

**A METHOD VALIDATION FOR ASSAY OF IMIPRAMINE
HYDROCHLORIDE IN IMPRESS TABLET 25 MG BY USING
HIGH PERFORMANCE LIQUID CHROMATOGRAPHY/
PHOTO DIODE ARRAY**

RADIATUL NADIAH BINTI RAZALI

**FACULTY OF SCIENCE
UNIVERSITI OF MALAYA
KUALA LUMPUR**

2014

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**A RESEARCH PROJECT REPORT SUBMITTED TO THE
DEPARTMENT OF CHEMISTRY, FACULTY OF SCIENCE,
UNIVERSITY OF MALAYA, IN PARTIAL FULFILMENT FOR THE
DEGREE OF MASTER OF SCIENCE (ANALYTICAL CHEMISTRY
AND INSTRUMENTAL ANALYSIS)**

**DEPARTMENT OF CHEMISTRY
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ORIGINAL LITERARY WORK DECLARATION

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Field of Study: **ANALYTICAL CHEMISTRY**

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ABSTRACT

Imipress Tablet 25 mg is one of the products of Chemical Company of Malaysia, Duopharma Sdn. Bhd, CCMD. The Active Pharmaceutical Ingredient is Imipramine Hydrochloride which contains 25 mg per tablet. Method validation was carried out in terms of specificity (force degradation), placebo analysis, linearity and range, accuracy, precision (repeatability and intermediate precision), robustness and solution stability. This method has been shown to be linear, accurate, precise, rugged and robust. The HPLC method is suitable for use as a stability indicating method for determination of Imipramine Hydrochloride content in Imipress Tablet 2 mg. Good linearity was established with R^2 of 1.000 with the mean accuracy of 99.83%. All the RSD (%) were less than 2% and 1% for %RSD of standard peak area and %RSD of retention time, respectively. The analytical method developed and validated for assay by HPLC is suitable for the accurate and precise determination of Imipramine Hydrochloride content in Imipress Tablet 2 mg.

ABSTRAK

Imipress Tablet 25 mg adalah salah satu produk *Chemical Company of Malaysia, Duopharma Sdn. Bhd, CCMD*. Ramuan Farmaseutikal Aktif Imipramine Hidroklorida mengandungi 25 mg untuk sebiji pil. Pengesahan kaedah telah dijalankan dari segi kekhususan (memaksa degradasi), analisis plasebo, kelinearan dan pelbagai, ketepatan (kebolehulangan dan ketepatan perantaraan), kemantapan dan kestabilan penyelesaian. Kaedah ini telah terbukti linear, tepat, tahan lasak dan teguh. Kaedah HPLC adalah sesuai untuk digunakan sebagai kestabilan kaedah yang menunjukkan untuk penentuan kandungan Imipramine Hidroklorida dalam *Imipress Tablet 2 mg*. Kelinearan baik telah ditubuhkan dengan R^2 1.000 dengan ketepatan purata sebanyak 99.83%. Semua sisihan piawai relatif (%) adalah kurang daripada 2% dan 1% untuk% sisihan piawai relatif luas permukaan untuk *standard* dan% sisihan piawai relatif untuk *retention time*. Kaedah analisis yang dibangunkan dan disahkan untuk cerakin oleh HPLC sesuai untuk penentuan secara tepat dan tepat kandungan Imipramine Hidroklorida dalam *Imipress Tablet 2 mg*.

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LIST OF ABBREVIATIONS

API	Active Pharmaceutical Ingredients
CCM	Chemical Company of Malaysia
CCMD	Chemical Company of Malaysia Duopharma
cGMP	Current Good Manufacturing Practice
FDA	Food and Drug Administration
GCMS	Gas Chromatography Mass Spectrometry
GMP	Good Manufacturing Practice
HPLC	High Performance Liquid Chromatography
ICH	International Conference on Harmonization
IH	Imipramine Hydrochloride
Ion-SME	Ion Selective Membrane electrode
IUPAC	International Union and Pure of Applied Chemistry
MDD	Major Depressive Disorder
NPCB	National Pharmaceutical Control Bureau
PDA	Photo Diode Array
QC	Quality Control
R ²	Correlation coefficient
RSD	Relative Standard Deviation
R _t	Retention time
SD	Standard deviation
TLC	Thin Layer Chromatography
Uv-Vis	Ultra Violet

CHAPTER 1

INTRODUCTION

1.1 Background of Study and Statement of Problem

1.1.1 Analytical Method Validation in Pharmaceutical Industry

Analytical method validation is just one type of validation required during drug development and manufacturing. To comply with the requirement of current GMP, pharmaceutical companies should have an overall validation policy which documents how validation will be performed. This will include the validation of production processes, cleaning procedures, analytical method, in process control test procedure and computerized system. The purpose of this validation is to show that processes involved in the development and manufacturer of drugs, such as production, cleaning and analytical testing can be performed an effective and reproducibility manner.^[7]

The reason that validation is included in cGMP in this way is to ensure that quality is built in at every step and not just tested for at the end. Validation is intended to provide assurance of the quality for the design, manufacturer and use of the system or process that cannot be found by simple testing alone.^[7]

Once a method has been developed and validated it may be used for routine analysis as shown in Figure 1. However, changes may occur which make it necessary to evaluate whether the method is still suitable for its intended use. The change may be covered or may result in revalidation and in some cases, redevelopment of the method followed by validation of the new method.

