Sudan University of Science and Technology

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Stability Evaluation of Ciprofloxacin Drug
تقييم ثبات دواء السبروفلوركساسين

A Thesis Submitted in Partial Fulfillment for the Requirement of the Degree Master of Science in Chemistry

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قال تعالى:

(وَمَا أُوتِيتُمْ مِنَ الْعِلْمِ إِلَّا قَلِيلا)

الاسراء (85)

Dedication

I dedicate this work to my;

Parents, husband, brother and sister.

الاهداء

Acknowledgment

I would like to thank Allah, who gives me strength and good health while doing this work, and hope to give me more to be continuing in my way.

Thanks for my subjected teacher Dr. Mai Makki Mahmoud, who never failed to teach and guide me.

Special thanks to all staff at Shanghai, and Blue Nile companies.

Abstract:

This study carried out the assay of the active material in ciprofloxacin drug with Shanghai and Blue Nile companies were found to be 96.8% and 100.56%, respectively.

In this work the dissolution testing achieved for six samples of ciprofloxacin drug (1) (2) (3) (4) (5) (6) for two different companies (Shanghai and Blue Nile), the percentage of dissolved of ciprofloxacin in water are (96.6, 96, 100.3, 95.7, 96.9, 95.9) % respectively for Shanghai company and (97.2, 97.3, 99.8, 95.7, 97.5, 96.5) % respectively for Blue Nile company.

And some physical properties tests were achieved. The hardness, thickness, disintegration, weight variation, and friability, for Shanghai company 19.7kgf, 6.18 mm, 2:56 minute, (721.74g – 720.25g), and 0.1% respectively, and for Blue Nile company are 17.2kgf, , 4.7 mm, and 2:23 minute, (721.74g – 720.25g), and 0.98% respectively.

المستخلص:

هذه الدراسة توضح النسبة المئوية للمادة الفعالة في Ciprofloxacin drug في شركتي شنغهاي والنيل الازرق وكانت النتيجة 80، 96%، 100.56% على التوالي.

في هذا العمل تم إجراء اختبار الذوبانية لستة عينات من Ciprofloxacin (1,2,3,4,5,6 drug,) وكانت النسب المئوية لازابة هذا الدواء في الماء هي:

(96.6, 96.0, 100.3, 95.7, 96.9, 95.9)%على التوالي لشركة شنغهاي و (97.20, 97.3, 99.8, 95.7 97.5 96.5) %. لشركة النيل الازرق على التوالي.

أجريت الخواص الفيزيائية لهذا الدواء وهي تباين الوزن, الصلابة, الهشاشة, الانحلال, السماكة وكانت النتيجة (720.25-721.74 g), 19.7kgf, 0.1%, 2:56 minutes, 6.18mm, على التوالي لشركة شنغهاي و (720.27g,-721.74 17.2kgf, 0.98%, 2:23minutes, 4.7mm, على التوالي لشركة النيل الأزرق.

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Chapter One
Introduction

1. Introduction:

Stability testing of pharmaceutical products is a complex set of procedures involving considerable cost, time consumption and scientific expertise in order to build in quality, efficacy and safety in a drug formulation. Scientific and commercial success of a pharmaceutical product can only be ensured with the understanding of the drug development process and the myriad tasks and mile stones that are vital to a comprehensive development plan. The most important steps during the developmental stages include pharmaceutical analysis and stability studies that are required to determine and assure the identity, potency and purity of ingredients, as well as those of the formulated products (Singh et al., 2000). Stability of a pharmaceutical product may be defined as the capability of a particular formulation in a specific container/closure system to remain within its physical, chemical, microbiological, toxicological, protective and informational specifications (Kommanaboyina *et al*., 1999). In other words, it is the extent to which a product retains, within the specified limits, throughout its period of storage and use, the same properties and characteristics possessed at the time of its packaging. Stability testing thus evaluates the effect of environmental factors on the quality of the a drug substance or a formulated product which is utilized for prediction of its shelf life, determine proper storage conditions and suggest labeling instructions. Moreover, the data generated during the stability testing is an important requirement for regulatory approval of any drug or formulation (Singh et al., 2000).

Stability testing is termed as a complex process because of involvement of a variety of factors influencing the stability of a pharmaceutical product. These factors include stability of the active ingredient(s); interaction between active ingredients and excipients, manufacturing process followed, type of dosage form, container/closure system used for packaging and light, heat and moisture conditions encountered during shipment, storage and handling. In addition,

degradation reactions like oxidation, reduction, hydrolysis or racemization, which can play vital role in stability of a pharmaceutical product, also depend on such conditions like concentration of reactants, pH, radiation, catalysts etc., as well as the raw materials used and the length of time between manufacture and usage of the product. A pharmaceutical product may undergo change in appearance, consistency, content uniformity, clarity (solution), moisture contents, particle size and shape, pH, package integrity thereby affecting its stability. Such physical changes may be because of impact, vibration, abrasion, and temperature fluctuations such as freezing, thawing or shearing etc. The chemical reactions like solvolysis, oxidation, reduction, racemization etc. that occur in the pharmaceutical products may lead to the formation of degradation product, loss of potency of active pharmaceutical ingredient (API), loss of excipient activity like antimicrobial preservative action and antioxidants etc. (Carstensen *et al*., 2000). Stability of a pharmaceutical product can also be affected because of microbiological changes like growth of microorganisms in non sterile products and changes in preservative efficacy (Matthews et al., 1999). Potential adverse effects of instability in pharmaceutical products have been given in Table 1.

Table (1.1): Potential Adverse Effects of Instability in Pharmaceutical Products: (Carstensen *et al*, 2000)

Potential Adverse Effect	**Explanation/ Reason**	**Example**	**Stability Parameter Tested**
Loss of Active Ingredient	Degradation of API in product resulting in less than 90% drug as claimed on label - unacceptable quality	Nitroglycerine tablets	Time elapsed before the drug content no longer exceeds 90%
Increase in concentration of active Ingredient	Loss of vehicle perfusion bags sometimes allow solvent to escape and evaporate so that the product within the bag shows an increase in concentration.	Lidocaine gel, products in perfusion bags	Stability in final container
Alteration in	Changes in rate and extent of		Dissolution/releas

bioavailability	absorption on Storage		e studies
Loss of content uniformity	Loss of contents as a function of time	Suspension	Ease of re-dispersion or sedimentation Volume
Decline of microbiological status	Increase in number of viable microorganisms already present in the product. Contamination because of compromised package integrity during distribution/ storage	Multiuse cream	Total bioburden after storage
Loss of pharmaceutical elegance and patient acceptability	Speckling caused by the interaction of the drug containing amine group with a minor component in the lactose resulting in the formation of a chromatophore	Slight yellow or brown speckling on the surface of tablet containing spray-dried lactose	Visual Examination
Formation of toxic degradation products	Degradation of the drug component	Formation of epianhydrotetracycline from tetracycline, Protein drugs	Amount of degradation products during shelf life
Loss of package integrity	Change in package integrity during storage or distribution	Plastic screw cap losing back-off-torque	Specific package integrity tests
Reduction of label quality	Deterioration of label with time and cause the ink to run and thus adversely affect legibility	Plasticizer from plastic bottle migrates into the label	Visual examination of the label
Modification of any factor of functional relevance	Time-dependent change of any functionally relevant attribute of a drug product that adversely affects safety, efficacy, or patient acceptability or ease of use	Adhesion ageing of transdermal patches	Monitoring changes

1.1 Importance of Stability Testing

The primary reason for stability testing is the concern for the well-being of the patient suffering from the disease for which the products is designed. Apart from degradation of the unstable product into toxic decomposition products, loss of activity up to a level of 85% of that claimed on the label may lead to failure of the therapy resulting in death e.g. nitroglycerine tablets for angina and cardiac arrest.

Because of this concern, it has become a legal requirement to provide data for certain types of stability tests for the regulatory agencies before approval of a new product. Second important concern is to protect the reputation of the manufacturer by assuring that the product will retain fitness for use with respect to all functionally relevant attributes for as long as they are on the market. Other benefits of stability studies at the developmental stage or of the marketed products are to provide a database that may be of value in selection of adequate formulations, percipients and container closure systems for development of a new product, to determine shelf life and storage conditions for development of a new product, preparation of registration dossier, to substantiate the claimed shelf life for the registration dossier and to verify that no changes have been introduced in the formulation or manufacturing process that can adversely affect the stability of the product. (Singh *et al*, 2000; Carstensen *et al*, 2000)

1.2 Stability Testing Methods

Stability testing is a routine procedure performed on drug substances and products and is employed at various stages of the product development. In early stages, accelerated stability testing (at relatively high temperatures and/or humidity) is used in order to determine the type of degradation products which may be found after long-term storage. Testing under less rigorous conditions i.e. those recommended for long-term shelf storage, at slightly elevated temperatures is used to determine a product's shelf life and expiration dates. The major aim of pharmaceutical stability testing is to provide reasonable assurance that the products will remain at an acceptable level of fitness/quality throughout the period during which they are in market place available for supply to the patients and will be fit for their consumption until the patient uses the last unit of the product (Kommanaboyina *et al*, 1999).

1.3 Real-Time Stability Testing

Real-time stability testing is normally performed for longer duration of the test period in order to allow significant product degradation under recommended storage conditions. The period of the test depends upon the stability of the product which should be long enough to indicate clearly that no measurable degradation occurs and must permit one to distinguish degradation from inter-assay variation. During the testing, data is collected at an appropriate frequency such that a trend analysis is able to distinguish instability from day-to-day ambiguity. The reliability of data interpretation can be increased by including a single batch of reference material for which stability characteristics have already been established. Stability of the reference material also includes the stability of reagents as well as consistency of the performance of the instrument to be used throughout the period of stability testing. However, system performance and control for drift and discontinuity resulting from changes in both reagents and instrumentation must be monitored (Anderson *e tal,* 1991).

1.4 Accelerated Stability Testing

In accelerated stability testing, a product is stressed at several high (warmer than ambient) temperatures and the amount of heat input required to cause product failure is determined. This is done to subject the product to a condition that accelerates degradation. This information is then projected to predict shelf life or used to compare the relative stability of alternative formulations. This usually provides an early indication of the product shelf life and thus shortening the development schedule. In addition to temperature, stress conditions applied during accelerated stability testing are moisture, light, agitation, gravity, pH and package (Kommanaboyina *et al,* 1999). In accelerated stability testing the samples are subjected to stress, refrigerated after stressing, and hen assayed simultaneously. Because the duration of the analysis is short, the likelihood of instability in the measurement system is reduced in comparison to the real-time stability testing. Further, in accelerated stability testing, comparison of the unstressed product with

stressed material is made within the same assay and the stressed sample recovery is expressed as percent of unstressed sample recovery. For statistical reasons, the treatment in accelerated stability projections is recommended to be conducted at four different stress temperatures. However, for thermolabile and proteinaceous components, relatively accurate stability projections are obtained when denaturing stress temperatures are avoided (Anderson *et al.*, 1991). The concept of accelerated stability testing is based upon the Arrhenius equation (1) and modified Arrhenius equation (Anderson *et al.*, 1991), (Connors *et al.*, 1973) (2):

Where K = degradation rate/s, A = frequency factor/s, ΔE = activation energy (kJ/mol), R = universal gas constant (0.00831 kJ/mol), T=absolute temperature

$$\log\left(\frac{k_2}{k_1}\right) = \frac{-E_a}{2.303R}\left(\frac{1}{T_2} \quad \frac{1}{T_1}\right) \qquad (2)$$

Where *K1* and *K2* are rate constants at temperatures *T*1 and *T*2 expressed in degree kelvins; *Ea*is the activation energy; R is the gas constant.

These equations describe the relationship between storage temperatures and degradation rate. Using Arrhenius equation, projection of stability from the degradation rates observed at high temperatures for some degradation processes can be determined. When the activation energy is known, the degradation rate at low temperatures may be projected from those observed at “stress” temperatures (Connors *et al.*, 1973; Lachman *etal.*, 1976; Bott *etal.*, 2007). The stress tests used in the current International Conference on Harmonization (ICH) guideline (e.g., 40% for products to be stored at controlled room temperature) were developed from a model that assumes energy of activation of about 83 kJ per mole (Anderson *et al.*, 1991). A common practice of manufacturers in pharmaceutical industries

was to utilize various shortcuts such as *Q* rule and bracket tables for prediction of shelf life of the products but these methods are not official either in ICH or FDA. The *Q* rule states that a product degradation rate decreases by a constant factor *Q*10 when the storage temperature is decreased by 10°C. The value of *Q*10 is typically set at 2, 3 or 4 because these correspond to reasonable activation energies. This model falsely assumes that the value of Q does not vary with temperature. The bracket table technique assumes that, for a given analytic, the activation energy is between two limits (e.g., between 10 and 20 kcal). As a result, a table may be constructed showing days of stress at various stress temperatures. The use of a 10 to 20 kcal bracket table is reasonable because broad experience indicates that most analytic and reagents of interest in pharmaceutical and clinical laboratories have activation energies in this range (Kommanaboyina *et al.,* 1999; Anderson *et al.,* 1991).

1.5 Retained Sample Stability Testing

This is a usual practice for every marketed product for which stability data are required. In this study, stability samples, for retained storage for at least one batch a year are selected. If the number of batches marketed exceeds 50, stability samples from two batches are recommended to be taken. At the time of first introduction of the product in the market, the stability samples of every batch may be taken, which may be decreased to only 2% to 5% of marketed batches at a later stage. In this study, the stability samples are tested at predetermined intervals i.e. if a product has shelf life of 5 years, it is conventional to test samples at 3, 6, 9, 12,18, 24, 36, 48, and 60 months. This conventional method of obtaining stability data on retained storage samples is known as constant interval method (Kommanaboyina *et al.,* 1999; Carstensen *et al.,* 1993). Stability testing by evaluation of market samples is a modified method which involves taking samples

already in the market place and evaluating stability attributes. This type of testing is inherently more realistic since it challenges the product not just in the idealized retained sample storage conditions, but also in the actual marketplace, (1999).

