87.9	89.9	91.5
11	83.2	91.0	93.8	96.2	97.0	98.4
12	81.7	85.0	87.0	88.8	90.4	91.8
Mean	**83.0**	**87.6**	**89.9**	**91.7**	**93.3**	**94.4**
RSD	**6.3**	**5.1**	**4.9**	**4.3**	**3.8**	**3.8**
Minimum	**73.7**	**79.2**	**81.9**	**85.0**	**88.3**	**89.2**
Maximum	**90.7**	**94.2**	**95.7**	**96.9**	**98.5**	**99.0**

Table B10- Average dissolved percentage values of the test batch **200349.**

Unit	Baclofen Tablets 25 mg Batch 200349 Dissolution Profile					
	5 min (%)	10 min (%)	15 min (%)	20 min (%)	25 min (%)	30 min (%)
1	80.8	86.5	89.6	91.8	93.1	94.5
2	90.0	95.5	97.8	98.3	99.3	100.6
3	85.2	91.0	92.5	94.2	95.2	96.4
4	88.2	91.7	94.0	95.3	96.5	98.2
5	87.7	92.1	94.5	96.8	97.8	98.7
6	85.8	89.8	92.6	93.6	95.4	96.0
7	87.8	91.7	93.8	94.9	96.1	97.2
8	87.8	93.8	95.5	96.8	98.3	99.4
9	91.6	94.6	96.9	98.4	99.0	100.2
10	85.0	89.3	91.2	93.1	94.2	95.5
11	94.5	98.4	99.9	101.5	102.4	103.0
12	83.9	87.0	89.2	90.5	92.0	92.8
Mean	**87.4**	**91.8**	**94.0**	**95.4**	**96.6**	**97.7**
RSD	**4.2**	**3.8**	**3.4**	**3.3**	**3.0**	**3.0**
Minimum	**80.8**	**86.5**	**89.2**	**90.5**	**92.0**	**92.8**
Maximum	**94.5**	**98.4**	**99.9**	**101.5**	**102.4**	**103.0**

Table B11- Average dissolved percentage values of the test batch **200350.**

Unit	Baclofen Tablets 25 mg Batch 200350 Dissolution Profile					
	5 min (%)	10 min (%)	15 min (%)	20 min (%)	25 min (%)	30 min (%)
1	78.5	84.5	85.8	87.3	88.5	89.3
2	82.7	86.5	88.9	89.9	91.1	91.2
3	89.5	93.6	95.2	97.1	98.6	99.2
4	84.3	87.7	90.0	91.9	92.4	94.2
5	79.5	84.8	88.5	91.1	92.4	94.2
6	81.9	86.4	88.6	89.8	90.4	92.4
7	86.3	89.2	92.0	93.0	93.7	95.1
8	85.3	89.3	90.7	92.9	93.9	94.1
9	86.5	89.9	91.5	93.2	94.3	95.0
10	78.4	83.8	85.9	87.5	88.2	90.1
11	80.6	84.8	88.3	89.9	90.9	93.0
12	77.2	80.9	83.4	85.1	86.8	87.3
Mean	**82.6**	**86.8**	**89.1**	**90.7**	**91.8**	**92.9**
RSD	**4.7**	**3.9**	**3.5**	**3.6**	**3.5**	**3.4**
Minimum	**77.2**	**80.9**	**83.4**	**85.1**	**86.8**	**87.3**
Maximum	**89.5**	**93.6**	**95.2**	**97.1**	**98.6**	**99.2**

Table B12- Average dissolved percentage values of the test batch **200532.**

Unit	Baclofen Tablets 25 mg Batch 200532 Dissolution Profile					
	5 min (%)	10 min (%)	15 min (%)	20 min (%)	25 min (%)	30 min (%)
1	86.8	90.5	92.4	93.7	95.5	95.5
2	86.1	88.8	90.7	92.3	92.4	93.4
3	83.2	86.4	88.5	91.1	91.7	93.2
4	88.2	92.0	93.9	95.5	96.9	97.7
5	76.2	82.1	84.3	86.0	86.8	87.5
6	71.9	75.9	78.3	79.9	81.2	82.8
7	87.7	89.7	92.7	94.0	94.8	96.0
8	85.8	90.1	90.4	92.7	93.7	95.1
9	88.8	92.7	94.3	95.2	96.4	97.3
10	76.8	80.3	82.8	85.1	85.4	86.6
11	82.9	86.9	88.7	89.9	91.7	92.3
12	83.8	87.4	89.5	91.1	92.4	94.0
Mean	**83.2**	**86.9**	**88.9**	**90.5**	**91.6**	**92.6**
RSD	**6.5**	**5.8**	**5.4**	**5.1**	**5.2**	**5.0**
Minimum	**71.9**	**75.9**	**78.3**	**79.9**	**8.2**	**82.8**
Maximum	**88.8**	**92.7**	**94.3**	**95.5**	**96.9**	**97.7**

3. **Dissolution Media: Phosphate Buffer pH 6.8**

Table B13- Average dissolved percentage values of the commercial batch **3751.**

Unit	Baclofen Tablets 25 mg Batch 3751 Dissolution Profile					
	5 min (%)	10 min (%)	15 min (%)	20 min (%)	25 min (%)	30 min (%)
1	79.1	86.4	88.7	90.8	92.1	93.9
2	85.6	89.0	90.8	92.2	93.9	94.4
3	85.2	88.6	90.5	91.4	92.6	93.9
4	86.6	91.7	93.0	94.2	96.0	96.5
5	86.2	90.6	92.9	94.4	95.2	96.2
6	83.1	87.6	89.2	91.2	92.2	93.0
7	81.6	88.7	91.2	94.0	94.2	94.7
8	80.3	84.2	86.1	87.8	89.6	89.3
9	72.3	82.8	87.9	89.5	89.8	90.9
10	80.6	87.1	89.3	91.5	92.1	92.8
11	69.8	74.4	77.5	80.7	83.0	84.9
12	84,4	87.3	90.5	90.6	91.7	93.2
Mean	**81.2**	**86.5**	**89.0**	**90.7**	**91.9**	**92.8**
RSD	**6.6**	**5.2**	**4.6**	**4.1**	**3.7**	**3.4**
Minimum	**69.8**	**74.4**	**77.5**	**80.7**	**83.0**	**84.9**
Maximum	**86.6**	**91.7**	**93.0**	**94.4**	**96.0**	**96.5**