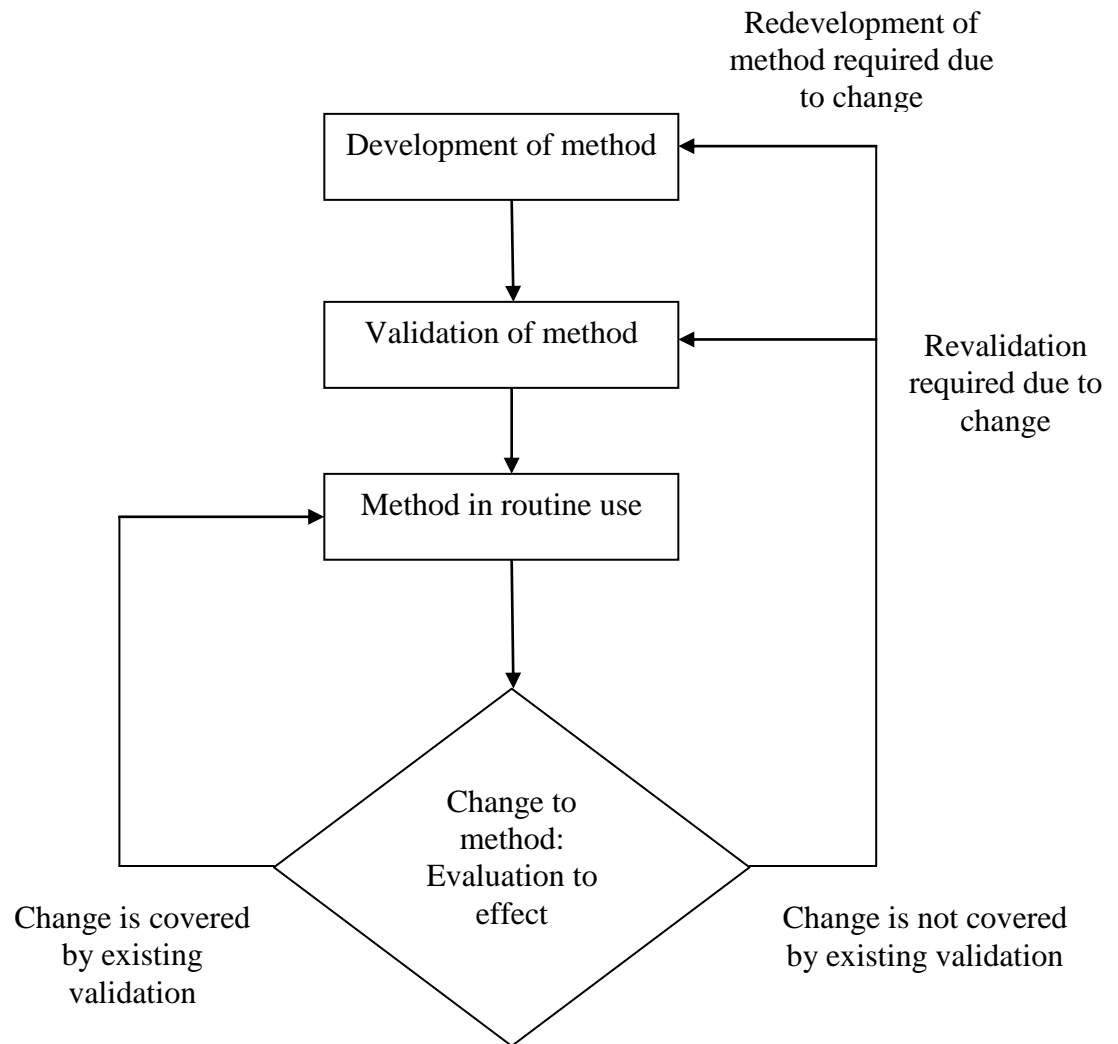


Figure 1: The cycle of analytical method validation

1.1.2 Active Pharmaceutical Ingredient: Imipramine Hydrochloride

Imipress Tablet 25 mg is one of the products of Chemical Company of Malaysia, Duopharma Sdn. Bhd, CCMD. The API is Imipramine Hydrochloride, IH, which contain 25 mg per tablet. The total weight of one individual Imipress Tablet 25 mg is 140 mg, which contain 115 mg of placebo. The placebo (also known as excipient) consist of Magnesium Stearate (2.1 mg), Lactose (98.578 mg), Aerocil (0.28 mg), Corn Starch (7 mg), Polyvinylpyrrolidone (2.8 mg), Carmoisine (0.042 mg) and Promigel (4.2 mg).

IH is a tricyclic antidepressant of the dibenzazepine group. IH also known as *Tofranil* as the trade name. ^[11] The molecular structure and the characteristic of IH had shown in Figure 2 and Table 1, respectively.

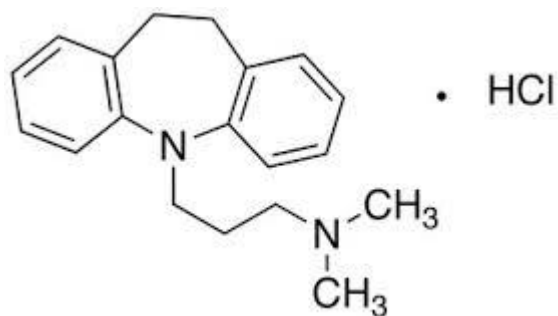


Figure 2: Imipramine Hydrochloride

Systematic	3-(10,11-dihydro-5 <i>H</i> -dibenzo[<i>b,f</i>]azepin-5-yl)- <i>N,N</i> -
IUPAC name	dimethylpropan-1-amine
Formula	C ₁₉ H ₂₅ ClN ₂
Molar Mass	316.5 g/mol
Characters	A white or slightly yellow and crystalline powder
Solubility	Freely soluble in water and in alcohol

Table 1: Imipramine Hydrochloride

The metabolism of IH within the body, it is converted to desipramine which belong to another type of Tricyclic antidepressant.^[13] The side effects of IH include the central nervous system such as dizziness, drowsiness, headache, weakness, insomnia, nightmares and increase psychiatric symptoms. For gastrointestinal, the patient will have for dry mouth, nausea, vomiting, increase appetite, cramps, jaundice and taste change. Moreover, the symptoms and the treatment of an Imipramine overdose are largely the same for the other tricyclic antidepressants. Cardinal symptoms are cardiac and neurological disturbances. Any ingestion by children should be considered as serious and potentially fatal.^[11]

1.2 Significant of Study

This study focuses on the extraction of the IH, determination of analytical characteristics method and percent assay of IH in Imipress Tablet 25 mg. The concerns about the percent assay of this API contain in the medicine have promoted for the Assay studies. The use of analytical methods during development and manufacturing provides information on potency which can relate directly to the requirements of known dose; impurities which can relate to the safety profile of the drug, evaluation of key drug characteristics such as crystal form, drug release, drug uniformity and properties which can compromise bioavailability; degradation products methods need to be stability indicating and effect of key manufacturing parameter to ensure that the production of drug substance and drug product is consistent.^[8]

The validation which is performed on the method which generate the data needs to demonstrate that they can do so reliability and consistently. Hence, this method validation is use full for routine analysis in QC and to demonstrate that the test method of IH in Imipress Tablet 25 mg is suitable for the intended use.

1.3 Objectives of the study

An analytical method details the steps necessary to perform an analysis. This may include preparation of samples, standards and reagents, use of apparatus, generation of the calibration curve, use of the formula for calculation.^[8] The intention of this study is to validate the assay test method by high performance liquid chromatographic analytical method in quantitating IH in Imipress Tablet 25 mg.