1.6 Cyclic Temperature Stress Testing

This is not a routine testing method for marketed products. In this method, cyclic temperature stress tests are designed on knowledge of the product so as to mimic likely conditions in market place storage. The period of cycle mostly considered is 24 hours since the diurnal rhythm on earth is 24 hour, which the marketed pharmaceuticals are most likely to experience during storage. The minimum and maximum temperatures for the cyclic stress testing is recommended to be selected on a product by-product basis and considering factors like recommended storage temperatures for the product and specific chemical and physical degradation properties of the products. It is also recommended that the test should normally have 20 cycles (Kommanaboyina *et al.,* 1999; Carstensen *et al.,* 2000).

1.7 Guidelines for Stability Testing

To assure that optimally stable molecules and products are manufactured, distributed and given to the patients, the regulatory authorities in several countries have made provisions in the drug regulations for the submission of stability data by the manufacturers. Its basic purpose was to bring in uniformity in testing from manufacturer to manufacturer. These guidelines include basic issues related to stability, the stability data requirements for application dossier and the steps for their execution. Such guidelines were initially issued in 1980s. These were later harmonized (made uniform) in the International Conference on Harmonization (ICH) in order to overcome the bottleneck to market and register the products in other countries.

The ICH was a consortium formed with inputs from both regulatory and industry from European commission, Japan and USA. The World Health Organization (WHO), in 1996, modified the guidelines because the ICH guidelines did not

address the extreme climatic conditions found in many countries and it only covered new drug substances and products and not the already established products that were in circulation in the WHO umbrella countries. In June 1997, US FDA also issued a guidance document entitled ‘Expiration dating of solid oral dosage form containing Iron. WHO, in 2004, also released guidelines for stability studies in global environment (WHO, 2004). ICH guidelines were also extended later for veterinary products. A technical monograph on stability testing of drug substances and products existing in India has also been released by India Drug Manufacturers Association (Singh *et al,* 2000). Further, different test condition and requirements have been given in the guidance documents for active pharmaceutical ingredients, drug products or formulations and percipients. The codes and titles covered under ICH guidance have been outlined in the Table 2.

Table: (1.2) Codes and titles used in ICH Guidelines: (Singh *et al*, 2000)

ICH Code	Guide line title
Q1A	Stability testing of new Drugs Substances and Products (Second Revisions)
Q1B	Stability testing: Photo stability testing of new Drugs Substances and Products
Q1C	Stability testing of new Dosage Forms
Q1D	Bracketing and Matrixing Designs for stability testing of Drugs Substances and Products
Q1E	Evaluation of stability data
Q1F	Stability data package for Registration Applications in Climatic Zones III and IV
Q5C	Stability testing of Biotechnological/Biological Products

Series of guidelines related to stability testing have also been issued by the Committee for Proprietary Medicinal Products (CPMP) under the European Agency for the Evaluation of Medicinal Products (EMEA) to assist those seeking marketing authorization for medicinal products in European Union. These relisted in Table 3.

Table. (1.3) CPMP Gudelines for Stability:
(Singh *et al*,2000: CPMP/QWP/122/02,2003)

CPMP Code	Guide Line Title
CPMP/QWP/ 576/96 Rve. 1	Guide line on Stability Testing for Applications for Variations to a Marketing Authoritation
CPMP/QWP/ 6142/03	Guide line on Stability Testing for Active Subustances and Medicinal Products Manufactured in Climatic Zones III and IV to be marketed in the EU
CPMP/QWP/ 609/96 Rev.1	Note for Guidance Declaration of Storage Conditions for Medicinals Products Particulars and Active Substances.
CPMP/QWP/ 122/02 Rev.1	Note for Guidance on Stability Testing of Exitance Active Substances and Relared Finished Products
CPMP/QWP/ 072/96	Note for Guidance on Start of Shelf Life of the Finished Dosage Form

1.8 Climatic Zones for Stability Testing

For the purpose of stability testing, the whole world has been divided into four zones (I - IV) depending upon the environmental conditions the pharmaceutical products are likely to be subjected to during their storage. These conditions have been derived on the basis of the mean annual temperature and relative humidity data in these regions. Based upon this data, long-term or real-time stability testing conditions and accelerated stability testing conditions have been derived. The standard climatic zones for use in pharmaceutical product stability studies have been presented in the Table 4. The break-up of the environmental conditions in each zone and also the derived long-term stability test storage conditions, as given by WHO have also been presented. The stability conditions have also been harmonized and adjusted to make them more practical for industry application and rugged for generalized application (Singh *et al.,* 2000; ICH Q1A (R2), 2003).

Table: (1.4) ICH Climatic Zones and Long Term Stability Conditions:
(Singh et al, 2000; ICHQ1A (R2), 2003; Grimm et al, 1998.

Climatic Zone	Climate/ Definition	Major Countries /Region	MAT*/MEAN annual partial water vapour pressure	Long-term testing conditions
I	Temperate	United Kingdom Northern Europe Russia	≤15ºC/≤11 hPa	21 ºC/45%RH
II	Subtropical and Mediterranean	United States Japan Southern Europe	>15-22ºC />11-18 hPa	25 ºC/60%RH
IV	Hot and Dry	Iraq India	>22 ºC/≤ 15 hPa	30 ºC/35%RH
IVa	Hot and humid	Iran Egypt	>22 ºC />15-27 hPa	30 ºC/65%RH
IVb	Hot and very humid	Brazil Singapore	>22 ºC /> 27 hPa	30 ºC/75%RH

*MAT: mean annual temperature in open air.

1.9 Batches

Stability studies at developmental stages are generally carried out on a single batch while studies intended for registration of new product or unstable established product are done on first three production batches, while for stable and well-established batches, even two are allowed. If the initial data is not on a full scale production batch, first three batches of drug product manufactured post-approval should be placed on long-term studies using the same protocol as in approved drug application. Data on laboratory scale batches obtained during development of pharmaceuticals are not accepted as primary stability data but constitute supportive information. In general, the selection of batches should constitute a random sample from the population of pilot or production batches (Singh *et al.*, 2000).

1.10 Containers and Closures

The testing is done on the product in immediate containers and closures proposed for marketing. The packaging materials include aluminum strip packs, blister packs, Alu-Alupacks, HDPE bottles etc. This may also include secondary packs, but not shippers. Products in all different types of containers/closures, whether meant for distribution or for physician and promotional samples, are to be tested separately. However, for bulk containers, testing in prototype containers is allowed, if it simulates the actual packaging (Singh *et al.*, 2000).

1.11 Sampling Plan

Sampling plan for stability testing involves, planning for the number of samples to be charged to the stability chambers and sampling out of the charged batch so as to cover the entire study. The first step should be the development of the sampling time points followed by the number of samples needed to be drawn at each pull point for complete evaluation of all test parameters and finally adding up to get the total number of samples. For example there would be a requirement of

about 100 tablets per pull out in a long term or accelerated stability studies including 10 each for assay, hardness and moisture determination, 6 each for dissolution and disintegration and 50 for friability. This multiplied by the total number of pull outs will give the total number of tablets required for a study. This is followed by the development of a sampling plan, which includes the selection of the containers representing the batch as a whole but in an unbiased manner. A stratification plan has been suggested whereby from a random starting point every *nth* container is taken from the filling or packaging line (*n* is chosen such that the sample is spread over the whole batch), (Singh *et al.*, 2000).

1.12 Test Storage Conditions

The storage conditions to be selected are based upon the climatic zone in which the product is intended to be marketed or for which the product is proposed to be filed for regulatory approval. General recommendations on the storage conditions have been given by ICH, CPMP and WHO. The abridged/indicative ICH and WHO storage conditions for drug products have been given in Table 6.

Table: (1.5) Stability test storage conditions for drug products:
(Cha *et al*, 2001; ICH Q1A (R2), 2003; WHO, 2004)

Intended storage condition	**Stability Test Method**	**ICH Test temperature and humidity (Period in months)**	**WHO Test temperature and humidity (Period in months)**
Room temperature	Long term	25± 2ºC/60 ± 5% RH (12)	25±2ºC/60±5% RH or 25±2ºC/65±5% RH 25±2ºC/75±5% RH (12)
	Intermediate	25±2ºC/65±5% RH (6)	25±2ºC/65±5% RH (6) 40±2ºC/75±5%

	Accelerated	40±2ºC/75±5% RH (6)		RH (6)
Refrigerated	Long term	5ºC/ (12)	ambient	5±3 ºC
	Accelerated	25±2ºC/60±5% RH (6)		25±2ºC/60±5% RH OR 25±2ºC/60±5% RH
Freezer	Long term	-20ºC/ (12)	ambient	-20ºC±5 ºC

1.13 Test Parameters

The stability test protocol should define the test parameters that would be used for evaluation of the stability samples. The tests that monitor the quality, purity, potency, and identity which could be expected to change upon storage are chosen as stability tests. Therefore appearance, assay, degradation products, microbiological testing, dissolution, and moisture are standard tests performed on stability test samples. Microbiological tests include sterility, preservative efficacy and microbial count as applicable e.g. for liquid inject able preparations. The batches used for stability study must meet all the testing requirements including heavy metals, residue on ignition, residual solvents etc. Some of these are required at the time of product release but not required to be repeated during stability testing (Cha *et al.,* 2001).

1.14 Acceptance Criteria

All analytical methods are required to be validated before initiating the stability studies. Similarly, the acceptance criteria for the analytical results as well as that for the presence of degradation products should also be fixed beforehand. The acceptance criteria for each test in the stability study is fixed in the form of numerical limits for the results expressed in quantitative terms e.g., moisture pick-

up, viscosity, particle size, assay, degradation products, etc. and pass or fail for qualitative tests e.g., odors, color, appearance, cracking, microbial growth, etc. These acceptance criteria should also include individual and total upper limits for degradation products. ICH guideline Q3B (R2) related to impurities in new drug products addresses degradation products in new drug formulations.

The degradation products of the active or interaction products from the active ingredients and excipients and/or active and container component should be reported, identified, and/or qualified when the proposed thresholds are exceeded. The reporting threshold of impurities is based upon the intended dose. If the maximum daily dose is less than or equal to 1gm, the limit is 0.1% and if greater than 1, the limit is 0.05%. The identification threshold of impurities is between 1.0-0.1% for the maximum daily dose ranging between 1mg and 2gm (Singh *et al.*, 2000; Cha *et al*, 2001).

1.15 Expiration Date/Shelf Life

An expiration date is defined as the time up to which the product will remain stable when stored under recommended storage conditions. Thus, an expiration date is the date beyond which it is predicted that the product may no longer retain fitness for use. If the product is not stored in accordance with the manufacturer's instructions, then the product may be expected to degrade more rapidly. Shelf life is the time during which the product, if stored appropriately as per the manufacturer's instructions, will retain fitness for use (>90% of label claim of potency). The expiration date is also defined as the date placed on the container/labels of a drug product designating the time during which a batch of the product is expected to remain within the approved shelf life specifications, if stored under defined conditions and after which it should not be used (Kommanaboyina *et al.*, 1999).

1.16 Estimation of Shelf Life

The shelf life is determined from the data obtained from the long term storage studies. The data is first linearized and test for goodness of fit is applied. The linearized data is then analyzed to see that the slope and the intercepts are matching. Table 7 gives the different possibilities in the pattern of the concentration-time data of the three batches. The data is pooled accordingly and used for estimation of the common slope (Singh *et al.,* 2000; Singh *et al.,* 1999).

Table: (1.6) Pattern of concentration-time data and pooling decision:

Slope	Intercept	Variation Factor	Pooling
Identical	Identical	Nil	Yes
Identical	Different	Batch e.g. unequal initial drug concentrations	No
Different	Identical	Storage e.g. difference in the rate of the drug loss	No
Different	Different	Interactive Forces-Both batch and storage factor	No

For determination of significance of difference in case of slope or intercept, statistical tests like t-test should be applied. The data is available in the form of only five data points i.e. 0, 3, 6, 9 and 12 months, either pooled from the three batches or from the three individual batches if they are not fit for pooling. In case data is not fit for pooling, stability estimates are to be made on the worst batch. The shelf life/expiry date is determined from the regression line of this five point data based on calculation of 95% one-sided confidence limit. For reading the expiry date, 90% drug concentration is considered as the lowest specification limit and the point where the extension line cuts the 95% confidence limit line is taken as an expiry date. Because shelf life derived from the intersection of the lower 90% confidence bound and 90% potency value has a 95% confidence level, therefore

there is only a 5% chance that our estimate of the shelf life will be too high (Ali *et al.,*

2008). For new drugs, it is a general practice to grant only two-year expiry initially, which is based on satisfactory one year long-term and 6 months accelerated stability data. The expiry date for third and later years is allowed only on production of real-time data for the subsequent years (Singh *et al.*, 2000). Most pharmaceutical products are characterized by only one shelf life. However, in some cases a product may have two e.g. a freeze-dried (lyophilized) protein product may have only 1 shelf life, say 2 years, for the product stored in the dry condition and a 2nd shelf life, say 2 days, for the product when it has been reconstituted with the appropriate vehicle and is ready for injection (Carstensen *et al.*, 2000).

1.17 Photo Stability Testing

USA FDA in 1996 issued ICH guidance for industry and stated that “the intrinsic photo stability characteristics of new drug substances and products should be evaluated to demonstrate that, as appropriate, light exposure does not result in unacceptable change”. In this photo stability testing is recommended to be carried out on a single batch of material; however these studies should be repeated if certain variations and changes are made to the product (e.g., formulation, packaging). While D65 is the internationally recognized standard for outdoor daylight as defined in ISO 10977 (1993), ID65 is the equivalent indoor indirect daylight standard.

Following this, testing of drugs in photo stability chamber is now increasingly being followed as a standard procedure, especially for the products intended to be marketed in Asian countries (ICH Q1B, 1996; Singh *et al*., 2000).

1.18 Stability Test Equipment

The equipment used for stability testing is called stability chamber. These are specialized environmental chambers that can simulate the storage condition and

enable evaluation of product stability based on real-time, accelerated and long-term protocols.