Table B14- Average dissolved percentage values of the commercial batch **3843.**

Unit	Baclofen Tablets 25 mg Batch 3843 Dissolution Profile					
	5 min (%)	10 min(%)	15 min (%)	20 min (%)	25 min (%)	30 min (%)
1	80.8	86.6	88.0	89.6	91.9	98.1
2	86.6	90.0	91.3	92.0	92.9	98.0
3	91.6	95.7	96.4	97.3	98.0	92.4
4	79.0	86.0	87.6	89.1	90.5	94.0
5	89.0	90.9	92.6	94.0	94.8	99.0
6	87.0	89.5	91.2	92.2	93.1	91.6
7	79.3	88.5	94.5	96.0	97.8	99.1
8	87.3	91.4	92.8	94.0	94.3	95.1
9	81.8	88.3	91.0	92.1	94.2	95.5
10	77.8	81.9	84.2	86.2	86.4	87.4
11	80.1	84.0	86.1	87.6	90.0	91.0
12	82.0	84.7	86.9	88.4	90.3	92.4
Mean	**83.5**	**88.1**	**90.2**	**91.5**	**92.9**	**94.5**
RSD	**5.4**	**4.3**	**4.1**	**3.8**	**3.6**	**3.9**
Minimum	**77.8**	**819**	**84.2**	**86.2**	**86.4**	**87.4**
Maximum	**91.6**	**95.7**	**96.4**	**97.3**	**98.0**	**99.1**

Table B15- Average dissolved percentage values of the commercial batch **3861.**

Unit	Baclofen Tablets 25 mg Batch 3861 Dissolution Profile					
	5 min (%)	10 min(%)	15 min (%)	20 min (%)	25 min (%)	30 min (%)
1	87.4	91.0	91.7	93.1	94.0	94.7
2	88.4	92.3	94.7	95.0	95.7	96.3
3	75.1	79.7	80.7	81.9	84.1	84.9
4	77.2	83.1	85.1	86.6	87.3	87.6
5	72.2	81.0	83.4	89.3	88.1	89.6
6	87.1	89.3	91.6	92.8	94.2	95.2
7	92.0	95.5	97.4	98.6	99.3	101.0
8	83.4	87.0	89.1	90.3	91.3	92.0
9	89.2	94.0	94.5	95.2	96.6	96.9
10	86.8	91.9	92.9	94.0	94.7	95.1
11	81.2	86.6	90.7	93.2	94.8	96.5
12	80.9	86.3	89.1	90.7	91.7	93.3
Mean	**83.4**	**88.1**	**90.1**	**91.7**	**92.7**	**93.6**
RSD	**7.4**	**5.8**	**5.5**	**4.8**	**4.7**	**4.7**
Minimum	**72.2**	**79.7**	**80.7**	**81.9**	**84.1**	**84.9**
Maximum	**92.0**	**95.5**	**97.4**	**98.6**	**99.3**	**101.0**

Table B16- Average dissolved percentage values of the test batch **200349.**

Unit	Baclofen Tablets 25 mg Batch 200349 Dissolution Profile					
	5 min (%)	10 min (%)	15 min (%)	20 min (%)	25 min (%)	30 min (%)
1	84.1	87.3	90.4	93.8	94.2	96.1
2	89.3	92.5	94.2	95.6	94.8	96.3
3	79.2	84.4	86.3	87.9	90.4	90.3
4	84.6	87.1	90.0	90.5	91.9	92.7
5	79.3	82.6	85.4	86.7	87.7	89.2
6	79.5	83.2	83.6	84.8	86.0	86.1
7	77.8	85.7	88.2	91.3	93.4	94.9
8	86.5	90.9	92.5	93.1	93.3	94.8
9	83.7	86.8	88.2	88.6	88.9	90.3
10	86.4	87.8	89.4	89.8	91.8	92.9
11	84.8	87.3	90.5	91.8	92.9	94.0
12	79.7	82.4	85.1	86.7	86.0	87.6
Mean	**82.9**	**86.5**	**88.7**	**90.1**	**91.0**	**92.1**
RSD	**4.4**	**3.6**	**3.6**	**3.6**	**3.4**	**3.7**
Minimum	**77.8**	**82.4**	**83.6**	**84.8**	**86.0**	**86.1**
Maximum	**89.3**	**92.5**	**94.2**	**95.6**	**94.8**	**96.3**

Table B17- Average dissolved percentage values of the test batch **200350.**

Unit	Baclofen Tablets 25 mg Batch 200350 Dissolution Profile					
	5 min (%)	10 min (%)	15 min (%)	20 min (%)	25 min (%)	30 min (%)
1	72.6	77.6	79.7	81.9	83.8	84.0
2	80.5	85.3	86.9	88.2	89.0	90.1
3	83.6	87.3	89.0	90.7	90.9	92.8
4	89.6	92.6	94.0	96.7	97.9	97.0
5	84.3	86.8	87.7	91.3	90.8	93.0
6	84.0	90.1	89.4	86.8	92.1	93.2
7	78.8	85.4	88.5	92.0	93.8	95.2
8	90.9	95.5	96.7	97.4	97.7	98.7
9	84.0	87.8	88.5	90.0	91.7	92.4
10	79.0	83.0	87.8	82.0	86.9	87.8
11	82.3	86.7	84.0	89.4	90.6	92.0
12	86.0	89.5	91.3	92.8	93.9	95.3
Mean	**83.0**	**87.3**	**88.6**	**89.9**	**91.6**	**92.6**
RSD	**6.0**	**5.2**	**4.9**	**5.4**	**4.4**	**4.3**
Minimum	**72.6**	**77.6**	**79.7**	**81.9**	**83.8**	**84.0**
Maximum	**90.9**	**95.5**	**96.7**	**97.4**	**97.9**	**98.7**