CHAPTER 2

LITERATURE REVIEW

2.1 **Functionality of Imipramine Hydrochloride**

The primary function of IH is to treatment of mental depressive disorder, MDD is a mental disorder characterized by a pervasive and persistent low mood that is accompanied by low self-esteem and by a loss of interest or pleasure in normally enjoyable activities. MDD is a disabling condition that adversely affects a person's family, work or school life, sleeping and eating habits and general health.^[9]

2.2 **Therapeutic Uses of Imipramine Hydrochloride**

IH also used to treatment of enuresis which refers to repeated inability to control urination which usually limited to describing old enough to be expected to exercise such control.^[10] IH is used in the treatment of depression such as associated with agitation or anxiety and has similar efficacy to the antidepressant drug moclobemide.^[12]

2.3 **Mechanism of Action of Imipramine Hydrochloride**

IH affects numerous neurotransmitter systems known to be involved in the etiology of depression, anxiety, enuresis and numerous other mental and physical conditions. IH is similar in structure to some muscle relaxants and has a significant analgesic effect that is very use full in some pain conditions.^[11]

2.4 Analytical determination of Imipramine Hydrochloride

A variety of procedures have been developed for the analysis of IH. IH has been determined by a variety of analytical technique such as spectrophotometry, spectofluorimetry, conductimetry and flow injection methods. However, many of these methods are limited in their applications or rather tedious and time consuming.^[15]

HPLC has been applied to measure small amounts of IH and its major metabolite, desipramine in serum. A computerized GCMS technique has also been proposed for quantitatively determination of IH. TLC method has been used for the separation and detection of IH.^[14] Ion-SME can be applied successfully for the determination of IH in substance and in pharmaceuticals.

The recommended method from monograph which includes United State Pharmacopia, USP 36 and British Pharmacopia, BP 2013 for the quantitating percent assay of “*Imipramine Hydrochloride Tablet*” is by using UV-Vis Spectrophotometer. This method is simple and sensitive for the determination of IH in Imipress Tablet 25 mg.

However, in this study, the method validation of Imipramine Hydrochloride in Imipress Tablet 25 mg uses an in-house method as the analytical test method. The method originally adopted from USP 36, Volume 2 under “Imipramine Hydrochloride”, page 3888 (refer to Figure which using the HPLC method. This method considered as in-house method because the validation of IH are base on raw material method rather than choosing finish product method.

In CCMD, there were three different departments in laboratory side which include QC Department, Validation Department and Stability Department. In order to standardize the analytical test method for IH in Imipress Tablet 25 mg for all departments, only one establishes method will be chosen as the validated method.

For stability department, the use of HPLC method are more recommended because recent days, the current guidelines and regulatory requirements are very stringent in terms of estimation and quantification of residual impurities in any synthetic route or process, due to this reason the separation of all compounds in single run or during analysis, LC methods was taken preference over regular conservative mode methods of analysis. The advantages of LC methods are that the developed analytical methods were posses’ greater selectivity, sensitivity, accurate, precise and robust. Therefore, all most all methods used in Stability Department was developed by using HPLC.^[15]

2.5 Method Validation in according to ICH Guidelines

According to International Conference on Harmonization, ICH, Guidelines, the discussion of the validation of analytical procedures is directed to the four most common types of analytical procedures which are identification test, quantitative tests for impurities' content, limit tests for the control impurities and quantitative test of the API in samples of drug substance or drug or other selected components (s) in the drug product.^[2] The parameter to be validated for assay study will base on the Table 2.

Type of analytical procedure characteristics	Identification	Testing for Impurities (quantitative Limit)		Assay (dissolution, content and potency)
Accuracy	-	+	-	+
Precision	-	+	-	+
Repeatability	-	+	-	+
Intermediate	-	+ ⁽¹⁾	-	+ ⁽¹⁾
Precision				
Specificity	+	-	+	+
Detection Limit	-	- ⁽³⁾	+	-
Quantitation Limit	-	+	-	-
Linearity	-	+	-	+
Range	-	+	-	+

- Signifies that this characteristics is not normally evaluated

+ Signifies that this characteristics is normally evaluated

⁽¹⁾ In cases where reproducibility has been performed, intermediate precision is not needed.

⁽²⁾ Lack of specificity of one analytical procedure could be compensated by other supporting analytical procedures

⁽³⁾ May be needed in some cases.

Table 2: Type of analytical procedure characteristic

CHAPTER 3

METHODOLOGY

3.1 Chemicals and reagents

Imipramine Hydrochloride (IH) Working Standard was an in-house working standard. HPLC grade methanol and HPLC grade acetonitrile were purchased from Merck. Triethylamine, Perchloric acid (for adjusting pH) and Sodium Perchloride were purchased from Fluka. Deionized water was used throughout the study.

3.2 Instrumentation and chromatographic conditions

Quantitative analysis of IH was carried out using a HPLC unit that consisted of a degasser, Model: Shimadzu DGU-20A₃ Degasser; pump; Model: Shimadzu LC-20AT Prominence LC; Model: Shimadzu SIL-20A Prominence, Column oven; Model Shimadzu CTO – 10AS VP and computer; Model: DELL. The injection was done by using 20 μ L by auto sampler; Model: Shimadzu SIL-20A Prominence.

Separation was achieved using a 5 μ m Phenomenex C18, Gemini (150 mm length \times 4.6 mm diameter). The column temperature was set to 40°C. The mobile phase was 0.06 M Sodium Perchlorate: Acetonitrile: Triethylamine (625:375:1) with adjustment the pH 2.0 with Perchloric Acid and the flow rate was 1.0 mL min^{-1} . The detector was set at 269 nm.

3.3 Apparatus

The apparatus used in this study were 25 mL, 50 mL, 100 mL, 200 mL and 1000 mL volumetric flasks; 5 mL, 6 mL, 10 mL, 15 mL pipettes; sonicator; water bath and beaker.

3.4 Preparation of 0.06M Sodium Perchlorate

Sodium perchlorate about 7.34.64 g weight was transferred into 1000 mL volumetric flask. All chemical was dissolved and diluted with water and it will mix well. Mass was calculated using following calculation:

$$\text{Mole} = \text{Molarity} \times \text{Volume (L)}$$

$$= 0.06 \text{ M} \times 1 \text{ L}$$

$$= 0.06 \text{ mole}$$

$$\text{Mass} = \text{Mole} \times \text{Molecular weight}$$

$$= 0.06 \text{ mole} \times 136.086 \text{ g/mole}$$

$$= 3.402 \text{ g}$$

3.5 Preparation of diluent

The diluent was water and acetonitrile with the ratio 5:3. The use of the diluent is for dilution.

3.6 Preparation of standard (Imipramine Hydrochloride Working Standard)

30 mg of IH working standard was transferred into 100 mL volumetric flask and top up to volume using diluent. Then, label the solution as a **Standard Solution**. The **Standard Solution** contains approximately 0.3 mg/mL of IH. This **Standard Solution** was filtered through a 0.45 μ m membrane filter and inject into the HPLC system for analysis.