They are available in both walk-in and reach-in styles. Smaller chambers are preferred for accelerated testing, as the retention time of products is much less in these cabinets, while the walk-in chambers are preferred for long-term testing. Such chambers or rooms are engineered and qualified to ensure uniform exposure of the set conditions to all the samples in the chamber. These chambers are expected to be dependable and rugged because of the requirement of uninterrupted use for years. They are fitted with appropriate recording, safety and alarm devices. In addition,

Photo stability chambers are also available and utilized both with and without temperature and humidity control. Two types of light sources are usually employed in photo stability chambers, one is the combination of cool white and near UV fluorescent tubes, while second are the artificial daylight lamps, e.g., xenon or metal halide. It is required to obtain a total exposure of 1.2 millions lux hours. The visible light intensity is estimated using a lux meter. The calculation is made on how many hours of exposure is needed (Singh *et al.*, 2000).

1.19 The Three Stability of Drug

The stability of drug must be considered, Physical stability, Microbiological stability, and Chemical stability.

1.19.1 Physical Stability:

Physical instabilities possibilities are:

1- Crystal formation in pharmaceutical preparations:

Causes:

- Polymorphism phenomena: i.e. Chloramphenicol (change of amorphous to crystalline form.
- Saturated solution: by different temperature precipitation of solute may occur.

- In suspension: when very fine powder is used a part of suspending agent will dissolve then precipitate as crystal.

2. Loss of volatile substances from pharmaceutical dosage forms, like Aromatic waters, Elixirs, Spirits, and Some types of tablets which contain aromatic water (Nitroglycerin tablets).

3. Loss of water:

This can be seen in the following dosage forms:

- Saturated solution: by loss of water they become supersaturated and precipitate as crystals is formed
- Emulsions: Loss of water leads to separation of the two phases and change to other type.
- Creams: especially oil/water, they become dry by loss of water.
- Pastes.
- Ointments: especially aqueous base ointments.

4. Absorption of water:

These phenomena can be seen in the following pharmaceutical forms:

- Powders: Liquefaction and degradation may occur as a result of absorption of water.
- Suppositories which base made from hydrophilic substances as Glycerin, Gelatin, and polyethylene glycol.

The consistency of these forms becomes jelly-like appearance.

5. Change in crystalline form:

• Example: Cocoa butter which is capable of existing in four polymorphic forms.

(cst-kh.edu.pcs/steff/mabujamee/wp-content/…/unit-4-drug-stability.pdf.20.12.2014, 11:45 pm)

1.19.2 Microbiological Stability:

- Contamination from microorganisms is a big problem for all formulations containing moisture but it can be a bother in solid dosage forms also if some natural polymers are used because many natural polymers are fertile sources of microorganisms.

- In the type of hygienic manufacture carried out today where “Quality Assurance” is a prerequisite as per the GMP procedures, there are definite procedures to prevent microbial contamination in all formulations.

(cst-kh.edu.pcs/steff/mabujamee/wp-content/…/unit-4-drug-stability.pdf.20.12.2014, 11:45pm)

- ***Sources of Microbial Contamination:***

1. Water.
2. Air.
3. Raw materials, containers and closures.
4. Personnel.
5. Instruments and apparatus.

1.19.3 Chemical Stability:

- Chemical stability implies:

The lack of any decomposition in the chemical moiety that is incorporated in the formulation as the drug, preservatives or any other percipients.

This decomposition may influence the physical and chemical stability of the drug.

- **Mechanisms Of Degradation:**

1- Hydrolysis:

Hydrolysis means “splitting by water’’ Some Functional Groups Subject to Hydrolysis:

Table (1.7): Function Group in Drug and Example:

Drug type	Examples
Esters	Aspirin, alkaloids, Dexmethasne sodium phosphate, Nitroglycerin
Lactones	Pilocarpine, Spironolactone

Amides	Chloramphenicol
Lactams	Penicillins, Cephalosporins
Imides	Glutethimide
Malonic ureas	Barbiturates

2- Oxidation

Oxidation of inorganic and organic compounds is explained by a loss of electrons and the loss of a molecule of hydrogen.

Some Functional Groups Subject to Autoxidation:

Table (1.8): Function Group in Drug and Example

Functional group	Examples
Catechols	Catecholamines (dopamine)
Ethers	Diethylether
Thiols	Dimercaprol (BAL)
Thioethers	Chlorpromazine
Carboxylic acids	Fatty acids

3- Photolysis

It means: decomposition by light e.g. Sodium nitroprusside is administered by intravenous infusion for the management of acute hypertension. If the solution is protected from light, it is stable for at least 1 year; if exposed to normal room light, it has a shelf life of only 4 hours. Which is prevented by?

1- Suitable packing in amber colored bottles.

2- Cardboard outers.

3- Aluminum foil over wraps.

- **Factors Affecting Rates Of Degradation:**

1- pH:

The acidity or the alkalinity of a solution has a profound influence on the decomposition of drug compound.

- Aspirin buffered solution is maximum stable at a pH of 2.4, above a pH of 10 the decomposition rate rapidly increases.
- pH can also influence the rate of oxidation.
- The system is less readily oxidized when the pH is low.

2- Complexation:

- Complex formation reduces the rate of hydrolysis and oxidation.
- E.g. caffeine complexes with local anesthetics, such as benzocaine, procaine and tetracaime to cause a reduction in their rate of hydrolytic degradation.

3- Surfactants:

- Nonionic, cationic and anionic surfactants when added to solutions containing drugs form micelle and the drug particles become trapped in the micelle.
- The hydrolytic groups such as OH cannot penetrate this micelle cover and reach the drug particles, hence hydrolysis rate is decreased.

4- Presence of heavy metals:

- Heavy metals, such as copper, iron; cobalt and nickel increase the rate of formation of free radicals and enhance oxidative decomposition.

5- Light and humidity:

Light, especially ultraviolet light enhances photolysis and humidity enhances hydrolytic decomposition.

1.19.4 Stabilization of Drugs against Hydrolysis, Oxidation and Photolysis:

1- Temperature:

All the drug products are stored at suitable temperatures to avoid thermal acceleration of decomposition. Three varieties of temperatures are suggested for storage of drug products. Room temperature, cool storage and cold storage.

2- Light:

Light sensitive materials are stored in ambered color bottles.

3- Humidity:

Packing materials are chosen (usually glass and plastic) to prevent exposure of drug products to high humid condition.

4- Oxygen:

Proper packing keeping the oxygen content of the solution less and leaving very little head space in the bottle above the drug products are methods to fight against oxidation.

5- Chelating Agents:

Chelating agents form complexes with heavy metal ions and prevent them from catalyzing oxidative decomposition.

E.g. ethylenediaminetetracetic acid (EDTA) derivatives and salts, citric acid and tartaric acid.

6- Solvents:

By the addition of a suitable solvent hydrolysis rate may be decreased.

(uqu.edu.sa/.../file manager/files/4290183/**STABILITY** OF **DRUGS**.ppt 20.7.2014, 10:50am)

1.19.5 Packaging

• Packaging of the drug product is very important when its stability is being considered.

• The immediate container and closure are particularly important in affecting product stability.

• Glass, plastic, rubber (natural and synthetic) and metal are the four types of containers commonly utilized for packing drug products.

1. Glass:

• Glass is resistant to chemical and physical change and is the most commonly used material, but it has the limitations of:

1. Its alkaline surface may raise the pH of the product.

2. Ionic radicals present in the drug may precipitate insoluble crystals from the glass.

3. The clarity of the glass permits the transmission of high energy wavelength of light which may accelerate decomposition.

• All these limitations are overcome by the technologists in the following way:

1. The first problem is overcome by the use of Borosilicate glass which contains fewer reactive alkali ions.

2. Treatment of glass with chemicals or the use of buffers helps in overcoming the second problem.

3. Amber colored glass which transmits light only at wavelengths above 470 nm is used for photolytic drug products.

2. Plastics:

• Plastics include a wide range of polymers of varying density and molecular weight, each possessing different physicochemical characteristics. The problems with plastic are:

1. Migration of the drug through the plastic into the environment.

2. Transfer of environmental moisture, oxygen, and other elements into the pharmaceutical product.

3. Leaching of container ingredients into the drug.

4. Adsorption or absorption of the active drug or percipients by the plastic.

• For all these problems the solution is to suitably pretreat the plastic chemically. The drug product packed in the final container must be tested for stability.

3. Metals:

• Various alloys and aluminum tubes may be utilized as containers for emulsions, ointments, creams and pastes.

• They may cause corrosion and precipitation in the drug product.

• Coating the tubes with polymers or epoxy may reduce these tendencies.

4. Rubber:

• Rubber also has the problems of extraction of drug ingredients and leaching of container ingredients described for plastics.

• The use of neoprene, butyl or natural rubber, in combination with certain epoxy, Teflon or varnish coatings reduces drug-container interactions.

• The pretreatment of rubber vial stoppers and closures with water and steam removes surface blooms and also reduces potential leaching.

Preservatives:

• Extremely hygienic manufacture ensures a product that is free of contamination in the case of almond-sterile preparations and a sterile preparation in the case of all parenterals.

• There are two strategies followed in the manufacture of microbiologically stable, acceptable

1.20 The Instability Possibilities in Different Formulations:

- Oral solutions

Instability problems:

1. Loss of flavor.
2. Change in taste.
3. Presence of off flavors due to interaction with plastic bottle.
4. Loss of dye.
5. Precipitation.
6. Discoloration.

Effects:

Change in smell or feel or taste

Steps to prevent instability:

Use of proper percipients and suitable packing materials

-Parenteral solutions:

Physical instability occurs due to:

1. Interaction of the contents with the container.
2. Changes in Chemical composition.

Instability problems:

1. Discoloration due to photo chemical reaction or oxidation. Ex: thiamine hydrochloride.
2. Presence of precipitate due to interaction with container or stopper.
3. Presence of “whiskers”. If some small pinholes are present in the ampule due to improper sealing the solution wicks out, the liquid evaporates and the solid settles on the outside. It further helps in wicking out more solution and long lines of crystals form on the outside of the vial which are called whiskers. This may happen due too small whole going undetected or the crack developing during storage.
4. Clouds: A cloud will appear in the product due to:

a. Chemical changes (an ester eg: polysorbate may hydrolyse producing an acid which is poorly soluble).

b. Solubility product may be exceeded.

c. The original preparation of a supersaturated solution or the use of a metastable form (ex: calcium gluceptate).

Effects:

Change in appearance and in bioavailability.

Steps to prevent instability:

1. Use of antioxidants (0.5%) Acetylcystane or 0.02 – 1% Ascorbic acid) or Chelating agents (0.01 – 0.075 sodium edentate) to prevent discoloration.

2. Change in stopper or material of the container will eliminate the problem.

3. Checking of the manufacturing process Increasing solubility by the use of co solvents (e.g.: polyethylene glycol) or by other methods such as micellar approach or complexation will reduce clouding.

- Suspensions:

This instability occurs due to:

a. Particle diameter.

b. Concentration of responding agent.

c. Viscosity of surrounded media.

d. Temperature.

e. pH.

f. Presence of microbes.

Instability problems:

1. Settling.

2. Caking.

3. Crystal growth.

Effects:

Loss of drug content uniformity in different doses from the bottle and loss of elegance.

Steps to prevent instability:

Design of product based on proper pre-formulation studies.

-Emulsions:

This instability occurs due to:

a. Droplet diameter.

b. Viscosity.

c. Difference in Density.

d. Temperature.

e. pH.

f. Presence of microbes.

Instability problems:

1. Creaming.

2. Cracking.

Effects:

Loss of drug content uniformity in different doses from the bottle and loss of elegance.

Steps to prevent instability:

Design of product based on proper pre-formulation studies.

-Semisolids (Ointments and suppositories):-

Instability problems:

1. Changes in:

a. Particle size

b. Polymorphic state, or hydration or salvation state

c. Consistency

d. drug release rate

2. Caking or coalescence.

3. Bleeding

Effects:

Loss of drug content uniformity, loss of elegance and change in drug release rate.

Steps to prevent instability:

Design of product based on proper pre-formulation studies.

1.32.6 Tablets

Instability problems:

Change in

a. Disintegration time

b. Dissolution profile

c. Hardness

d. Appearance

Effects:

Change in drug release

Steps to prevent instability:

Design of product based on proper pre-formulation studies.

- Capsules

Instability problems:

Change in

a. Appearance

b. Dissolution

c. Strength

Effects:

Change in drug release

Steps to prevent instability:

Design of product based on proper pre-formulation studies.

(cst-kh.edu.pcs/steff/mabujamee/wp-content/…/unit-4-drug-stability.pdf.20.12.2014, 11:45pm)

1.21 Ciprofloxacin

Ciprofloxacin (INN) is an antibiotic that can treat a number of bacterial infections. It is a second-generation fluoroquinolone. Its spectrum of activity includes most strains of bacterial pathogens responsible for respiratory, urinary tract, gastrointestinal, and abdominal infections, including Gram-negative (Escherichia coli, Haemophilusinfluenzae, Klebsiellapneumoniae, Legionella pneumophila, Moraxella catarrhalis, Proteus mirabilis, and Pseudomonas aeruginosa), and Gram-positive (methicillin-sensitive, but not methicillin-resistant Staphylococcus aureus, Streptococcus pneumoniae, Staphylococcus epidermidis, Enterococcus faecalis, and Streptococcus pyogenes) bacterial pathogens. Ciprofloxacin and other

fluoroquinolones are valued for this broad spectrum of activity, excellent tissue penetration, and for their availability in both oral and intravenous formulations.

Ciprofloxacin is used alone or in combination with other antibacterial drugs in the empiric treatment of infections for which the bacterial pathogen has not been identified, including urinary tract infectionsand abdominal infections among others. It can also treat infections caused by specific pathogens known to be sensitive.

Ciprofloxacin is the most widely used of the second-generation quinolone antibiotics that came into clinical use in the late 1980s and early 1990s. In 2010, over 20 million outpatient prescriptions were written for ciprofloxacin, making it the 35th-most commonly prescribed drug, and the 5th-most commonly prescribed antibacterial, in the US. Ciprofloxacin was discovered and developed by Bayer A.G. and subsequently approved by the US Food and Drug Administration (FDA) in 1987. Ciprofloxacin has 12 FDA-approved human uses and other veterinary uses, but it is often used for unapproved uses (off-label).