Table B18- Average dissolved percentage values of the test batch **200532.**

Unit	Baclofen Tablets 25 mg Batch 200532 Dissolution Profile					
	5 min (%)	**10 min (%)**	**15 min (%)**	**20 min (%)**	**25 min (%)**	**30 min (%)**
1	85.4	88.4	90.7	92.8	93.9	95.4
2	78.0	83.7	86.0	89.7	88.7	90.6
3	84.3	88.2	90.6	90.1	92.1	93.2
4	88.7	93.9	95.8	97.3	98.5	100.3
5	70.6	76.5	77.4	80.3	81.2	81.3
6	77.9	81.8	82.7	83.4	84.9	85.9
7	79.1	82.1	84.5	86.7	87.7	88.2
8	83.7	87.5	89.1	90.8	91.9	92.9
9	87.3	90.5	92.1	93.0	94.5	95.0
10	82.7	86.0	88.0	89.4	90.7	91.4
11	78.0	81.6	83.9	85.1	86.7	87.4
12	74.3	78.1	80.7	81.7	82.5	84.0
Mean	**80.8**	**84.9**	**86.8**	**88.4**	**89.4**	**90.5**
RSD	**6.7**	**6.0**	**6.0**	**5.7**	**5.7**	**5.9**
Minimum	**70.6**	**76.5**	**77.4**	**80.3**	**81.2**	**81.3**
Maximum	**88.7**	**93.9**	**95.8**	**97.3**	**98.5**	**100.3**

Appendix C - Areas of Dissolved 10 mg Baclofen through multiple sampling points

1. **Dissolution Media: HCl 0.1N**

Table C1- Areas of dissolved 10 mg Baclofen of commercial batch **3827**

Unit	Baclofen 10 mg Areas of Batch 3827					
	5 min	10 min	15 min	20 min	25 min	30 min
1	95336	100722	101518	102262	102914	104748
2	101077	103843	104798	105546	106083	105728
3	101363	103125	103568	104123	104536	104581
4	101653	102538	102949	103085	102794	104357
5	97294	100076	100973	102376	102510	102634
6	100875	103174	104133	104491	104830	103859
7	100406	103478	104209	104794	104570	105968
8	101393	103540	104568	103757	105013	104721
9	101662	103449	104317	104574	105571	105390
10	96493	101458	102470	101346	103361	104343
11	94924	100345	101305	103031	103380	103510
12	98457	100754	101851	101762	102738	103659
Mean	**100641**	**102832**	**103259**	**103421**	**103958**	**104469**

Table C2- Areas of dissolved 10 mg Baclofen of commercial batch **3828**

Unit	Baclofen 10 mg Areas of Batch 3828					
	5 min	10 min	15 min	20 min	25 min	30 min
1	100969	102189	102983	103181	103970	104212
2	98206	99616	100738	100258	100454	101839
3	96882	97984	100177	101684	102340	103180
4	100213	102002	102438	119915	102596	103652
5	96207	98987	101115	102206	102641	102913
6	98319	99075	101100	101601	102055	102115
7	102412	103318	104210	105052	105377	104732
8	99978	102887	104103	103655	105529	104928
9	100010	103069	103531	104009	103767	104876
10	102357	102735	104024	104936	105231	104612
11	102124	103325	103673	104137	105248	105334
12	102348	103806	104207	103530	105270	105386
Mean	**100112**	**102462**	**103257**	**103593**	**103869**	**104412**

Table C3- Areas of dissolved 10 mg Baclofen of commercial batch **3830**

Unit	Baclofen 10 mg Areas of Batch 3830					
	5 min	10 min	15 min	20 min	25 min	30 min
1	94282	96772	97517	98747	99285	99481
2	95807	97233	97130	98436	99002	99821
3	96053	97795	98004	98818	100411	100344
4	100687	101019	102217	102546	103392	102766
5	97224	101139	100727	103038	103756	103917
6	98939	99253	101743	101859	101843	102320
7	102186	103036	103154	102612	103226	103420
8	106798	108667	108419	108253	107395	108473
9	100510	102883	102077	102812	103093	104358
10	104957	106379	106109	106648	105300	105948
11	100591	102895	103712	104091	102717	104252
12	101505	105351	104944	105377	106240	106472
Mean	**100551**	**102011**	**102147**	**102712**	**103160**	**103669**

Table C4- Areas of dissolved 10 mg Baclofen of commercial batch **200347**

Unit	Baclofen areas 10 mg 200347 Batch					
	5 min	10 min	15 min	20 min	25 min	30 min
1	104316	106398	109170	109950	107035	108490
2	112693	115392	115971	115876	112537	112860
3	112286	114441	115341	114351	112447	112687
4	115048	117867	118887	117826	115192	115950
5	96010	96748	99020	100286	99156	99942
6	113807	116281	116377	116515	113857	114282
7	107567	111740	112190	112217	113594	113420
8	119432	110878	111753	111997	111960	112103
9	108701	111220	111816	111720	111806	111820
10	113834	115992	115922	116501	116344	115383
11	101977	106389	107631	108681	109077	109091
12	110206	112594	112534	113073	112912	112585
Mean	**111246**	**112167**	**112362**	**112645**	**112492**	**112636**