3.7 Preparation of sample (Imipress Tablet 25 mg)

20 tables of Imipress Tablet 25 mg is weigh to get the average weight of this tablet. Grind the tablet sample into fine powder. Transfer about 168 mg (equivalent to 30 mg of IH) of sample into a 100 mL volumetric flask. Dilute to volume with diluent, mix well and label it as **Sample Solution**. The **Sample Solution** contains approximately 0.3 mg/mL of IH. Sample Solution is filter through a 0.45 μ m membrane filter and inject once into the HPLC system for analysis. Six replicate of sample preparation will be performing.

3.8 Method Validation Procedure

3.8.1 System Suitability

The Standard Solution from Section 3.6 is use for the system suitability.

The system suitability requirements for the above assay chromatographic procedure are shown in Table 3:

Parameters	Criteria
Tailing Factor	≤ 2
System precision	i) % RSD of Standard retention time $\leq 1\%$
	ii) % RSD of Standard peak area $\leq 2\%$
Theoretical Plate Count	>2000

Table 3: Parameter for system suitability

3.8.2 Placebo analysis

The placebo is prepared by mixing all the excipients and kept in an amber glass bottle. This placebo is a combination of all excipients for Imipress Tablet 25 mg as shown in Table 4. For the procedure, transfer about 138 mg of Placebo into a 100 mL volumetric flask, dilute to volume with diluent and mix well. Label this as **Placebo Solution**. The **Placebo Solution** contains approximately 1.38 mg/mL of Placebo. **Placebo Solution** is filter through a 0.45 μ m membrane filter and inject into the HPLC system for analysis. One (1) Imipress Tablet 25 mg contains 115 mg of placebo and 25 mg of IH. For assay sample preparation, the sample solution contains 0.3 mg/mL of IH and 1.38 mg/mL of placebo.

No	Excipients	Excipients (mg)	(w/w)	(w/w)%	Quantity of 300 tablets (mg)
1	Magnesium Stearate	2.1	0.0182	1.82	5.46
2	Aerosil	0.28	0.0024	0.24	0.72
3	Cornstarch	7	0.0608	6.08	18.24
4	Lactose	98.578	0.8572	85.72	257.16
5	Polyvinylpyrrolidone	2.8	0.0243	2.43	7.29
6	Carmoisine	0.042	0.0003	0.03	0.09
7	Promigel	4.2	0.0365	3.65	10.95
Total		115	0.9997	99.97	299.91

Table 4: Placebo for Imipress Tablet 25 mg

3.8.3 Specificity (Forced Degradation)

Forced degradation has been carried out by introducing chemical degradant into sample in three different routes.

3.8.3.1 Control

420 mg of powdered tablets was weight (equivalent to 75 mg of IH) and transferred into 50 mL volumetric flask, dissolved and diluted to volume with diluent. This solution was heated in water bath at 70°C for 2 hours. This solution was labeled as **Sample Solution (a1)**. 5 mL of **Sample Solution (a1)** was pipette into 25 mL volumetric flask and top up to volume with diluent. The solution was labeled as **Sample Solution (a2)**. This solution contains approximately 0.3 mg/mL of IH and 1.38 mg/mL of Placebo and injected with 20 µL of the resulting solution in the HPLC system.

3.8.3.2 Acid hydrolysis

420 mg of powdered tablets was weight (equivalent to 75 mg of IH) and transferred into 50 mL volumetric flask, dissolved and diluted to volume with 3M HCl. This solution was heated in water bath at 70°C for 2 hours. This solution was labeled as **Sample Solution (b1)**. 5 mL of **Sample Solution (b1)** was pipette into 25 mL volumetric flask. For neutralization, add 5 mL of 3M NaOH and top up to volume with diluent. This solution was labeled as **Sample Solution (b2)**. This solution contains approximately 0.3 mg/mL of IH and 1.38 mg/mL of Placebo and injected with 20 µL of the resulting solution in the HPLC system.

3.8.3.3 Base hydrolysis

420 mg of powdered tablets was weight (equivalent to 75 mg of IH) and transferred into 50 mL volumetric flask, dissolved and diluted to volume with 3M NaOH. This solution was heated in water bath at 70°C for 2 hours. This solution was labeled as **Sample Solution (c1)**. 5 mL of **Sample Solution (c1)** was pipette into 25 mL volumetric flask. For neutralization, add 5 mL of 3M HCl and top up to volume with diluent. This solution was labeled as **Sample Solution (c2)**. This solution contains approximately 0.3 mg/mL of IH and 1.38 mg/mL of Placebo and injected with 20 µL of the resulting solution in the HPLC system.

3.8.3.4 Oxidation degradation

420 mg of powdered tablets was weight (equivalent to 75 mg of IH) and transferred into 50 mL was heated in water bath at 70°C for 2 hours This solution was labeled as **Sample Solution (d1)**. 5 mL of **Sample Solution (d1)** was pipette into 25 mL volumetric flask and top up to volume with diluent. Label this as **Sample Solution (d2)**. This solution contains approximately 0.3 mg/mL of IH and 1.38 mg/mL of Placebo and injected with 20 µL of the resulting solution in the HPLC system.

The acceptance criteria that need to full fill were listed below:

- a) Analyte peak is well resolved from the other peaks, resolution > 1.5
- b) Analyte peak degraded at least 20% by one of the degradation method
- c) Peak purity index > 0.95

3.8.4 Linearity and Range

Standard Stock Solution was prepared by dissolving approximately 300 mg of Imipramine Hydrochloride in 200 mL volumetric flask with diluent (concentration is 1.5 mg/mL of Imipramine Hydrochloride). From this **Standard Stock Solution**, a series of five standards that span a range of 50-150% were prepared.

For 50% (0.15 mg/mL of Imipramine Hydrochloride), 5 mL of **Standard Stock Solution** was pipette into 50 mL volumetric flask and top up to volume with diluent. For 80% (0.24 mg/mL of Imipramine Hydrochloride), 4 mL of **Standard Stock Solution** was pipette into 25 mL volumetric flask and top up to volume with diluent. For 100% (0.3 mg/mL of Imipramine Hydrochloride), 10 mL of **Standard Stock Solution** was pipette into 50 mL volumetric flask and top up to volume with diluent. For 120% (0.36 mg/mL of Imipramine Hydrochloride), 6 mL of **Standard Stock Solution** was pipette into 25 mL volumetric flask and top up to volume with diluent. For 150% (0.45 mg/mL of Imipramine Hydrochloride), 15 mL of **Standard Stock Solution** was pipette into 50 mL volumetric flask and top up to volume with diluent. Each solution were injected three times into the HPLC system and record the chromatogram of each solution, at 50%, 80%, 100%, 120% and 150% according to the method as described in section 3.2.

The peak area ratio for each level versus concentration was plotted and performs a linear regression using the least square method on the resulting curve. The acceptance criteria that need to full fill are listed below:

- a) $R^2 > 0.999$
- b) %RSD of peak response ratio $\leq 2\%$.
- c) Magnitude of intercept against 100% working standard $\pm 2\%$.