Overall, the safety of ciprofloxacin and other fluoroquinolones appears to be similar to that of other antibiotics, but serious side effects occur on occasion. Some disagreement in the literature exists regarding whether fluoroquinolones produce serious adverse events at a higher rate than other broad-spectrum antibiotics. The U.S. FDA-approved label for ciprofloxacin includes a "black box" warning of increased risk of tendon damage and/or rupture and for exacerbation of muscle weakness in patients with the neurological disorder myasthenia gravis. Other side effects include nausea, vomiting, diarrhea and dizziness.

1.21.1 Chemical Properties of Ciprofloxacin 500 mg

Ciprofloxacin is 1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-7- (1-piperazinyl)-3-quinolinecarboxylic acid. Its empirical formula is C17H18FN3O3 and its molecular weight is 331.4 g/mol. It is a faintly yellowish to light yellow crystalline substance.

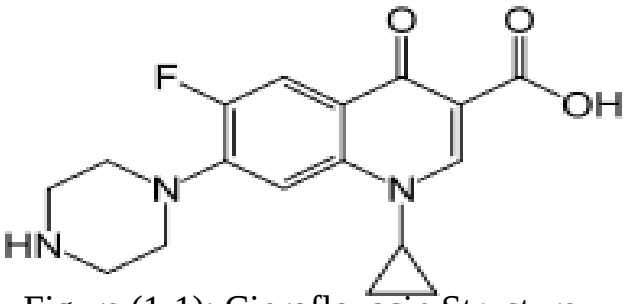


Figure (1-1): Ciprofloxacin Structure.

(-http://en.wikipedia.org/wiki/Ciprofloxacin)

1.22 Instrumental Used:

1.22.1 High-Performance Liquid Chromatography (HPLC):

Schematic representation of an HPLC unit. (1) Solvent reservoirs, (2) Solvent degasser, (3) Gradient valve, (4) Mixing vessel for delivery of the mobile phase, (5) High-pressure pump, (6) Switching valve in "inject position", (6') Switching valve in "load position", (7) Sample injection loop, (8) Pre-column (guard column), (9) Analytical column, (10) Detector (i.e. IR, UV), (11) Data acquisition, (12) Waste or fraction collecptor.

Figure: (1-2) High Performance Liquid Chromatography Instrument.

High-performance liquid chromatography (HPLC; formerly referred to as high-pressure liquid chromatography), is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture. It relies on pumps to

pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out the column.

HPLC has been used for medical (e.g. detecting vitamin D levels in blood serum), legal (e.g. detecting performance enhancement drugs in urine), research (e.g. separating the components of a complex biological sample, or of similar synthetic chemicals from each other), and manufacturing (e.g. during the production process of pharmaceutical and biological products) purposes. (Gerber,F *et al* 2004)

1.22.1.1 Operation:

The sample mixture to be separated and analyzed is introduced, in a discrete small volume (typically micro liters), into the stream of mobile phase percolating through the column. The components of the sample move through the column at different velocities, which are function of specific physical interactions with the sorbent (also called stationary phase). The velocity of each component depends on its chemical nature, on the nature of the stationary phase (column) and on the composition of the mobile phase. The time at which a specific analyte elutes (emerges from the column) is called its retention time. The retention time measured under particular conditions is considered an identifying characteristic of a given analyte.

Many different types of columns are available, filled with sorbents varying in particle size, and in the nature of their surface ("surface chemistry"). The use of smaller particle size packing materials requires the use of higher operational pressure ("backpressure") and typically improves chromatographic resolution (i.e. the degree of separation between consecutive analytes emerging from the column).

In terms of surface chemistry, sorbent particles may be hydrophobic or polar in nature.

Common mobile phases used include any miscible combination of water with various organic solvents (the most common are acetonitrile and methanol). Some HPLC techniques use water-free mobile phases (see Normal-phase chromatography below). The aqueous component of the mobile phase may contain acids (such as formic, phosphoric or trifluoroacetic acid) or salts to assist in the separation of the sample components. The composition of the mobile phase may be kept constant ("isocratic elution mode") or varied ("gradient elution mode") during the chromatographic analysis. Isocratic elution is typically effective in the separation of sample components that are not very different in their affinity for the stationary phase. In gradient elution the composition of the mobile phase is varied typically from low to high eluting strength. The eluting strength of the mobile phase is reflected by analyte retention times with high eluting strength producing fast elution (=short retention times). A typical gradient profile in reversed phase chromatography might start at 5% acetonitrile (in water or aqueous buffer) and progress linearly to 95% acetonitrile over 5–25 minutes. Periods of constant mobile phase composition may be part of any gradient profile. For example, the mobile phase composition may be kept constant at 5% acetonitrile for 1–3 min, followed by a linear change up to 95% acetonitrile.

The chosen composition of the mobile phase (also called eluent) depends on the intensity of interactions between various sample components ("analytes") and stationary phase (e.g. hydrophobic interactions in reversed-phase HPLC). Depending on their affinity for the stationary and mobile phasesanalytes partition between the two during the separation process taking place in the column. This partitioning process is similar to that which occurs during a liquid–liquid extraction but is continuous, not step-wise. In this example, using a water/acetonitrile gradient, more hydrophobic components will elute (come off the column) late,

once the mobile phase gets more concentrated in acetonitrile (i.e. in a mobile phase of higher eluting strength).

The choice of mobile phase components, additives (such as salts or acids) and gradient conditions depends on the nature of the column and sample components. Often a series of trial runs is performed with the sample in order to find the HPLC method which gives adequate separation.

1.22.1.2 Partition Chromatography:

Partition chromatography was the first kind of chromatography that chemists developed. The partition coefficient principle has been applied in paper chromatography, thin layer chromatography, gas phase and liquid–liquid applications. The 1952 Nobel Prize in chemistry was earned by Archer John Porter Martin and Richard Laurence Millington Synge for their development of the technique, which was used for their separation of amino acids. Partition chromatography uses a retained solvent, on the surface or within the grains or fibers of an "inert" solid supporting matrix as with paper chromatography; or takes advantage of some coulombic and/or hydrogen donor interaction with the stationary phase. Analyte molecules partition between a liquid stationary phase and the eluent. Just as in Hydrophilic Interaction Chromatography (HILIC; a sub-technique within HPLC), this method separates analytes based on differences in their polarity. HILIC most often uses a bonded polar stationary phase and a mobile phase made primarily of acetonitrile with water as the strong component. Partition HPLC has been used historically on unbounded silica or alumina supports. Each works effectively for separating analytes by relative polar differences. HILIC bonded phases have the advantage of separating acidic, basic and neutral solutes in a single chromatographic run.(Lindsay, S *et al* 1987)

1.22.1.3 Normal–phase chromatography:

Normal–phase chromatography was one of the first kinds of HPLC that chemists developed. Also known as normal-phase HPLC (NP-HPLC) this method separates analysts based on their affinity for a polar stationary surface such as silica, hence it is based on analytic ability to engage in polar interactions (such as hydrogen-bonding or dipole-dipole type of interactions) with the sorbent surface. NP-HPLC uses a non-polar, non-aqueous mobile phase (e.g. Chloroform), and works effectively for separating analytes readily soluble in non-polar solvents. The analyte associates with and is retained by the polar stationary phase. Adsorption strengths increase with increased analyte polarity. The interaction strength depends not only on the functional groups present in the structure of the analyte molecule, but also on steric factors. The effect of steric hindrance on interaction strength allows this method to resolve (separate) structural isomers.

The use of more polar solvents in the mobile phase will decrease the retention time of analytes, whereas more hydrophobic solvents tend to induce slower elution (increased retention times). Very polar solvents such as traces of water in the mobile phase tend to adsorb to the solid surface of the stationary phase forming a stationary bound (water) layer which is considered to play an active role in retention. This behavior is somewhat peculiar to normal phase chromatograhy because it is governed almost exclusively by an adsorptive mechanism (i.e. analytes interact with a solid surface rather than with the solvated layer of a ligand attached to the sorbent surface; see also reversed-phase HPLC below). Adsorption chromatography is still widely used for structural isomer separations in both column and thin-layer chromatography formats on activated (dried) silica or alumina supports.

Partition- and NP-HPLC fell out of favor in the 1970s with the development of reversed-phase HPLC because of poor reproducibility of retention times due to the presence of a water or protic organic solvent layer on the surface of the silica or alumina chromatographic media. This layer changes with any changes in the

composition of the mobile phase (e.g. moisture level) causing drifting retention times.

1.22.1.4 Reversed-Phase Chromatography (RPC):

Reversed phase HPLC (RP-HPLC) has a non-polar stationary phase and an aqueous, moderately polar mobile phase. One common stationary phase is a silica which has been surface-modified with RMe_2SiCl, where R is a straight chain alkyl group such as $C_{18}H_{37}$ or C_8H_{17}. With such stationary phases, retention time is longer for molecules which are less polar, while polar molecules elute more readily (early in the analysis). An investigator can increase retention times by adding more water to the mobile phase; thereby making the affinity of the hydrophobic analyte for the hydrophobic stationary phase stronger relative to the now more hydrophilic mobile phase. Similarly, an investigator can decrease retention time by adding more organic solvent to the eluent. RP-HPLC is so commonly used that it is often incorrectly referred to as "HPLC" without further specification. The pharmaceutical industry regularly employs RP-HPLC to qualify drugs before their release.

RP-HPLC operates on the principle of hydrophobic interactions, which originates from the high symmetry in the dipolar water structure and plays the most important role in all processes in life science. RP-HPLC allows the measurement of these interactive forces. The binding of the analyte to the stationary phase is proportional to the contact surface area around the non-polar segment of the analyte molecule upon association with the ligand on the stationary phase.

1.22.1.5 Isocratic and Gradient Elution:

A separation in which the mobile phase composition remains constant throughout the procedure is termed *isocratic* (meaning *constant composition*). The word was coined by Csaba Horvath who was one of the pioneers of HPLC.

The mobile phase composition does not have to remain constant. A separation in which the mobile phase composition is changed during the separation process is described as a *gradient elution*.(Lloyd.R et al 2006) One example is a gradient starting at 10% methanol and ending at 90% methanol after 20 minutes. The two components of the mobile phase are typically termed "A" and "B"; *A* is the "weak" solvent which allows the solute to elute only slowly, while *B* is the "strong" solvent which rapidly elutes the solutes from the column. In reversed-phase chromatography, solvent *A* is often water or an aqueous buffer, while *B* is an organic solvent miscible with water, such as acetonitrile, methanol, THF, or isopropanol.

In isocratic elution, peak width increases with retention time linearly according to the equation for N, the number of theoretical plates. This leads to the disadvantage that late-eluting peaks get very flat and broad. Their shape and width may keep them from being recognized as peaks.

Gradient elution decreases the retention of the later-eluting components so that they elute faster, giving narrower (and taller) peaks for most components. This also improves the peak shape for tailed peaks, as the increasing concentration of the organic eluent pushes the tailing part of a peak forward. This also increases the cpeak height (the peak looks "sharper"), which is important in trace analysis. The gradient program may include sudden "step" increases in the percentage of the organic component, or different slopes at different times – all according to the desire for optimum separation in minimum time. (Ronald E et al 2010; Leem.L. et al 2006; Lipsky. SR .et al 1967)

1.22.1.6 Pump Pressure:

Pumps vary in maximum pressure capacity, but their performance is measured on their ability to yield a consistent and reproducible flow rate. Modern HPLC pumps are available in versions which may attain pressures as high as 20,000 psi, or about 1,372 bars. Most HPLC pumps have a 400 or 600 bar pressure maximum and those which are capable of operating at or above the 600 bar level are sometimes referred to as "UHPLC" systems ("Ultra High Performance Liquid Chromatography"). The higher pressure ratings found in UHPLC systems may allow the use of sub 2 micron particles, which under some conditions, can result in higher resolution separations, (http://www.agilent.com/en-us-products/liquid-chromatography/lc-pumps-vacuum-degassers/1290-infinity-quaternary-pump#specifications).

The term "UPLC" is a trademark of the Waters Corporation for marketing their UHPLC systems.

1.22.1.7 Detectors:

HPLC most commonly uses a UV-Vis absorbance detector, however a wide range of other chromatography detectors can be used. In certain cases it is possible to use multiple detectors, for example LCMS normally combines UV-Vis with a Mass spectrometer. (en.m.wikiped.org)

1.22.2 Dissolution Testing

In the pharmaceutical industry, drug dissolution testing is routinely used to provide critical in vitro drug release information for both quality control purposes, i.e., to assess batch-to-batch consistency of solid oral dosage forms such as tablets, and drug development, i.e., to predict in vivo drug release profiles.(Ab Bai, G., Wang, Y., Armenante, P. M 2011)

In vitro drug dissolution data generated from dissolution testing experiments can be related to vivo pharmacokinetic data by means of in vitro-in vivo correlations (IVIVC). A well established predictive IVIVC model can be very helpful for drug

formulation design and post-approval manufacturing changes. (Kortejärvi H et al 2006)

The main objective of developing and evaluating an IVIVC is to establish the dissolution test as a surrogate for human studies, as stated by the Food and Drug Administration (FDA). Analytical data from drug dissolution testing are sufficient in many cases to establish safety and efficacy of a drug product without in vivo tests, following minor formulation and manufacturing changes (Qureshi and Shabnam, 2001).

Figure (1-3): Dissolution Tester.

Features:

- The 8/14 station microcontroller based instrument is designed to conduct the test simultaneously in all vessels in similar conditions as per IP/BP/USP Standards
- Equipped with a large graphic LCD, to display
- Speed of stirrer can be controlled and maintained by advanced microcontroller based system
- 150 Test Methods with 18 different time intervals can be programmed
- Water bath contains a non-corrosive tank pate

- Incorporated with circulation pump to maintain the constant temperature in water-bath
- Individual vessels centering is possible
- Password protection at different level
- Off line printing facility features selective printing as per date and product selection
- Facility to customize test reports with user’s company name and instrument number

1.23 Objectives of this Work

. To determine of assay of ciprofloxacin 500 mg by High prephormance Liquid chromatography (HPLC) in two company (blue Nile and shanghai).