Table C5- Areas of dissolved 10 mg Baclofen of commercial batch **200348**

Unit	Baclofen 10 mg Areas of Batch 200348					
	5 min	**10 min**	**15 min**	**20 min**	**25 min**	**30 min**
1	108096	111297	110693	111057	111055	111549
2	106767	108930	110601	111247	111450	111499
3	107357	108731	109030	109794	109521	110086
4	112213	113267	114051	115107	114447	114060
5	93027	96108	99517	101652	103742	104678
6	108065	107968	108951	108761	110834	110950
7	102987	106224	107613	109033	109771	110168
8	108137	109188	110148	110693	110769	110561
9	109807	111894	112620	114167	114960	115289
10	100078	106561	109406	109352	112058	112892
11	105007	106684	107653	108014	109118	107986
12	112498	113394	114212	114717	116483	115933
Mean	**107711**	**108831**	**109777**	**110244**	**110945**	**111225**

Table C6- Areas of dissolved 10 mg Baclofen of commercial batch **200531**

Unit	Baclofen 10 mg Areas of Batch 200351					
	5 min	**10 min**	**15 min**	**20 min**	**25 min**	**30 min**
1	98813	102206	103431	103678	104506	104520
2	104298	106924	106970	104502	109191	108882
3	100732	102391	102524	104742	104497	104048
4	98835	102505	103313	101535	105421	105801
5	100401	102943	104199	107865	104660	102422
6	95416	99623	100487	103852	102108	105313
7	98943	101103	101646	102506	102781	103608
8	104009	106239	107391	107076	107432	107591
9	98044	100532	101767	102275	102551	103045
10	103098	104448	104871	104817	105104	105880
11	105649	106658	107116	107715	108195	107690
12	105540	106611	106945	107604	108121	108251
Mean	**100567**	**102724**	**103815**	**104622**	**104882**	**105557**

2. **Dissolution Media: Acetate buffer pH 4.5**

Table C7- Areas of dissolved 10 mg Baclofen of commercial batch **3827**

Unit	Baclofen 10 mg Areas of Batch 3827					
	5 min	10 min	15 min	20 min	25 min	30 min
1	46116	49506	51142	51599	52426	53037
2	48618	50682	52975	54209	54479	54629
3	47380	50250	51036	51399	52488	53114
4	51497	52665	53634	54037	54362	54460
5	47917	50633	51488	52103	52970	53028
6	42254	45140	45998	46737	47595	47701
7	51103	53484	54080	54675	55077	55027
8	49246	51060	52122	52223	53050	53416
9	50181	52393	53518	53593	54112	54494
10	52677	53622	54734	54734	55224	54934
11	49910	51152	52190	52519	53579	53896
12	45913	48667	50180	50672	51760	52414
Mean	**48932**	**50871**	**52156**	**52371**	**53315**	**53656**

Table C8- Areas of dissolved 10 mg Baclofen of commercial batch **3828**

Unit	Baclofen 10 mg Areas of Batch 3828					
	5 min	10 min	15 min	20 min	25 min	30 min
1	47966	49647	51095	52177	52966	53776
2	47204	51362	52090	52866	53615	53840
3	48918	51730	52880	53059	53580	54120
4	47790	49729	51144	51413	51975	52320
5	49817	51828	52570	52862	53237	53744
6	46371	49197	49474	50839	51252	51982
7	47198	49196	50216	50974	51805	52942
8	48534	50447	51766	51945	52460	52726
9	49420	51460	52399	53979	53600	54313
10	49390	51530	52555	53077	53143	54067
11	45711	49049	49823	51353	51709	52378
12	44911	47911	49192	49927	50699	51360
Mean	**47878**	**50088**	**51455**	**52061**	**52713**	**53343**

Table C9- Areas of dissolved 10 mg Baclofen of commercial batch **3830**

Unit	Baclofen 10 mg Areas of Batch 3830					
	5 min	10 min	15 min	20 min	25 min	30 min
1	47856	51002	51209	51775	52206	52778
2	49843	51778	52818	53265	53707	54431
3	48882	50281	51303	52170	53215	53390
4	44944	47114	48350	49547	50136	50375
5	45056	48377	49855	51091	52064	52977
6	44841	47297	48945	49808	50191	51394
7	47957	49943	51213	52199	52250	53529
8	47554	50179	50978	51811	52345	52353
9	50818	53110	54292	54357	55303	55386
10	46740	50513	50975	51878	52170	52555
11	47826	49734	51253	51969	52521	52848
12	46182	48742	50077	50552	51490	51652
Mean	**47690**	**50061**	**51094**	**51845**	**52228**	**52813**

Table C10- Areas of dissolved 10 mg Baclofen of commercial batch **200347**

Unit	Baclofen areas 10 mg 200347 Batch					
	5 min	10 min	15 min	20 min	25 min	30 min
1	45487	49459	50943	52061	52342	52777
2	46036	49074	49808	50689	50909	51587
3	45948	48622	49079	50971	51351	51484
4	46059	50764	51414	51931	52459	53285
5	43013	46183	47106	48273	48666	49093
6	44518	48527	49873	51137	51008	51643
7	47745	50443	51404	51791	51795	52407
8	45421	48118	48967	49936	49876	49925
9	45784	48803	50558	51903	51399	52253
10	47133	50133	51005	51440	53521	51575
11	47010	48148	49264	49868	50492	51474
12	46758	48765	50218	51031	51613	51309
Mean	**45992**	**48784**	**50046**	**51084**	**51375**	**51581**

Table C11- Areas of dissolved 10 mg Baclofen of commercial batch **200348**

Unit	Baclofen areas 10mg 200348 Batch					
	5 min	10 min	15 min	20 min	25 min	30 min
1	46255	48712	49187	50084	50091	50484
2	46799	48946	49370	49379	50507	50237
3	46384	48093	48570	49655	50029	50034
4	46526	48440	49310	49691	50283	49806
5	42337	45708	46785	47095	47464	48103
6	47810	49534	51578	51153	50920	51739
7	45374	44048	48076	48409	48735	48683
8	48394	52610	51473	52271	52887	52760
9	47122	49517	50213	50860	51262	51361
10	48374	50847	50882	51988	51808	52623
11	44507	46339	46949	48383	49122	50063
12	47577	50349	51769	52417	52830	47672
Mean	**46663**	**48829**	**49340**	**49888**	**50395**	**50150**