3.8.5 Accuracy

For accuracy, **Standard Stock Solution** from Section 3.4.4 was used and placebo standard solution was prepared by dissolving 345 mg of placebo into 50 ml volumetric flask with diluent (concentration is 6.9 mg/mL of placebo). Accuracy is assessed by performing 3 separate replicate recovery experiments with 'spiked standard' at 3 levels; 50%, 100% and 150% of the working concentration of Imipramine Hydrochloride.

10 mL of placebo was transferred into 50 mL volumetric flask; 5 mL of standard stock solution was spiked into the solution and top up to volume with diluent. This solution contains 50% of spiked Imipramine Hydrochloride (0.15 mg/mL of Imipramine Hydrochloride and 1.38 mg/mL of placebo).

10 mL of placebo was transferred into 50 mL volumetric flask; 10 mL of standard stock solution was spiked into the solution and top up to volume with diluent. This solution contains 100% of spiked Imipramine

Hydrochloride (0.3 mg/mL of Imipramine Hydrochloride and 1.38 mg/mL of placebo).

10 mL of placebo was transferred into 50 mL volumetric flask; 15 mL of standard stock solution was spiked into the solution and top up to volume with diluent. This solution contains 150% of spiked Imipramine Hydrochloride (0.45 mg/mL of Imipramine Hydrochloride and 1.38 mg/mL of placebo). Each spiked sample was injected once into the HPLC system. For each sample, the sample concentration for Imipramine Hydrochloride using standards prepared was calculated. The percent recovery from the sample at each level was computed. The acceptance criteria that need to full fill were listed below:

- a) Overall mean accuracy within $100\% \pm 2\%$
- b) Overall %RSD for the measurement precision $\leq 2\%$
- c) Confidence limits at 95%, μ is $100\% \pm 2\%$

3.8.6 Precision

3.8.6.1 Repeatability

For repeatability test, 6 replicates of sample according to method as described in section 3.7 were prepared versus a freshly prepared standard as described in section 3.6. The precision between individual results was calculated and expressed as %RSD. The acceptance criteria that need to full fill were listed below:

- a) %RSD of standard retention time $\leq 1\%$

- b) %RSD for standard peak area $\leq 2\%$
- c) %RSD of sample result $\leq 2\%$
- d) Theoretical plate count > 2000
- e) Tailing factor ≤ 2

3.8.6.2 Intermediate Precision

Intermediate precision was carried out by different analyst on different day, using different HPLC instrument and also different standard and samples preparation. The samples were prepared in 6 replicates according to method described in section 3.7. The standard solution was prepared as described in section 3.6. The acceptance criteria that need to full fill were listed below:

- a) %RSD of standard retention time $\leq 1\%$
- b) %RSD of the standard peak area $\leq 2\%$
- c) %RSD of sample result $\leq 2\%$
- d) Difference of mean value for results between analysts within $\pm 2\%$.

3.8.7 Robustness

For robustness test, 6 replicates of sample were prepared according to the method as described in section 3.7 versus a freshly standard as described in section 3.6. The robustness analysis was performed under different conditions that vary slightly from the method parameters. Six replicate samples were analyzed at different flow rate, pH and different ratio of mobile phase, refer to Table 5:

Condition	Flow Rate (mL/min)	pH	Mobile Phase		Triethylamine	Remarks
			0.06M Sodium Perchlorate	Acetonitrile		
A	1.0	2.0	625	375	1	Original
B	1.2	2.0	625	375	1	Increase flow rate
C	0.8	2.0	625	375	1	Decrease flow rate
D	1.0	2.0	605	395	1	Increase organic mobile phase
E	1.0	2.0	645	355	1	Decrease organic mobile phase
F	1.0	2.2	625	375	1	Increase pH
G	1.0	1.8	625	375	1	Decrease pH

Table 5: Parameter for Robustness

The mean results for different conditions were compared against the mean result for the original condition. The acceptance criteria that need to full fill were listed below:

- a) System suitability for 7 conditions is met if:
 - i. Tailing factor of standard solution is ≤ 2
 - ii. Theoretical plate count of 7 injections of standard solution > 2000
 - iii. Precision of 6 injections of standard solution (%RSD) $\leq 2\%$
- b) Difference of mean values of assay results for 6 conditions compare to original conditions are not more than $\pm 2\%$
- c) % RSD of standard retention time for 7 conditions are $\leq 1\%$
- d) % RSD of sample assay for 7 conditions are $\leq 2\%$

3.8.8 Solution Stability

For solution stability test, 6 replicates of sample were prepared according to the method as described in section 3.7 versus a freshly prepared standard as described in section 3.6. The samples were kept in the HPLC auto sampler at room temperature and re-inject after 24 hours to determine whether the samples are stable within 24 hours. A fresh new standard solution was prepared and compared to the standard solution that kept for the specific hours at room temperature. The mean assay value for samples after 24 hours using new standard was compared against mean assay value for samples at initial injection. The acceptance criteria that need to full fill were listed below:

- a) Difference of mean values assay results $\leq 2\%$
- b) Difference of peak area for freshly prepared standard solution and standard solution kept for specific hours is between 98% to 102%

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Method Validation for Assay Test Method

Method Validation is the process to confirm that the analytical procedure employed for a specific test is suitable for its intended use.^[2] Methods need to be validated or revalidated before their introduction into routine use, whenever the conditions change for which the method has been validated, whenever the method is changed and the change is outside the original scope of the method, when quality control indicates an established method is changing with time and in order to demonstrate the equivalence between two methods.^[3]

The validity of this method has been demonstrated in laboratory experiments using samples and standards that are similar to the samples analyzed routinely.^[3] In this study, the method was validated in terms of system suitability, specificity force degradation placebo analysis, linearity and range; accuracy, intermediate precision, robustness and solution stability.

4.2 System Suitability

In many analytical procedures, the integral part is the system suitability testing. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such System Suitability test parameters to be established for a particular procedure depend on the type of procedure being validated. ^[1] The system suitability results and chromatogram of IH Working Standard in this study were showed in Table 6 and Chromatogram 1 (Appendix 2), respectively.

Parameters	Acceptance Criteria	Results
Tailing Factor	≤ 2	1.6
System precision	i) % RSD of Standard retention time $\leq 1\%$	0.4
	ii) % RSD of Standard peak area $\leq 2\%$	0.0
Theoretical Plate Count	> 2000	6676.8

Table 6: The system suitability results for IH Working Standard

4.2.1 Placebo analysis

Placebo also known as excipient was the inactive ingredient in drug product. The placebo analysis was performed in order to investigate whether the placebo peak will interfere with the analyte peak or not. In this study, it shows that the placebo in Imipress Tablet 25 mg peak not give any interference to the IH peak (Chromatogram 2 and 3 in Appendix 2).