. To determine of dissolution of ciprofloxacin 500 mg by dissolution apparatus usig UV/VIS-spectrophotometer as a detector in two company (Blue Nile).

. To characterize physical properties of ciprofloxacin.

Chapter Two
Methods and Materials

Methods and Materials

2.1 Chemical Used

Blue Nile Company

- Triethyle amine (Assay: 99.7%, Density: 0.73 g/cm^3, Manufactured by LOBA cheme).
- orthophosphoric acid (Assay: 85-88%, Manufactured by LOBA cheme).
- Acetonitril (Assay: 99.9%, Density: 0.782-0.783 g/ml, Manufactured by LOBA cheme).
- Distilled water.
- ciprofloxacin 500 mg.

Shanghai Company

- Triethyle amine (Assay: 99.5%, Density: 0.73 g/ml, Manufactured by SCHARLAU).
- orthophosphoric acid (Assay: 88%, Density:1.75 g/ml, Manufactured by CDH).
- Acetonitril (Assay: 99.9%, Density: 0.781 g/ml, Manufactured by Chemical Lab).
- Distilled water.
- ciprofloxacin 500 mg.

Instrument:

Blue Nile Company

- Analytical Balance ((Manufactured by Electro lab, India).
- Hardness Tester (Manufactured by Electro lab, India).
- Friabilator Device (Manufactured by Electro lab, India).
- Disintegration Tester (Manufactured by Electro lab, India).
- Vernier Caliper.
- High performance liquid chromatography HPLC (Manufactured by Shimadzu, Japan).
- Dissolutions Tester (Manufactured by Electro lab, India).
- UV/VIS- spectrophotometer Device (Manufactured by Shimadzu, Japan).
- Volumetric flask, pipate, beakers, washing bottle, etc.

Shanghai Company

- Analytical Balance ((Manufactured by KERN, China).

- Hardness Tester (Manufactured by XZH, China).
- Friabilator Device (Manufactured by FT-2000A, China).
- Disintegration Tester (Manufactured by TDEF, China).
- Vernier Caliper.
- High performance liquid chromatography HPLC (Manufactured by Shimadzu, Japan).
- Dissolutions Tester (Manufactured by TDEF, China).
- UV/VIS- spectrophotometer Device (Manufactured by Shimadzu, Japan).
- Volumetric flask, pipate, beakers, washing bottle, etc.

2.2 Procedures

Weight Variation: The USP weight variation test was run by weighing 20 tablets individually, calculating the average weight, and comparing the individual tablet weights to the average. The tablets met the USP tests that were not more than 2 tablets were outside the percentage limit and no tablets differed by more than 2 times the percentage limit.

Hardness: Hardness of the tablets was determined by breaking it between the second and third fingers with thumb being as a fulcrum. There was a sharp snap the tablet was deemed to have acceptable strength. Hardness of the tablets was determined by Hardness Tester.

Friability: The friability of tablets was determined by Friablator. 20 tablets were taken and weighed. After weighing the tablets were placed in the Roche Friablator and subjected to the combined effects of abrasion and shock by utilizing a plastic chamber that revolves at 25RPM for minutes dropping the from a distance of six inches with each revolution. After operation the tablets were de-dusted and reweighed.

Disintegration Test: Disintegration time is considered to be one of the important criteria in selecting the best formulation. For most tablets the first important step toward solution is break down of tablet into smaller particles or granules, a process

known as disintegration. One tablet was placed into each tube and the assembly was suspended into the 1000ml beaker containing water maintained at 37 ± 2oC and operate the apparatus was operated for 30 seconds. The assembly was removed from the liquid. The tablets were observed.

Thickness Test: Diameter of Tablet to be measured by Vernier (Digital Caliper).

- Procedures for Shanghai (USP pharmacopeia) company:

- High performance liquid chromatography HPLC:

Preparation of mobile phase:

5 ml of triethyl amine was added to 1000 ml of water, the pH was adjusted to 3 by orthophosphoric acid and filtered off, the filtrate was taken and 150 ml of acetonitrile was added.

Preparation of ciprofloxacin standard:

24.4 mg of ciprofloxacin standard was added to 50 ml of mobile phase, 5ml of the mixture was diluted by 50 ml of mobile phase.

Preparation of ciprofloxacin sample:

35.8 mg of sample was added to 50 ml of mobile phase, 5ml from the mixture solution was diluted by adding 50 ml of mobile phase.

- **Dissolution of ciprofloxacin 500 mg by UV/VIS** spectrophotometer **as a detector**

Procedure of standard:

14.6 mg of ciprofloxacin standard was dissolved in 25 ml of distilled water; 2 ml of this solution was transferred into 100 ml volumetric flask, this solution was filtered and the absorption was measured using UV spectrophotometer.

Procedure of sample:

500 mg of sample was dissolved in 900 ml of water and was put in a dissolution tester for 30 minute, 2 ml of this solution was transfer into 100 ml volumetric flask of water, this solution was filtered and the absorption was measured using UV spectrophotometer.

- **Procedures for Blue Nile (BP pharmacopeia) company**
- **High performance liquid chromatography HPLC:**

Procedure of Preparation of mobile phase

13 ml of acetonitrile and 87 ml of a 0.245% w/v solution of orthophosphoric acid were added to each other and the pH was adjusted to 3.0 with triehylamine.

Procedure of Preparation of standard:

58 mg of standard was prepared in 100 ml volumetric flask.

Procedure of Preparation of sample:

The powder of 2 tablets was added to 750 ml of the mobile phase. The solution was mixed with the aid of ultrasound for 20 minutes, a sufficient mobile phase was added to produce 1000 ml, and the solution was mixed and filtered. 5 ml of the filtrate was added to 20 ml of mobile phase.

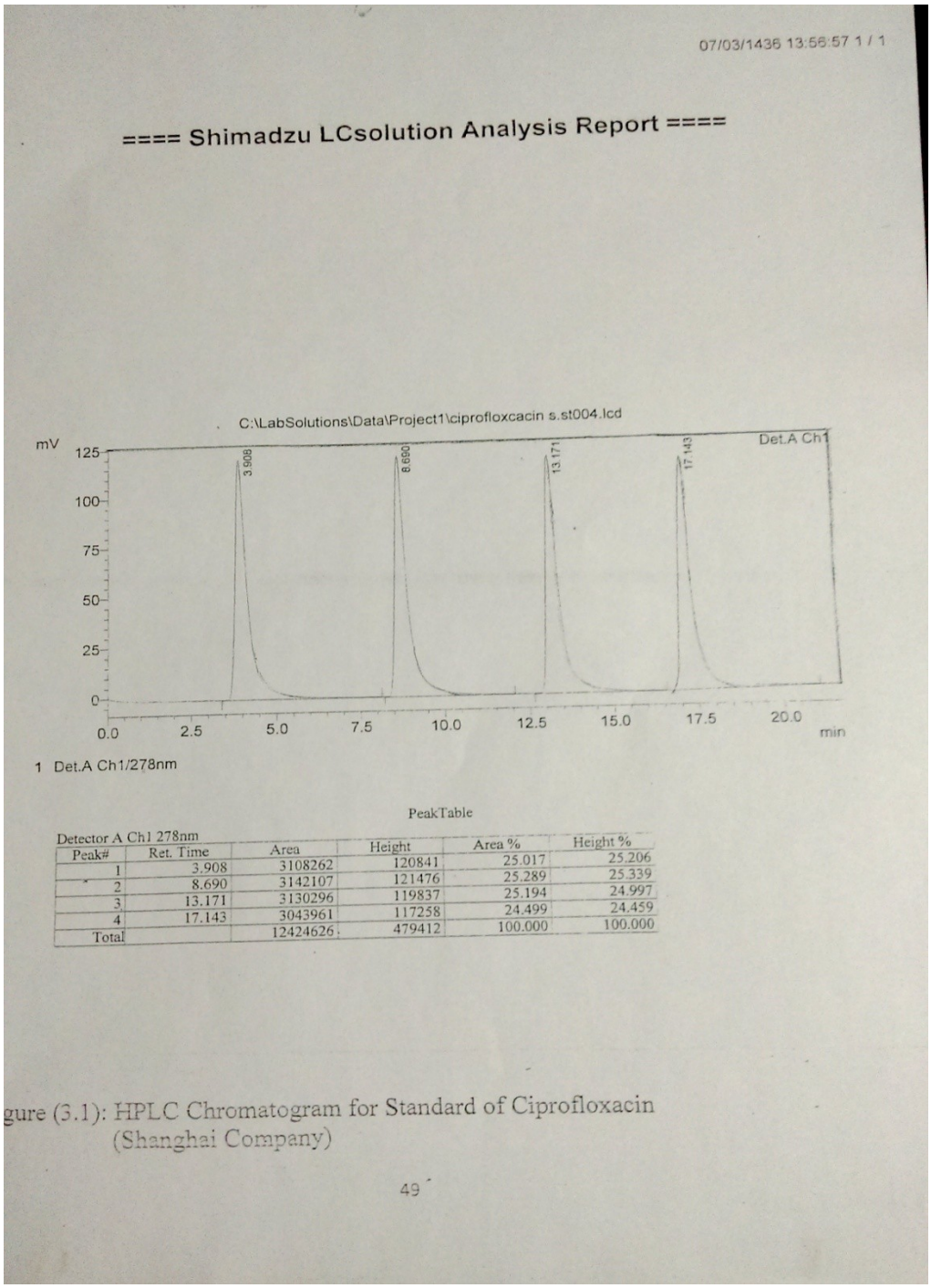
- **Dissolution of ciprofloxacin 500 mg by UV/VIS** spectrophotometer **as a detector**

Procedure of sample and standard of ciprofloxacin:

500 mg of standard and sample were weighted separately, dissolved in 900 ml of water and the two solutions were put separately in equipment, After 30 minutes, 10 ml was diluted with water and filtered and the absorption was measured by U.V spectrophotometer.

Chapter Three
Results and Discussions

Results and Discussion



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==== Shimadzu LCsolution Analysis Report ====

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Det.A Ch1

mV

125
100
75
50
25
0

3.908
8.690
13.171
17.143

0.0 2.5 5.0 7.5 10.0 12.5 15.0 17.5 20.0 min

1 Det.A Ch1/278nm

PeakTable

Detector A Ch1 278nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.908	3108262	120841	25.017	25.206
2	8.690	3142107	121476	25.289	25.339
3	13.171	3130296	119837	25.194	24.997
4	17.143	3043961	117258	24.499	24.459
Total		12424626	479412	100.000	100.000

gure (3.1): HPLC Chromatogram for Standard of Ciprofloxacin (Shanghai Company)

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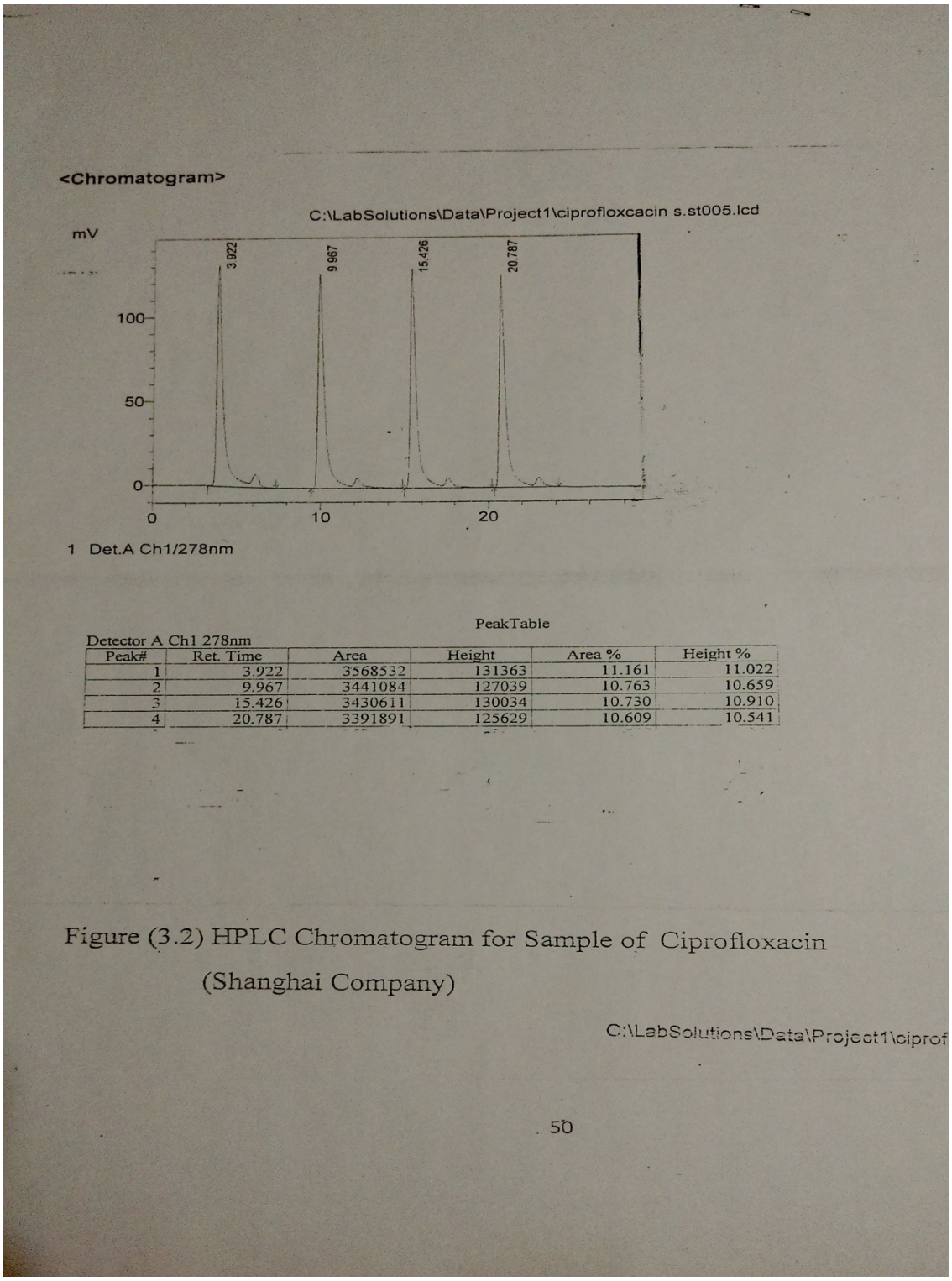
Figure (3.1): HPLC Chromatogram for Standard of Ciprofloxacin (Shanghai Company)