Table C12- Areas of dissolved 10 mg Baclofen of commercial batch **200531**

Unit	Baclofen Tablets 25mg batch 200351 Dissolution Profile					
	5 min	10 min	15 min	20 min	25 min	30 min
1	47700	49015	50019	50830	50766	53200
2	44432	46456	47691	48681	49019	49042
3	46935	48781	48923	49862	50450	51332
4	48731	52947	51408	51765	52365	53555
5	42546	45101	47689	48998	49396	49778
6	45597	47999	48935	49440	49975	49803
7	45191	47105	48777	48511	49243	49843
8	47006	49039	49610	50435	50747	51375
9	49443	51152	51435	51959	51539	55611
10	47595	50101	50324	50694	50445	53509
11	43413	45900	46783	48004	48106	48508
12	46301	48015	49572	52340	50946	54149
Mean	**46618**	**48398**	**49254**	**50149**	**50448**	**51354**

3. **Dissolution Media: Phosphate buffer pH 6.8**

Table C13- Areas of dissolved 10 mg Baclofen of commercial batch **3827**

Unit	Baclofen 10 mg Areas of Batch 3827					
	5 min	10 min	15 min	20 min	25 min	30 min
1	49174	50725	52063	52426	52848	53697
2	47206	49437	49808	50597	51377	51285
3	47830	50806	51844	52678	52676	53229
4	44909	50217	52993	53219	53527	54241
5	45726	51929	53311	53995	53411	53677
6	39562	43834	45415	46684	47613	47977
7	43949	48863	51410	51807	52276	52098
8	45092	47801	48556	49123	49911	50237
9	44815	48383	50263	50558	51818	52195
10	43720	47135	47810	48483	49196	49717
11	41681	49364	52007	52500	53112	53614
12	47929	49753	50722	51522	52394	52993
Mean	**45001**	**49401**	**51066**	**51665**	**52335**	**52594**

Table C14- Areas of dissolved 10 mg Baclofen of commercial batch **3828**

Unit	Baclofen 10 mg Areas of Batch 3828					
	5 min	10 min	15 min	20 min	25 min	30 min
1	46602	52395	54087	53884	54017	53537
2	40451	46953	49406	50758	51390	52753
3	41826	45268	46657	47064	47751	48363
4	34338	44748	48770	50378	51461	51666
5	51179	53687	54491	54830	54773	55191
6	44539	47292	48308	50073	49479	50757
7	37254	44683	48695	50668	51361	52130
8	41549	46408	48642	49422	50181	50644
9	45178	51376	52537	52984	54227	53880
10	45073	51258	52032	52536	52572	52509
11	40449	52971	53608	54107	54028	54340
12	52554	45267	48824	50736	51809	52687
Mean	**43183**	**47123**	**49115**	**50747**	**51635**	**52598**

Table C15- Areas of dissolved 10 mg Baclofen of commercial batch **3830**

Unit	Baclofen 10 mg Areas of Batch 3830					
	5 min	10 min	15 min	20 min	25 min	30 min
1	45310	51633	53289	45125	54229	54028
2	47225	48945	49500	53827	50463	51212
3	46350	52358	54233	50122	55441	46595
4	45354	51500	55733	54517	54661	54850
5	48036	52530	53537	54446	55589	56063
6	31443	41236	54130	55205	47288	48742
7	47843	49633	50439	50527	52080	51965
8	47762	51232	51698	52589	52425	53058
9	47071	49033	49661	50048	50581	50855
10	50541	51874	52785	52676	53544	53940
11	47272	48206	49265	50458	50933	51250
12	45241	48259	49317	49991	50521	51018
Mean	**47148**	**50433**	**52242**	**51558**	**52253**	**51608**

Table C16- Areas of dissolved 10 mg Baclofen of commercial batch **200347**

Unit	Baclofen areas 10 mg 200347 Batch					
	5 min	10 min	15 min	20 min	25 min	30 min
1	32153	41509	45725	47695	50031	50305
2	44493	48260	50215	50005	51236	50466
3	43153	47678	49451	50750	51152	52163
4	33790	42967	44769	46924	47946	48462
5	47520	50719	51622	51851	52330	52311
6	39740	42461	43978	44415	45463	45669
7	44548	49316	51287	52241	51416	51229
8	41863	46897	48505	49922	50822	51335
9	30280	48378	51357	51886	51631	52548
10	21714	40782	45072	48737	50997	51747
11	43438	49404	50976	52156	53065	53201
12	39855	44796	46784	48262	49034	49717
Mean	**40859**	**47288**	**48978**	**49964**	**51075**	**51282**

Table C17- Areas of dissolved 10 mg Baclofen of commercial batch **200348**

Unit	Baclofen areas 10mg 200348 Batch					
	5 min	**10 min**	**15 min**	**20 min**	**25 min**	**30 min**
1	44722	47084	48422	49620	49487	50078
2	46336	49056	50099	50768	50986	51385
3	44766	46582	47277	48503	48642	49229
4	35898	46688	48558	48986	49248	50066
5	44006	46258	49372	49665	50512	50362
6	45270	48092	49046	50092	50353	50786
7	40188	42884	44390	45299	45925	47044
8	43387	45702	47178	48468	48772	48573
9	46123	48482	47768	49126	49172	50055
10	37612	42403	43298	44489	45595	44954
11	29327	48410	52551	52960	53546	53351
12	36551	41414	42912	43947	44362	45520
Mean	**43697**	**46635**	**48095**	**49056**	**49210**	**50061**