4.2.2 Specificity (Forced Degradation)

Forced degradation usually involved the exposure of representative samples of drug product to the relevant stress conditions of heat, humidity, acid or base hydrolysis and oxidation. These testing play an important role in the drug development process. The results of force degradation studies can facilitate stability indicating method development, drug formulation design, selection of storage conditions and packaging, better understanding of potential liabilities of the drug molecule chemistry and solving of stability related problems. [4]

In this study, 3M Hydrochloric Acid and 3M Sodium Hydroxide were represented the chemical degradant for acid hydrolysis degradation and base hydrolysis degradation, respectively. While, 3% Hydrogen Peroxide was the chemical degradant for oxidation degradation. The result for stress conditions and it chromatogram were show in Table 7 and Chromatogram 4 to 7 (Appendix 2), respectively. From acid hydrolysis degradation condition, it shows no interference of IH peak. The percent (%) degradation for acid hydrolysis degradation, base hydrolysis degradation and oxidation degradation were 31.47%, 82.43% and 10.96%. The % degradation was calculated using the following formula:

$$\% \text{ Degradation} = \frac{R_{\text{analyte}} \text{ in diluent (Control)} - R_{\text{analyte}} \text{ in degradation}}{R_{\text{analyte}} \text{ in diluent (Control)}}$$

In which;

R_{analyte} is the peak area of IH

Diluent act as a control

Degradation including acid and base hydrolysis and oxidation degradation

Peak purity index were used to detect the presence of an impurity that is co eluting with the analyte peak. ^[5] As showed in Table 8 and Figure 6 to 9 (Appendix 2), all of the chromatogram shows peak purity index 1.000. Method was able to separate IH from possible degradation peaks. The test showed that no peak overlapped with IH peak after degradation.

Table 7: Results for stress conditions for IH in Imipress Tablet 25 mg

Stress Condition	Acceptance criteria	Results
Acidic 3M HCl / 70°C/ 2 hours		<ul style="list-style-type: none"> i. No interference of IH peak ii. % degradation is 31.47%
Basic 3M NaOH / 70°C/ 2 hours	<ul style="list-style-type: none"> i. Analyte peak is well resolved from the other peaks, resolution > 1.5 ii. Analyte peak degraded at least 20% by one of the degradation method 	<ul style="list-style-type: none"> i. Analyte peak is well resolved from the other peaks which has resolution 10.99 ii. % degradation is 82.43%
Oxidation 3% H ₂ O ₂ / 70°C/ 2 hours		<ul style="list-style-type: none"> i. Analyte peak is well resolved from the other peaks which have resolution 16.39 and 1.59 ii. % degradation is 10.96%

Table 8: Peak Purity for stress conditions for IH in Imipress Tablet 25 mg

Stress Condition	Acceptance criteria	Peak Purity Index
Sample in diluent (control)	> 0.95	1.000
Sample in 3M HCl / 70°C/ 2 hours	> 0.95	1.000
Sample in 3M NaOH / 70°C/ 2 hours	> 0.95	1.000
Sample in 3% H ₂ O ₂ / 70°C/ 2 hours	> 0.95	1.000

4.2.3 Linearity and Range

A linear relationship should be evaluated across the range of the analytical procedure. Linearity should be evaluated by visual inspection of a plot of signals as a function of analyte concentration. If there is a linear relationship, test results should be evaluated by appropriate statistical methods. The correlation coefficient, y-intercept, slope of the regression line and residual sum of squares should be measured. For establishment of linearity, a minimum of five concentrations is recommended. ^[2]

The specified range is normally derived from linearity studies and depends on the intended application of the procedure. It is established by conforming that the analytical procedure provides an acceptable degree of linearity, accuracy and precision when applied to samples containing amount of analyte within or at the extremes of the specified range of the analytical procedure. ^[2]

The calibration curve obtained was a plot of peak area (μV per seconds) as a function of concentration (mg/mL) as shown in Figure 4. A range of IH working standards at concentration ranges from 50% to 150% (5 points calibration) was selected in order to determine limit of linearity of the method. The chromatogram of each concentration can be seen in Chromatogram to 8 to 12. The data obtained, the calibration curve and the peak response were shown in Table 9, Table 10, Figure 4 and Figure 5. R^2 value of 1.000 in Figure 5 proves that it is a linear calibration curve and this range was suitable with the purpose of this study. The %RSD for peak response was 0.3% which is less than 2%. The peak response and peak response ratio were calculated base on following formula:

$$\text{Peak Response} = \frac{\text{Peak Area of IH}}{\text{Each Concentration of IH}}$$

$$\text{Peak Response Ratio} = \frac{\text{Peak Response of each concentration of IH}}{\text{Mean peak response of IH}}$$

Table 9: IH Standard Stock use in Linearity and range

Imipramine Hydrochloride	
Weight of standard	300.63 mg
Concentration of standard stock solution	1.50315 mg/mL
Purity of IH	100.00%

Table 10: Regression Analysis on Linearity of IH

Level	Value No.	X = Conc.(mg/mL)	Y = Peak Area	Peak response	Peak Response ratio
50%	1	0.15032	3561685.7	23694812.0	1.00
80%	2	0.24050	5671178.5	23580391.5	1.00
100%	3	0.30063	7097547.4	23608912.5	1.00
120%	4	0.36076	8561019.9	23730776.1	1.00
150%	5	0.45095	10659158.0	23637379.2	1.00
			Mean	23650454.3	1.00
			SD	61694.1655	0.0026
			%RSD	0.3	0.3