PeakTable

Detector A Ch1 278nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.922	3568532	131363	11.161	11.022
2	9.967	3441084	127039	10.763	10.659
3	15.426	3430611	130034	10.730	10.910
4	20.787	3391891	125629	10.609	10.541

Figure (3.2) HPLC Chromatogram for Sample of Ciprofloxacin
(Shanghai Company)

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Figure (3.2) HPLC Chromatogram for Sample of Ciprofloxacin
(Shanghai Company)

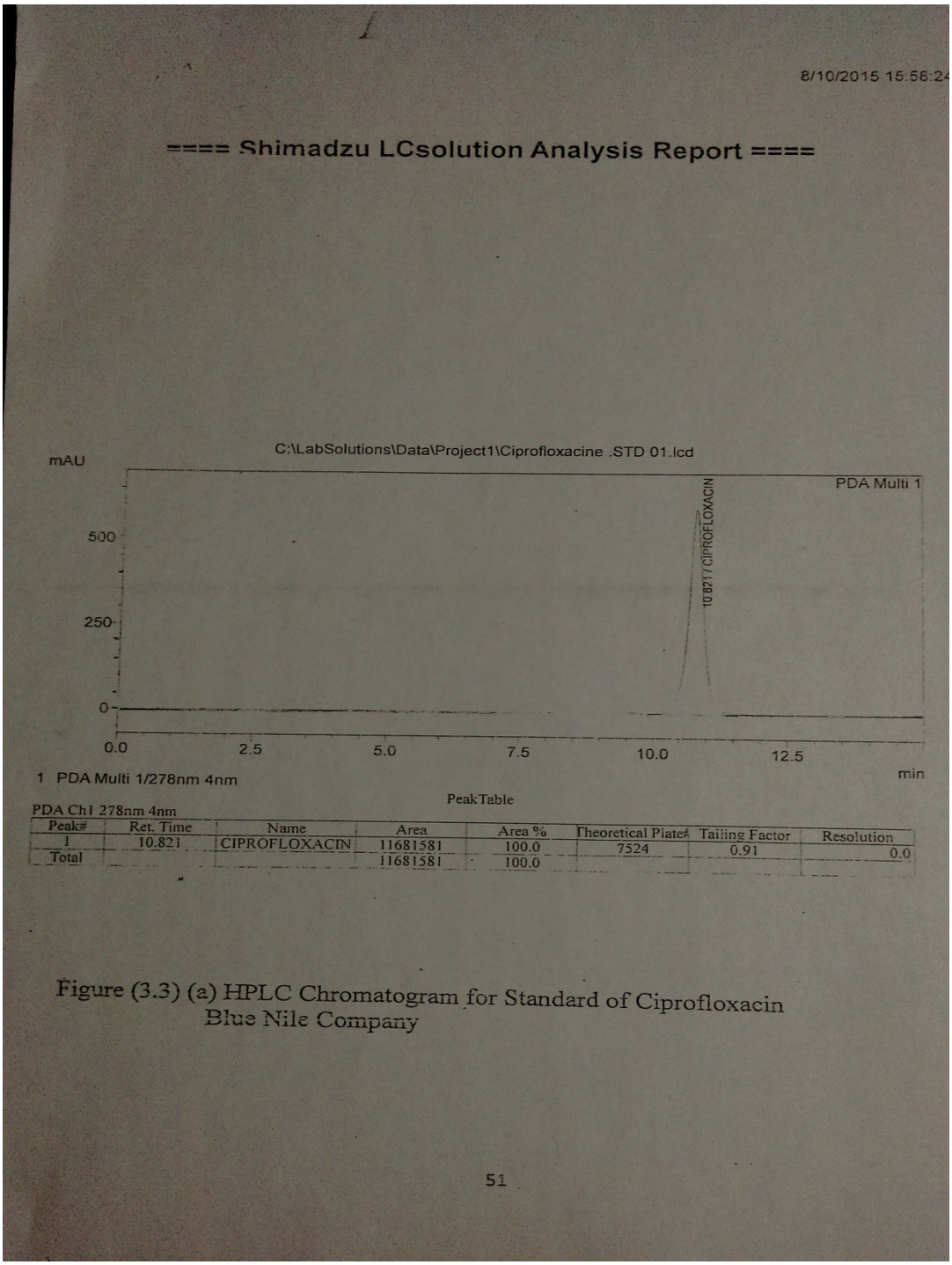


Figure (3.3) (a) HPLC Chromatogram for Standard of Ciprofloxacin
Blue Nile Company

Figure (3.3) (a) HPLC Chromatogram for Standard of Ciprofloxacin

(Blue Nile Company)

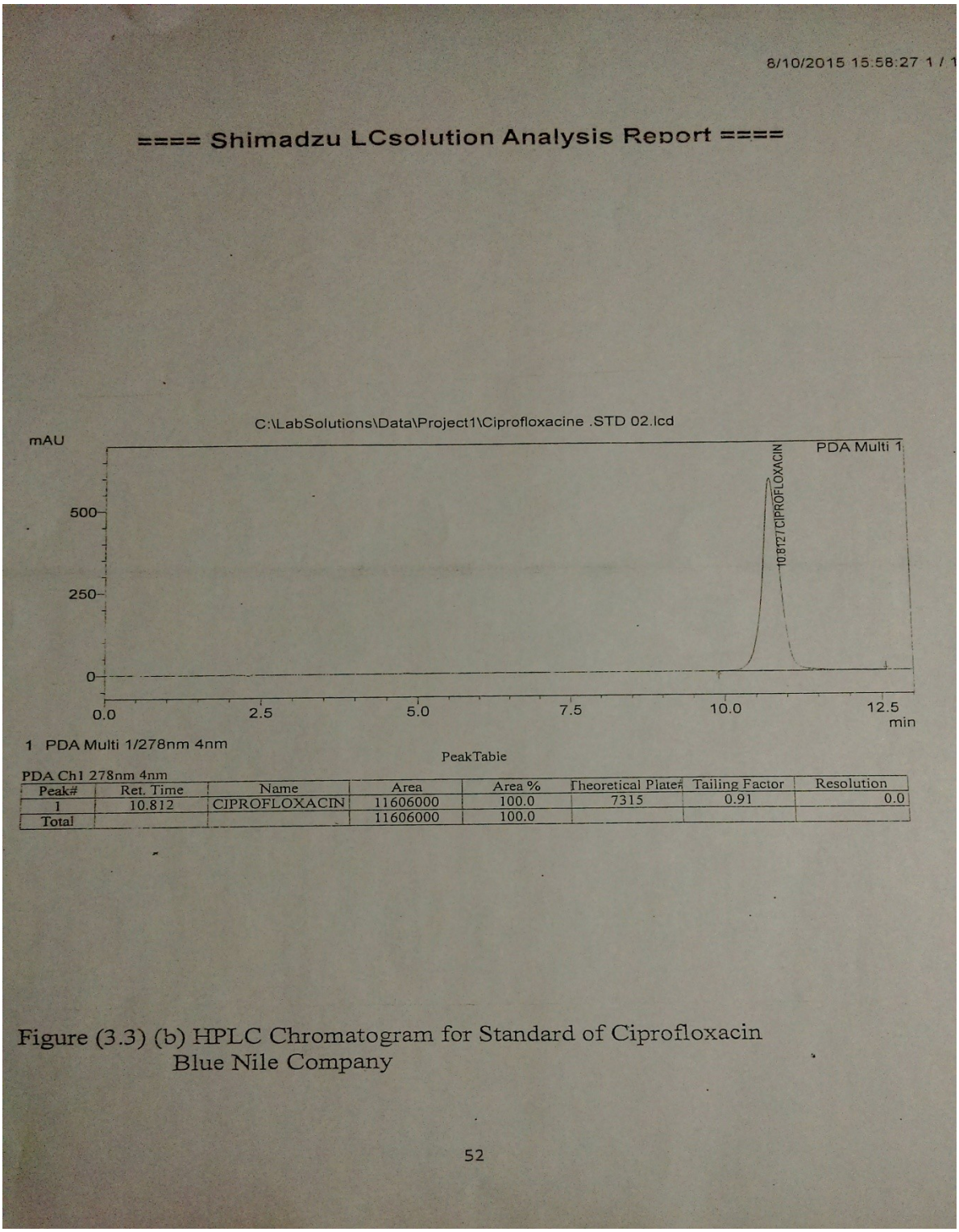


Figure (3.3) (b) HPLC Chromatogram for Standard of Ciprofloxacin
Blue Nile Company

Figure (3.3) (b) HPLC Chromatogram for Standard of Ciprofloxacin
(Blue Nile Company)

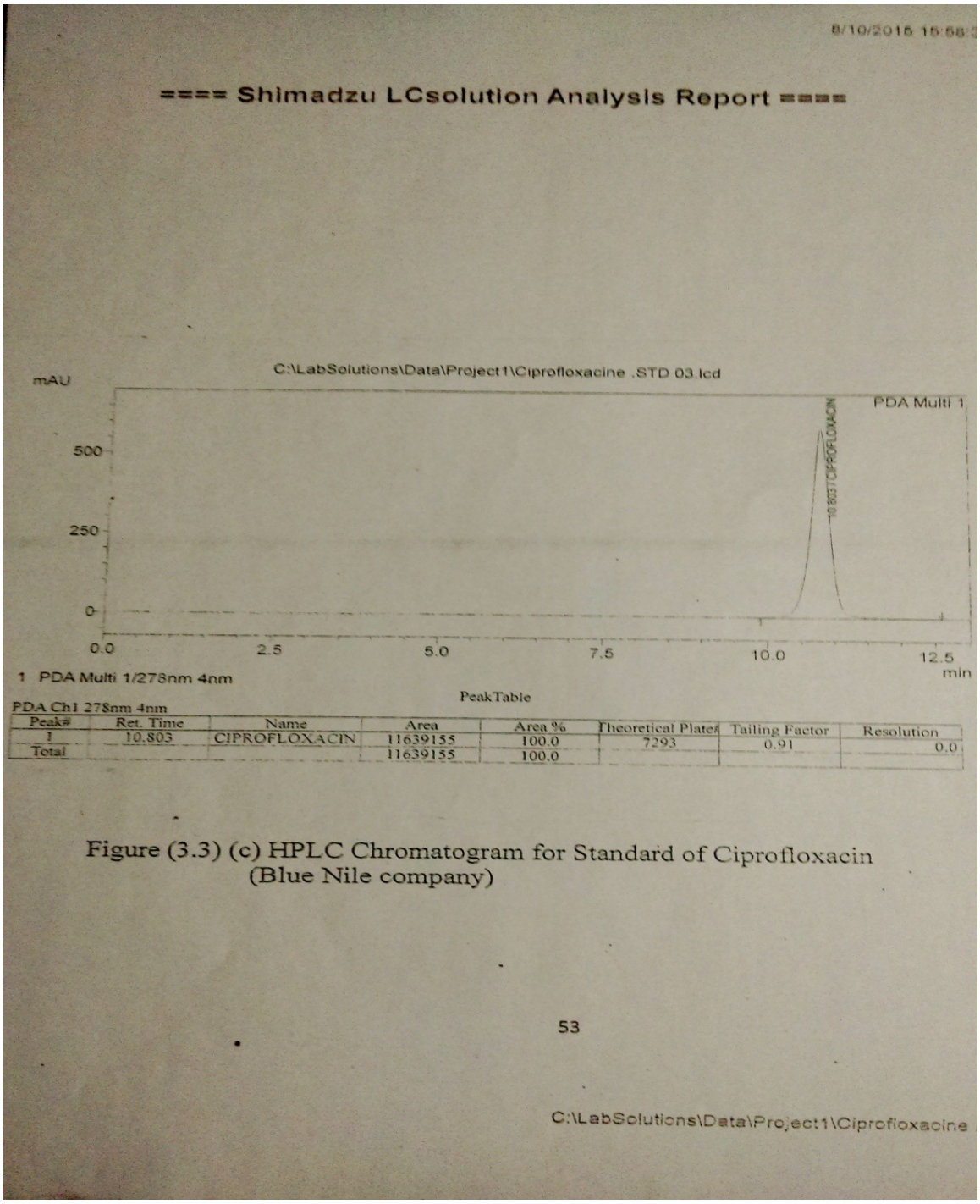


Figure (3.3) (c) HPLC Chromatogram for Standard of Ciprofloxacin
(Blue Nile company)

Figure (3.3) (c) HPLC Chromatogram for Standard of Ciprofloxacin
(Blue Nile Company)