Table C18- Areas of dissolved 10 mg Baclofen of commercial batch **200531**

Unit	Baclofen Tablets 25mg batch 200351 Dissolution Profile					
	5 min	**10 min**	**15 min**	**20 min**	**25 min**	**30 min**
1	42385	46925	48481	49539	49820	50783
2	41092	45094	46755	47452	48694	48930
3	32115	42644	46469	47737	48941	49578
4	48296	50723	51575	52774	52762	53184
5	38351	45886	48175	50885	50879	50745
6	41946	44838	47129	47900	48131	49616
7	45110	47630	49074	50178	50797	50971
8	44949	48660	50074	51330	51508	51254
9	48221	43357	53873	53674	53744	53925
10	35013	44400	47708	49287	50213	50105
11	34618	46178	50765	52592	53361	54554
12	37916	42338	43552	45435	46751	48188
Mean	**41519**	**45490**	**48328**	**49859**	**50505**	**50764**

Appendix D - Areas of Dissolved 25 mg Baclofen through multiple sampling points

1. **Dissolution Media: HCl 0.1N**

Table D1- Areas of dissolved 25 mg Baclofen of commercial batch **3751.**

Unit	Baclofen 25mg Areas of batch 3751					
	5 min	10 min	15 min	20 min	25 min	30 min
1	219292	228860	231897	233576	235143	237921
2	216114	225659	230066	232382	235501	236196
3	227121	236771	241136	241836	246004	243727
4	166683	187933	194301	201191	205614	206623
5	214942	222443	224366	229806	231447	233330
6	189630	200402	205624	209855	214382	216465
7	97952	104294	107330	108424	109969	112248
8	116695	120016	120016	123320	123635	124009
9	113343	117274	118440	119536	120400	121245
10	109727	114829	116323	117641	116884	118866
11	106830	112137	114354	115940	115884	118129
12	109677	112330	115525	117009	116786	119614
Mean	**141689**	**153975**	**157159**	**162256**	**164625**	**165316**

Table D2- Areas of dissolved 25 mg Baclofen of commercial batch **3843.**

Unit	Baclofen 25mg Areas of 3843 batch					
	5 min	10 min	15 min	20 min	25 min	30 min
1	227964	233906	237982	241698	241192	242550
2	220500	233779	239409	244232	243706	246447
3	239145	236557	239397	240328	242666	242752
4	229889	236095	240422	240915	242322	245254
5	204930	215718	221163	224485	228240	233137
6	226594	233577	239795	241678	248324	240284
7	103292	109161	111340	112959	114680	110539
8	111568	115435	116737	117241	118784	107070
9	106615	110649	113176	115633	117227	108742
10	111779	113825	115879	116567	99303	103049
11	111018	115244	118569	119061	115996	118809
12	102783	106442	108653	111158	111653	116027
Mean	**158355**	**165577**	**169866**	**171773**	**173512**	**175973**

Table D3- Areas of dissolved 25 mg Baclofen of commercial batch **3861.**

Unit	Baclofen 25mg Areas of 3861 batch					
	5 min	**10 min**	**15 min**	**20 min**	**25 min**	**30 min**
1	241977	245669	248573	253035	254318	255687
2	231158	241448	246365	250282	251375	251801
3	199916	216537	221561	228544	233569	236715
4	228816	235854	242037	245668	249335	248142
5	231372	240178	245183	246365	250374	251856
6	232316	238734	243798	248617	250116	251975
7	114617	116129	118530	119189	119683	119731
8	95003	101776	104331	108027	109947	110958
9	113143	115885	116891	117629	119132	119598
10	108569	115832	115548	117624	117863	117696
11	93875	98228	102647	104811	106107	107069
12	98030	101355	103226	103933	105802	107395
Mean	**157267**	**166333**	**170046**	**173867**	**176626**	**178223**

Table D4- Areas of dissolved 25 mg Baclofen of commercial batch **200349.**

Unit	Baclofen 25mg Areas of 200349 batch					
	5 min	**10 min**	**15 min**	**20 min**	**25 min**	**30 min**
1	260449	268222	274482	274857	278356	277537
2	248889	259336	265257	268489	269447	273363
3	274390	280625	282445	284529	285959	286135
4	248925	258484	264232	268773	271202	271164
5	256237	265957	269292	270765	274225	275188
6	243013	251264	257745	262086	264734	266138
7	260398	266779	270546	270915	273739	271499
8	243175	253561	259113	261794	263164	265455
9	250429	260379	261595	267695	266124	266539
10	253902	256762	261820	264139	263666	265082
11	274469	277788	281971	279697	280372	278233
12	270432	273543	276206	276008	276364	278692
Mean	**255070**	**263168**	**267275**	**269769**	**272471**	**272431**

Table D5- Areas of dissolved 25 mg Baclofen of commercial batch **200350.**

Unit	Baclofen 25mg Areas of 200350 batch					
	5 min	**10 min**	**15 min**	**20 min**	**25 min**	**30 min**
1	251738	257436	259741	260388	262251	263418
2	232044	240444	246313	248469	251939	254640
3	248210	253950	257765	260107	261126	262726
4	255850	263173	266321	267358	267665	267865
5	231824	239810	243956	249495	252151	254420
6	243230	251728	257194	259944	263313	265993
7	231400	241124	246469	249952	252791	254196
8	251281	255802	260048	260638	261635	261778
9	238620	243805	249800	252118	254814	255919
10	253384	260266	261651	264903	266302	267075
11	254663	262625	266722	268653	271676	271408
12	231440	242196	244911	251150	252219	256542
Mean	**245720**	**252839**	**257480**	**260026**	**261381**	**262252**