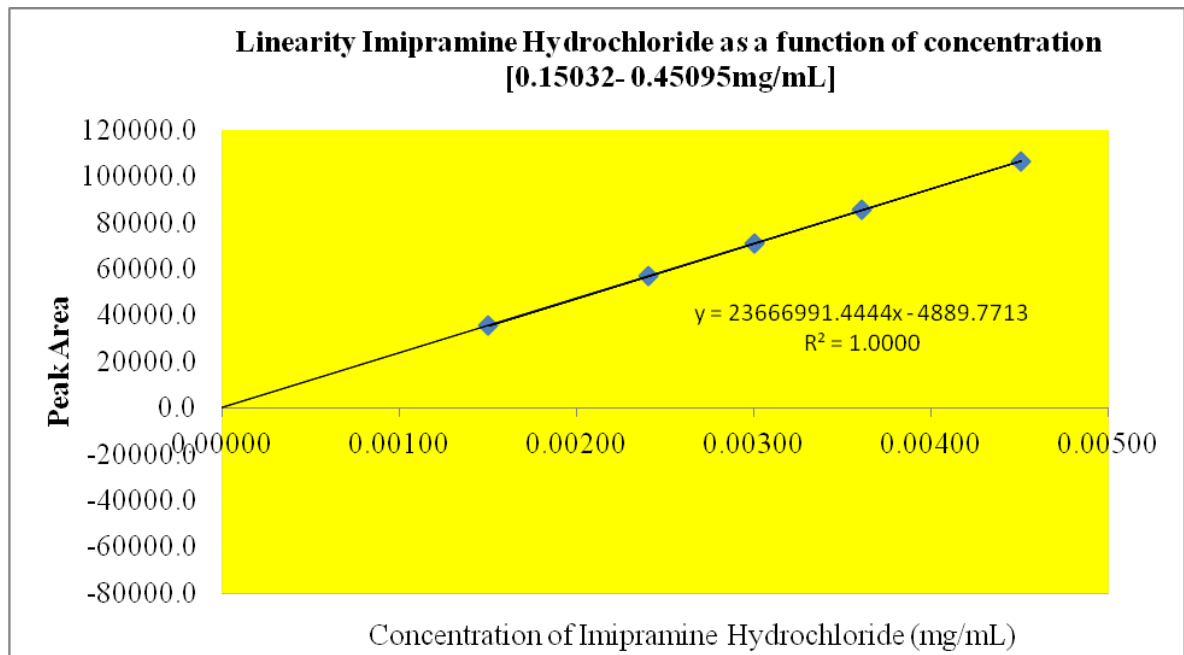


Figure 4: Calibration curve for IH

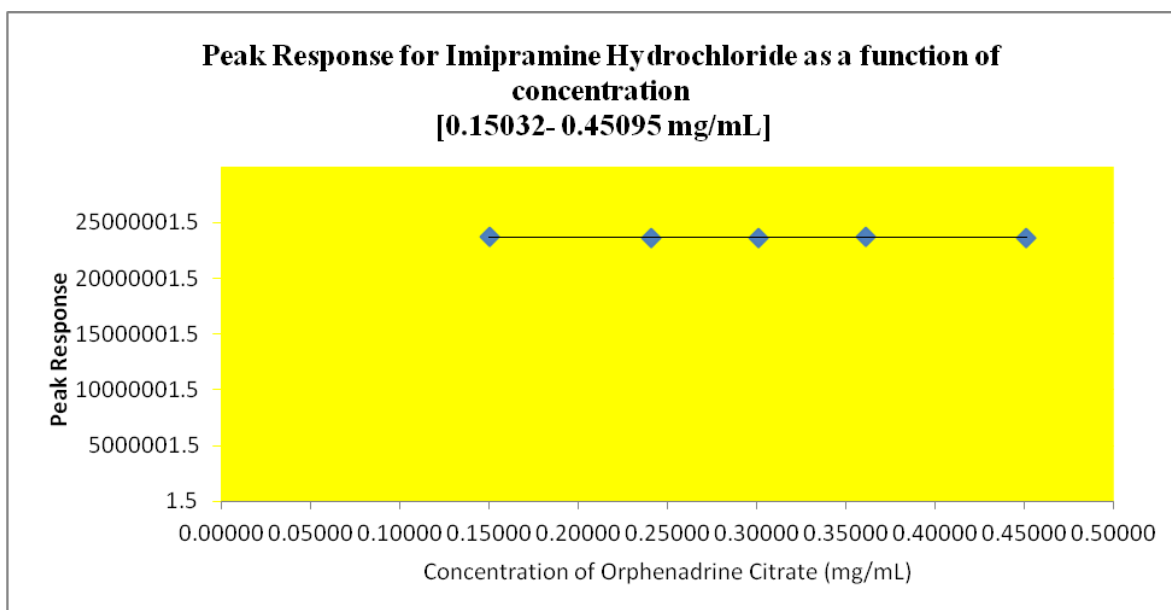


Figure 5: Peak Response for IH

Table 11: Acceptance Criteria for linearity and range

Parameters	Acceptance criteria	Results
correlation coefficient, R^2	> 0.999	1.000
%RSD of peak response	$\leq 2\%$	0.3%

The linearity conforms to all acceptance criteria (Table 11). Therefore the range of analysis was 0.15032 mg/mL to 0.45095 mg/mL for IH.

4.2.4 Accuracy

The accuracy of an analytical method is the extent to which test results generated by the method and the true value agree. Concentration of IH involved 50% concentration (0.150315 mg/mL), 100% concentration (0.300630 mg/mL) and 150% concentration (0.450945 mg/mL). The data for accuracy was tabulated in Table 12 and the chromatograms have shown in Chromatogram 13 to 15 in Appendix 2. The Found concentration (Y) of IH in mg/mL was calculated based on the formula from linearity and range curve:

$$y = 23666991.4444x - 4889.7713$$

In which;

y = Peak Area of IH

x = Concentration of IH (mg/mL)

For this accuracy analysis, the peak area for IH was obtained from HPLC analysis. To calculate the found concentration of IH said Y obtains from the analysis. Therefore,

$$Y = (y - 4889.7713) / 23666991.4444$$

In which;

Y = Found concentration in mg/mL

y = Peak area of IH

The method has shown to be accurate for IH determination in Imipress Tablet 25 mg and passed all the acceptance criteria (Table 13).

Table 12: Recovery results for IH (of finished product form)

Level (Expected content in mg/mL)	Replicate	Peak Area	Y (Found concentration in mg/mL)	%Recovery (100* Y/X)
50% X = (mg/mL) 0.150315	1	3545303.4	0.1495929	99.52
	2	3574339.3	0.1508197	100.34
	3	3565414.3	0.1504426	100.08
	Mean	3561685.7	0.1502851	99.98
	%RSD	0.4	0.4	0.4
100% X = (mg/mL) 0.300630	1	7099833.1	0.2997822	99.72
	2	7093459.0	0.2995129	99.63
	3	7099350	0.2997618	99.71
	Mean	7097547.4	0.2996856	99.69
	%RSD	0.1	0.1	0.1
150% X = (mg/mL) 0.450945	1	10658558.2	0.4501488	99.82
	2	10692079.1	0.4515652	100.14
	3	10626836.6	0.4488085	99.53
	Mean	10659158.0	0.4501742	99.83
	%RSD	0.3	0.3	0.3
Average mean				99.83
SD				0.290
%RSD				0.3
Confidence limit				0.22

Table 13: Acceptance Criteria for accuracy

Parameters	Acceptance criteria	Results
Overall mean accuracy	100% ±2%	99.83
Overall % RSD	≤ 2%	0.3%
Confidence limits at 95%	100% ± 2%	99.62% - 100.05%

4.2.5 Precision (Repeatability and Intermediate Precision)

Validation of tests for assay includes an investigation of precision. ^[2] The measures standard deviation can be obtained into repeatability and intermediate precision. Repeatability is obtained when the analysis is carried out in one laboratory by one operator using one piece of equipment over a relatively short time span. Repeatability was assessed using six determinations at 100% of the test concentration (0.3 mg/mL of IH). In Chromatogram 16 and 17 (Appendix 2) shows the chromatogram for Imipramine Hydrochloride Working Standard and IH in Imipress Tablet 25 mg, respectively. The acceptance criteria for precision depend very much on the type of analysis. While the compound analysis in pharmaceutical quality a control precision of better than 1% RSD is easily achieved. ^[3] Table 14, 15 and 16 show the results for repeatability. Repeatability result also have been use to evaluate the intermediate precision for first analyst.