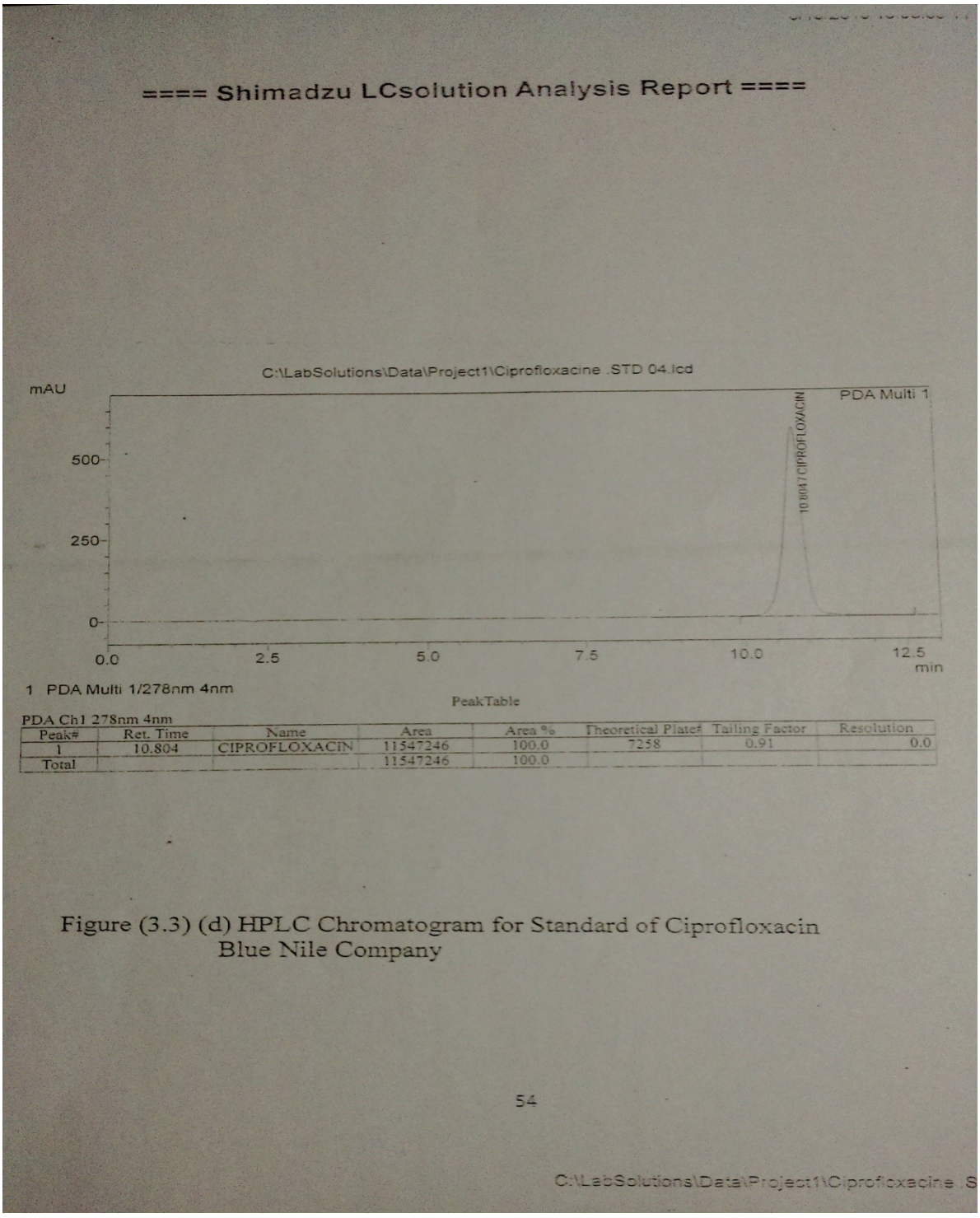


Figure (3.3) (d) HPLC Chromatogram for Standard of Ciprofloxacin
Blue Nile Company

Figure (3.3) (d) HPLC Chromatogram for Standard of Ciprofloxacin
(Blue Nile Company)

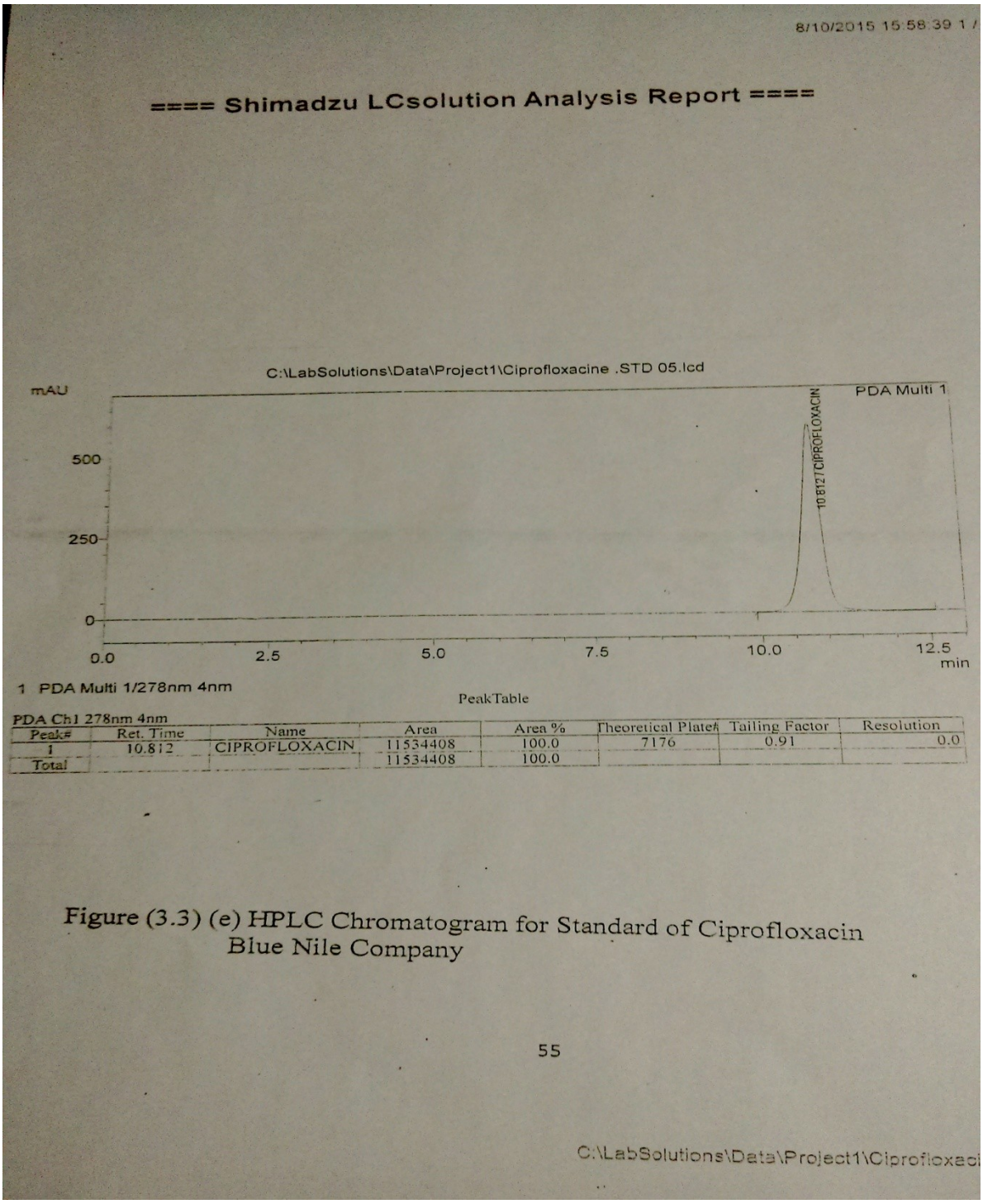


Figure (3.3) (e) HPLC Chromatogram for Standard of Ciprofloxacin
Blue Nile Company

55

Figure (3.3) (e) HPLC Chromatogram for Standard of Ciprofloxacin
(Blue Nile Company)

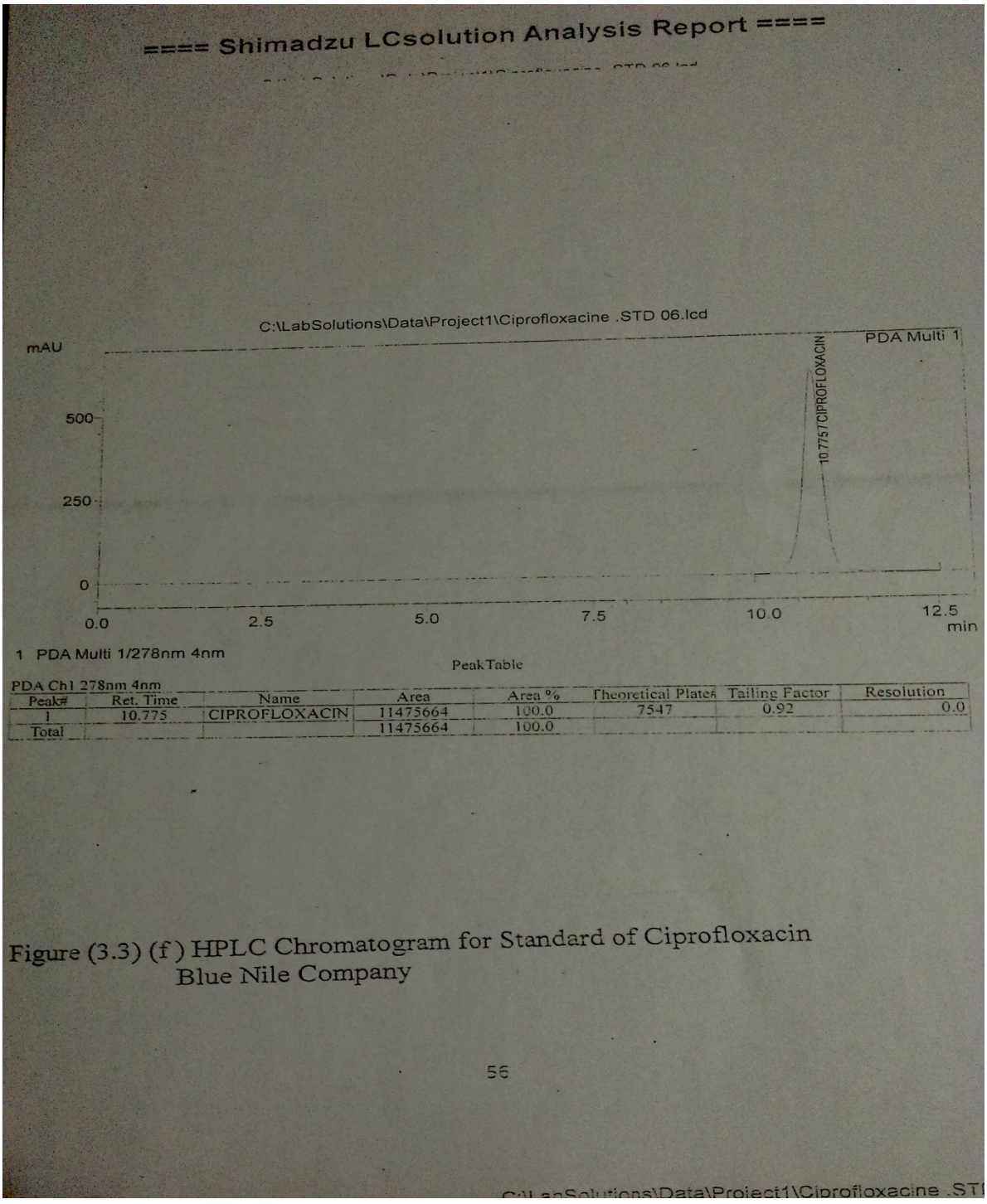


Figure (3.3) (f) HPLC Chromatogram for Standard of Ciprofloxacin
Blue Nile Company

Figure (3.3) (f) HPLC Chromatogram for Standard of Ciprofloxacin
(Blue Nile Company)

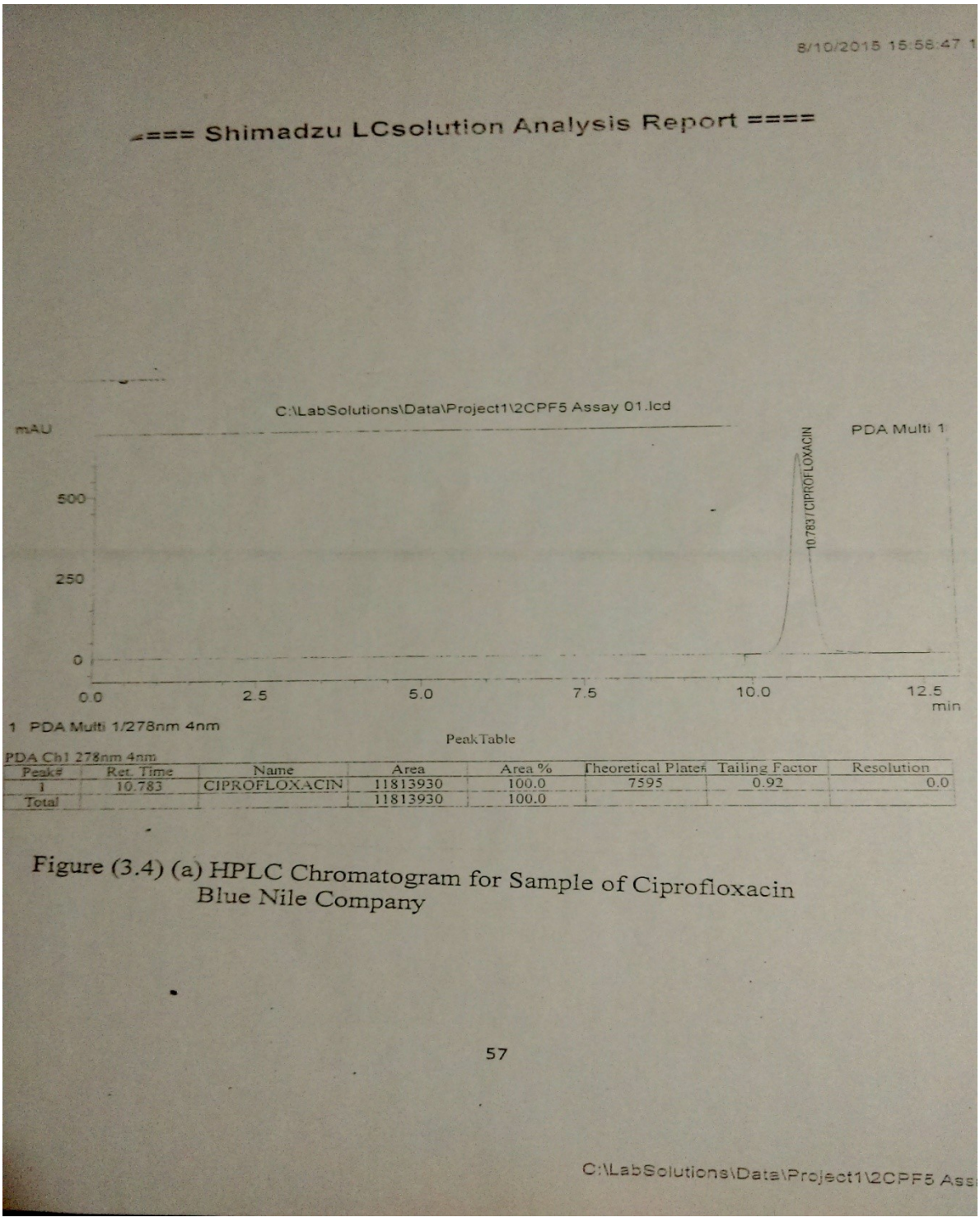
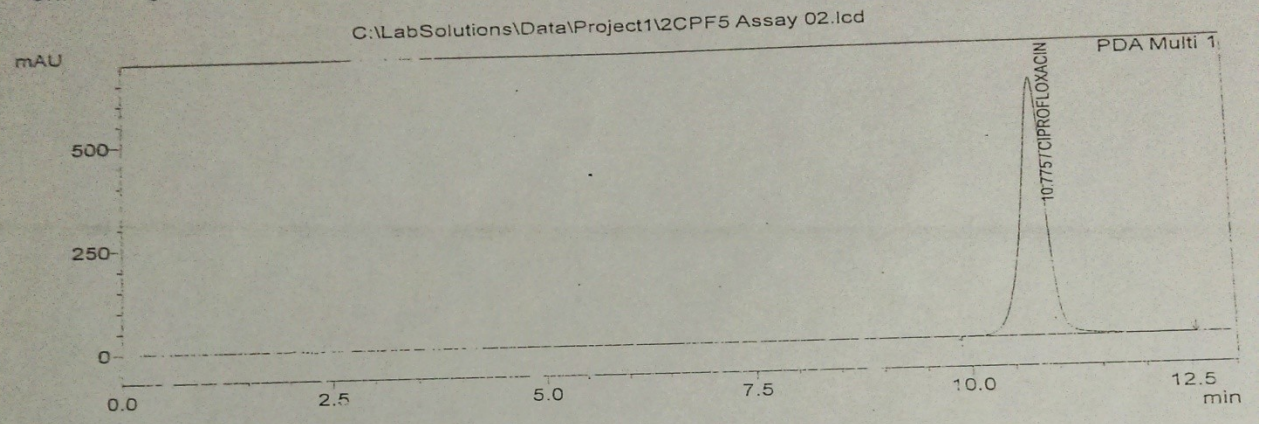