Table D6- Areas of dissolved 25 mg Baclofen of commercial batch **200532.**

Unit	Baclofen 25mg Areas of 200532 batch					
	5 min	**10 min**	**15 min**	**20 min**	**25 min**	**30 min**
1	211987	228682	233547	238267	241742	246426
2	207387	217120	225543	227682	232797	236623
3	238964	248581	249085	252835	255271	256612
4	238090	250626	251722	255497	258851	257199
5	249595	255778	257963	258674	262599	262983
6	244368	251601	253645	257589	258737	259538
7	244577	253709	256132	259759	258507	261923
8	181828	205284	214198	218917	222981	227722
9	243660	249693	251573	254835	258004	258390
10	240670	250405	255042	256480	257554	261123
11	240303	247270	251838	254665	257746	258478
12	239547	246141	249481	252725	253949	256552
Mean	**239925**	**249137**	**251648**	**254750**	**257650**	**257795**

2. **Dissolution Media: Acetate buffer pH 4.5**

Table D7- Areas of dissolved 25 mg Baclofen of commercial batch **3751.**

Unit	Baclofen 25mg Areas of 3751 batch					
	5 min	10 min	15 min	20 min	25 min	30 min
1	116058	119430	121699	122898	125104	126791
2	119346	125422	128257	129051	130196	133026
3	106681	116287	123037	127659	130102	132846
4	100224	107351	108640	111616	112284	115092
5	102340	112894	118256	121107	124385	126712
6	98328	105505	110793	114646	119294	121016
7	107018	112532	116204	118314	120136	121731
8	100511	110307	113413	115333	117091	119120
9	100343	108379	113208	116032	117673	119303
10	114315	119292	122494	123203	125412	128552
11	108120	116773	123093	126845	128132	130955
12	97217	104211	107800	110132	113283	114857
Mean	**104511**	**112713**	**117230**	**119711**	**122261**	**124222**

Table D8- Areas of dissolved 25 mg Baclofen of commercial batch **3843.**

Unit	Baclofen 25mg Areas of 3843 batch					
	5 min	10 min	15 min	20 min	25 min	30 min
1	116677	121548	123431	125594	127123	127283
2	103381	112498	116057	118760	120921	122957
3	100262	112982	116985	119559	122511	122782
4	114297	120441	122545	122916	125391	126450
5	116615	121526	123197	125430	126702	128061
6	108446	113463	116225	118678	120639	122546
7	123113	128406	130619	133099	133599	134650
8	107141	115077	117521	119891	120168	122300
9	94822	105911	110132	112632	114038	117062
10	94183	101339	105219	106906	109686	111114
11	98718	109923	115765	117954	121288	123569
12	91951	100611	105022	105666	108357	110582
Mean	**105261**	**113223**	**116605**	**119160**	**121105**	**122870**

Table D9- Areas of dissolved 25 mg Baclofen of commercial batch **3861.**

Unit	Baclofen 25mg Areas of 3861 batch					
	5 min	**10 min**	**15 min**	**20 min**	**25 min**	**30 min**
1	118516	121916	126018	127111	129616	130093
2	102623	110495	113905	116976	118717	119890
3	114869	122855	125633	127623	130291	132065
4	119585	123465	126491	128704	129013	130932
5	99109	106447	110081	114322	119203	120365
6	110891	115379	118879	120897	122873	124759
7	113062	117251	120314	123493	125141	128331
8	112810	118631	121488	123074	124999	125527
9	121885	126687	128671	130272	132409	133117
10	103368	113064	115207	118226	120857	123105
11	111850	122302	126147	129406	130403	132384
12	109878	114227	116935	119378	121511	123393
Mean	**112330**	**117941**	**120901**	**123284**	**125070**	**126929**

Table D10- Areas of dissolved 25 mg Baclofen of commercial batch **200349.**

Unit	Baclofen 25mg Areas of 200349 batch					
	5 min	**10 min**	**15 min**	**20 min**	**25 min**	**30 min**
1	108185	115832	119975	122898	124738	126505
2	120537	127881	130967	131640	132999	134781
3	114068	121791	123837	126177	127500	129064
4	118144	122737	125838	127563	129192	131488
5	117437	123339	126575	129636	131025	132245
6	114898	120257	124035	125287	127782	128609
7	117555	122779	125634	127104	128651	130189
8	117589	125560	127891	129633	131638	133112
9	122654	126715	129719	131733	132581	134214
10	113751	119602	122158	124711	126204	127959
11	126569	131792	133740	135866	137176	137936
12	112310	116473	119426	121128	123255	124217
Mean	**117496**	**122758**	**125736**	**127334**	**128922**	**130839**

Table D11- Areas of dissolved 25 mg Baclofen of commercial batch **200350.**

Unit	Baclofen 25mg Areas of 200350 batch					
	5 min	**10 min**	**15 min**	**20 min**	**25 min**	**30 min**
1	104921	112878	114728	116653	118246	119393
2	110506	115597	118797	120128	121792	121841
3	119618	125132	127284	129798	131743	132662
4	112595	117152	120243	122786	123546	125916
5	106305	113291	118268	121796	123549	125882
6	109478	115474	118361	120086	120832	123535
7	115346	119227	122925	124237	125271	127066
8	113937	119284	121192	124215	125535	125769
9	115628	120131	122294	124585	125989	126976
10	104746	111949	114773	117003	117879	120462
11	107672	113340	118055	120225	121510	124334
12	103213	108180	111524	113740	116025	116753
Mean	**109992**	**115536**	**118579**	**121011**	**122669**	**125052**

Table D12- Areas of dissolved 25 mg Baclofen of commercial batch **200532.**

Unit	Baclofen 25mg Areas of 200532 batch					
	5 min	**10 min**	**15 min**	**20 min**	**25 min**	**30 min**
1	116838	121751	124328	126074	128518	128533
2	115852	119449	122125	124241	124310	125668
3	111956	116261	119144	122556	123454	125413
4	118686	123786	126384	128540	130468	131455
5	102606	110499	113503	115778	116796	117747
6	96802	102163	105367	107603	109328	111429
7	117961	120765	124759	126450	127648	129243
8	115470	121204	121709	124812	126077	128038
9	119502	124786	126948	128085	129811	130999
10	103360	108009	111497	114569	114971	116611
11	111506	116990	119360	120984	123427	124233
12	112827	117684	120443	122650	124367	126574
Mean	**114149**	**118567**	**121076**	**123446**	**124339**	**126121**