Figure (3.4) (a) HPLC Chromatogram for Sample of Ciprofloxacin
(Blue Nile Company)

==== Shimadzu LCsolution Analysis Report ====

<Chromatogram>



1 PDA Multi 1/278nm 4nm

PeakTable

PDA Ch1 278nm 4nm

Peak#	Ret. Time	Name	Area	Area %	Theoretical Plate#	Tailing Factor	Resolution
1	10.775	CIPROFLOXACIN	11853037	100.0	7484	0.92	0.0
Total			11853037	100.0			

Figure (3.4).(b) HPLC Chromatogram for Sample of Ciprofloxacin
Blue Nile Company

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Figure (3.4) (b) HPLC Chromatogram for Sample of Ciprofloxacin
(Blue Nile Company)

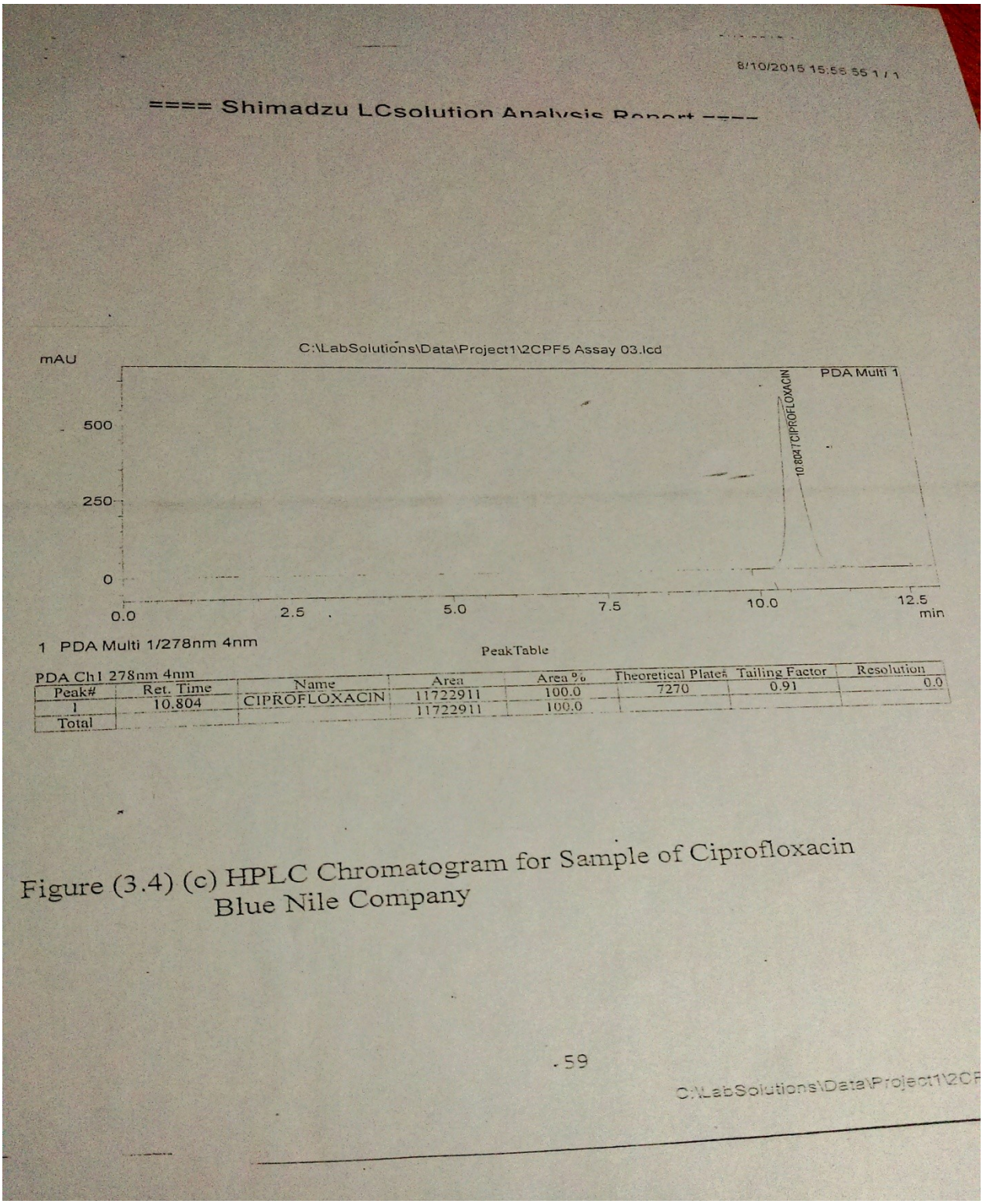

Figure (3.4) (c) HPLC Chromatogram for Sample of Ciprofloxacin
(Blue Nile Company)

3.1 Result of Assay for Shanghai and Blue Nile Companies

Table (3.1) Result of assay for Shanghai and Blue Nile companies:

Number	Shanghai peak area	Blue Nile peak area

	standard	sample	standard	Sample
1	3108262	3568532	11681581	11813930
2	3142107	3441084	1160630	11853037
3	3130296	3430611	11639155	11722911
4	3043961	3391891	11547246	-
-	-	-	11534408	-
-	-	-	11475664	-
Average of peak area	3106156.5	3458029.5	11580676	11796626

Calculation assay of shanghai company:

Average of sample = 0.75407

Factor = 331.34 / 385.82

Purity of standard = 98.5 %

Concentration of standard = .0488 mg / ml

Concentration of sample = 0.0716 mg / ml

Assay % of sample of Ciprofloxacin 500 mg

= Area of sample ×concentration of standard ×average of sample × purity of standard × factor

Area of standard × concentration of sample × label amount

% = 3458029.5×0.0488×0.75407×331.34×98.5 = 96.8%

3106156.5 0.0716 ×0.5 ×385.82

Calculation Assay of Blue Nile Company:

Factor =0.910332, Concentration of standard = 0.584 mg / ml, Concentration of sample = 0.5 mg / ml, Purity of standard = 93.8 %

Assay % of sample of Ciprofloxacin 500 mg=

= **Area of sample** * Concentration of standard * Purity of standard* Factor

Area of standard Concentration of sample

= (11796626/11580676)* (0.584/0.5) * 93.8 * 0.901033 = 100.56

Assay % of sample of Ciprofloxacin 500 mg with Shanghai and blue Nile companies were found to be 96.8% and 100.56%, respectively. That indicate the active material in ciprofloxacin drug will not affected by physicals, chemicals, and biological degradation.

3.2 Result of Dissolution by UV/Vis Equipment of Shanghai and Blue Nile Companies:

Table (3.2) Result of dissolution by UV/Vis Equipment of Shanghai and Blue Nile Companies:

Number	Shanghai absorption		% *	Blue Nile absorption		%*
	absorption			absorption		
	Standard	sample		standard	sample	
1		1.146	97.6		0.5843	94.24
2		1.291	100.2		0.5893	95.05
3	0.955	1.140	97.1	0.620	0.5463	88.11
4		1.185	100.9		0.5447	87.85
5		1.181	100.5		0.5523	89.08
6		1.180	100.4		0.561	90.48

Calculation of dissolution by u.v Equipment for Shanghai Company:

Concentration of standard = 0.01168 mg / ml

Concentration of sample = 0.0111 mg / ml

Purity of standard = 99.9 %

%* = ABS of sample× concentration of standard × purity of standard × factor

ABS of standard × concentration of sample

Calculation of dissolution by u.v Equipment for Blue Nile Company:

%* = ABS of sample/ ABS of stander * 100%

For the result obtained from the dissolution testing, which achieve for six samples of ciprofloxacin drug using dissolution apparatus and UV/Vis- spectroscopy as the detector to determine the amount of light absorbed by the dissolved molecules were found to be the concentration averages of ciprofloxacin drug dissolved approximately 100%, that indicate the ciprofloxacin drug undergo dissolved completely.

3.3 Result of Hardness, Thickness, and Disintegration Tests for Shanghai and Blue Nile Companies:

Table (3.3) Hardness, Thickness, and Disintegration Tests for Shanghai and Blue Nile Companies:

Test	Shanghai	Blue Nile
Hardness	19.7 kgf	17.2 kgf
Thickness	6.18 mm	4.7 mm
Disintegration	2:56 minute	2:23 minute

The hardness, thickness, and disintegration for shanghai company are 19.7 kgf, 6.18 mm, and 2:56 minute respectively, and for Blue Nile company are 17.2 kgf, 4.7 mm, and 2:23 minute respectively, which are acceptable results.

3.4 Result of Weight Variation Test for Shanghai and Blue Nile Companies

Table (3.4) Weight of Table:

Weigh per gram		
Number	**Shanghai**	**Blue Nile**
1	0.7274	0.718
2	0.7379	0.715 (min)
3	0.7287	0.722
4	0.7289	0.727
5	0.7384	0.726
6	0.7419	0.729
7	0.7302	0.719
8	0.7380	0.718
9	0.7476 (max)	0.732
10	0.7430	0.735
11	0.7367	0.725
12	0.7401	0.720
13	0.7366	0.737 (max)
14	0.7345	0.718
15	0.7461	0.719
16	0.7360	0.719

17	0.7464	0.729
18	0.7237	0.731
19	0.7269	0.737
20	0.7118 (min)	0.732
total	14.7008	14.450

Calculation of weight variation of shanghai company:

Average of weight = total weight/number of sample = 14.7008/20 = 0.7259

Weight variation (max) = ((max weight - average weight)/average weight) *100%

= ((0.7476 - 0.73504)/ 0.73504)* 100%

= +1.71%

Weight variation (min) = ((min weight - average weight)/average weight) *100%

= ((0.7118 - 0.73504)/ 0.73504) * 100%

= - 3.16%

Weight variation = (721.74 – 720.25) g

Calculation of weight variation of Blue Nile Company:

Average of weight = total weight/number of sample = 14.450/20 = 0.7225

Weight variation (max) = ((max weight - average weight)/average weight) *100%

= ((0.737 - 0.7225)/ 0.7225)* 100%

= +2.01%

Weight variation (min) = (| min weight - average weight | /average weight) *100%

= ((0.715 - 0.7225)/ 0.7225) * 100%

= 1.04%

Weight variation = (721.74 - 720.27) g

The weight variation of shanghai, and Blue Nile companies are lie in the limit determined, [721.00mg ± 5% (684.950mg-757.05mg)].

3.5 Result of Friability for Shanghai and Blue Nile Companies:

Table (3.5) Friability for Shanghai and Blue Nile Companies:

	Shanghai	Blue Nile

Weight of 20 tablet before the testing (w_b) per g	14.7008 g	14.450 g
Weight of 20 tablet after the testing (w_f) per g	14.6861 g	14.30839 g
Friability=((wb-wf)/wb)*100%	**0.1%**	**0.98%**

The friability of shanghai, and Blue Nile companies are lie in the limit determined, 1%.

3.6 Conclusion

- The result of assay ciprofloxacin drug in the two companies shanghai and blue Nile was found to be 96.8% and 100.56% respectively, that indicated for non-compose of drug.

- The result of dissolution tests of all samples of two companies was found to be 100% approximately, that indicated for very high solubility of ciprofloxacin drug in water and in the human body.

The result of hardness, thickness, disintegration, weight variation, and friability, for shanghai company were found to be 19.7 kgf, 6.18 mm, 2:56 minute, (721.74 – 720.25) g, and 0.1% respectively, and for Blue Nile company are 17.2 kgf, , 4.7 mm, and 2:23 minute, (721.74 – 720.27), and 0.98% respectively.

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