3. **Dissolution Media: Phosphate buffer pH6.8**

Table D13- Areas of dissolved 25 mg Baclofen of commercial batch **3751.**

Unit	Baclofen 25 mg Areas of Batch 3751					
	5 min	10 min	15 min	20 min	25 min	30 min
1	104271	113839	116893	119652	121372	123883
2	112876	117310	119722	121570	123768	124420
3	112346	116737	119305	120483	122132	123832
4	114131	120819	122550	124150	126571	127172
5	113576	119424	122421	124466	125545	126846
6	109569	115471	117533	120267	121568	122568
7	109080	118625	121986	125750	126033	126728
8	107387	112614	115144	117500	119837	119513
9	96727	110783	117645	119799	120222	121707
10	107826	116498	119491	122407	123227	124220
11	93307	99524	103715	107963	111093	113679
12	112848	116819	121116	121206	122676	124668
Mean	**109325**	**116618**	**119398**	**120845**	**122404**	**124052**

Table D14- Areas of dissolved 25 mg Baclofen of commercial batch **3843.**

Unit	Baclofen 25 mg Areas of Batch 3843					
	5 min	10 min	15 min	20 min	25 min	30 min
1	106778	114471	116381	118498	121576	122108
2	114492	119005	120731	121640	122862	124334
3	121058	126483	127482	128658	129590	130925
4	104414	113677	115813	117806	119687	121044
5	117631	120116	122399	124238	125380	126217
6	115020	118365	120564	121887	123076	124571
7	107012	119380	127604	129593	131983	133812
8	117812	123396	125167	126868	127229	128369
9	110419	119205	122758	124318	127186	128909
10	105023	110494	113598	116336	116618	117965
11	108043	113336	116171	118212	121536	122884
12	110615	114309	117296	119295	121817	124706
Mean	**110517**	**118685**	**120648**	**121764**	**122969**	**124639**

Table D15- Areas of dissolved 25 mg Baclofen of commercial batch **3861.**

Unit	Baclofen 25 mg Areas of Batch 3861					
	5 min	10 min	15 min	20 min	25 min	30 min
1	116926	121720	122705	124478	125729	126704
2	118262	123424	126632	127069	128059	128826
3	100485	106548	107982	109610	112513	113631
4	103208	111130	113863	115828	116792	117165
5	96597	108418	111552	119542	117856	119937
6	116469	119506	122481	124147	126074	127421
7	121579	126269	128807	130355	131336	133493
8	110193	115041	117863	119351	120688	121609
9	117869	124253	124984	125910	127704	128077
10	114696	121548	122882	124292	125218	125678
11	107397	114469	119881	123301	125342	127643
12	106905	114032	117760	119881	121296	123337
Mean	**112445**	**117274**	**121181**	**123724**	**125280**	**126191**

Table D16- Areas of dissolved 25 mg Baclofen of commercial batch **200349.**

Unit	Baclofen 25 mg Areas of Batch 200349					
	5 min	10 min	15 min	20 min	25 min	30 min
1	113434	117858	122006	126664	127129	129744
2	120517	124824	127125	128968	127989	130017
3	106802	113835	116495	118574	122065	121864
4	114149	117545	121445	122170	124093	125099
5	106995	111431	115314	116996	118391	120434
6	107291	112301	112744	114493	116029	116227
7	104946	115673	119002	123225	126120	128120
8	116682	122594	124810	125687	125856	128004
9	112906	117103	119041	119612	119959	121823
10	116538	118526	120676	121224	123942	125341
11	114486	117840	122146	123934	125379	126943
12	107548	111195	114831	117067	116052	118262
Mean	**113170**	**117324**	**119859**	**121697**	**124018**	**125220**

Table D17- Areas of dissolved 25 mg Baclofen of commercial batch **200350.**

Unit	Baclofen 25 mg Areas of Batch 200350					
	5 min	**10 min**	**15 min**	**20 min**	**25 min**	**30 min**
1	97967	104761	107557	110635	113143	113454
2	108725	115180	117342	119094	120183	121635
3	112885	117845	120210	122485	122722	125309
4	120959	125086	126857	130627	132243	131053
5	113812	117150	118428	123293	122662	125669
6	113450	121595	120690	117186	124403	125868
7	106380	115285	119460	124233	126720	128534
8	122734	128936	130599	131553	131968	133288
9	113455	118567	119549	121484	123850	124839
10	106697	112123	118602	110737	117321	118568
11	111083	117061	113411	120718	122379	124299
12	116175	120794	123327	125314	126803	128739
Mean	**113168**	**117498**	**119505**	**121985**	**123286**	**125489**

Table D18- Areas of dissolved 25 mg Baclofen of commercial batch **200532.**

Unit	Baclofen 25 mg Areas of Batch 200532					
	5 min	**10 min**	**15 min**	**20 min**	**25 min**	**30 min**
1	116023	120027	123118	125981	127574	129617
2	105921	113724	116754	121894	120527	123096
3	114484	119816	123068	122403	125145	126643
4	120388	127449	130070	132176	133777	136253
5	95914	103830	105100	109134	110271	110445
6	105816	111082	112340	113284	115337	116653
7	107368	111528	114770	117687	119151	119868
8	113629	118754	120981	123379	124755	126183
9	118486	122907	125077	126259	128300	129007
10	112233	116782	119470	121392	123146	124160
11	105976	110755	113883	115534	117758	118679
12	100932	106089	109580	110981	112052	114120
Mean	**109801**	**115253**	**118112**	**121643**	**121837**	**123628**