Table 14: IH Standard use in repeatability

Imipramine Hydrochloride	
Weight of standard	30.71 mg
Concentration of standard	0.3071 mg/mL
Weight of 20 Imipress Tablets 25 mg	2798.36 mg
Purity of IH	100.00%

Table 15: Repeatability for standard and sample injections

No	Retention Time Standard	Area Standard	Retention Time Sample	Area Sample	Weight of sample	Assay	%Assay
1	7.85	7144443.2	7.78	6949239.2	168.35	24.82	99.26
2	7.82	7147948.4	7.80	6990946.0	168.50	24.94	99.77
3	7.80	7148073.9	7.81	6958568.6	168.54	24.82	99.28
4	7.79	7148737.2	7.83	6992220.7	168.26	24.98	99.93
5	7.78	7148939.2	7.84	7000723.6	168.25	25.01	100.06
6	7.76	7146315.3	7.84	6997234.1	168.25	25.00	100.01
Mean	7.80	7147409.5	7.82	6981488.7	168.36	24.93	99.72
SD	0.032	1722.255	0.024	21853.497	0.131	0.090	0.358
%RSD	0.4	0.02	0.3	0.3	0.1	0.4	0.4

Table 16: Acceptance Criteria for repeatability

Parameters	Acceptance criteria	Results
%RSD of standard retention time	$\leq 1\%$	0.4%
%RSD for standard peak area	$\leq 2\%$	0.0%
%RSD of sample result	$\leq 2\%$	0.4%
Theoretical plate count	> 2000	6676.8
Tailing factor	≤ 2	1.6

Intermediate Precision is a term that has been define by ICH guidelines ^[2] as the long-term variability of the measurement process and is determined by comparing the results of a method run within a single laboratory over a number of weeks. A method's intermediate precision may reflect discrepancies in results obtained by different analyst, from different instruments, with different column, different sample and standard preparation and different day.

The objective of intermediate precision validation is to verify that in the same laboratory the method will provide the same results once the development phase is over. ^[3] The result for first analyst was obtained from repeatability testing. The result for second analyst was performed from a new sample and standard which prepared by different analyst (Table 17 and 18). In intermediate precision, the comparison of two analyst's result, the acceptance criteria were tabulated in Table 19 and 20, respectively. The chromatograms for first and second analyst have shown in Chromatogram 19 to 20. The difference in % between two analysts is within the acceptance criteria, hence the method is rugged.

Table 17: IH Standard use in intermediate precision (second analyst)

Imipramine Hydrochloride	
Weight of standard	30.69 mg
Concentration of standard	0.3069 mg/mL
Weight of 20 CCM Tablets	2798.36 mg
Purity of Imipramine Hydrochloride	100.00%

Table 18: Intermediate precision of standard and sample injections (second analyst)

No	Retention Time Standard	Area Standard	Retention Time Sample	Area Sample	Weight of sample	Assay	%Assay
1	8.20	7164573.2	8.05	6846088.0	168.10	24.48	97.93
2	8.19	7166265.2	8.05	6878338.3	168.50	24.54	98.16
3	8.10	7170866.3	8.04	6901685.0	168.40	24.64	98.55
4	8.07	7158157.1	8.03	6849749.3	168.20	24.48	97.93
5	8.05	7194894.2	8.03	6932935.9	169.30	24.62	98.47
6	8.05	7143083.2	8.04	6893588.3	168.50	24.59	98.38
Mean	8.11	7166306.5	8.04	6883730.8	168.50	24.56	98.24
SD	0.06	15528.41	0.01	30114.97	0.42	0.07	0.27
%RSD	0.77	0.22	0.10	0.44	0.25	0.28	0.28

Table 19: Influence of two different analyst, different HPLC column, different HPLC instrument and different day on the analysis results

Active Pharmaceutical Ingredient, API	Event	Assay of 6 samples (%)	Precision of sample assay (%RSD)	Difference of mean value between analysts (%)	Precision of standard injections (%RSD)	Precision of standard retention time (%RSD)
Imipramine Hydrochloride	D1.A1.I1.C1	99.72	0.4	(+)1.48	0.0	0.4
	D2.A2.I2.C2	98.24	0.2		0.2	0.7
Acceptance criteria			≤ 2%	± 2%.	≤ 2%	≤ 1%

Table 20: The difference in % between 2 analysts is within the acceptance criteria, hence the method is rugged.

D1	Day 1	D2	Day 2
A1	Analyst 1 [Radiatul Nadiah]	A2	Analyst 2 [Paremala]
C1	Column 1: Phenomenex Gemini, C18 4.6 x 150mm, 5µm S/N:56680-20	C2	Column 1: Phenomenex Gemini, C18 4.6 x 150mm, 5µm S/N:56679-18
I1	Instrument 1 (Shimadzu Prominence) High Performance Liquid Chromatograph – Shimadzu Prominence, Shimadzu PDA Detector and LC Solutions Software for instrument control.(L20234915442)	I2	Instrument 2 (Shimadzu Prominence) High Performance Liquid Chromatograph – Shimadzu Prominence, Shimadzu PDA Detector and LC Solutions Software for instrument control. (L20154604090)

4.2.6 Robustness

The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters. If measurements are susceptible to variations in analytical conditions, the analytical conditions should be suitably controlled or a precautionary statement should be included in the procedure. One consequence of the evaluation of robustness was to ensure that the validity of the analytical procedure is maintained whenever used. ^[2] ICH guidelines defined robustness as a measure of the method's capability to remain unaffected by small, but deliberate variations in method parameters. ^[6] In this study, the parameters that have changed were flow rate, mobile phase composition and pH in mobile phase. The difference in % between the different in mobile phase composition, pH and flow rate is within the acceptance criteria, hence the method is robust. (Refer to Chromatogram 21 to 35).

Table 21: IH standard and sample use in robustness (flow rate)

Imipramine Hydrochloride	
Weight of standard	30.71 mg
Concentration of standard	0.3071 mg/mL
Weight of sample (mg)	168.35mg,168.50mg,168.54mg,168.26mg, 168.25mg, 168.25mg

Table 22: Results of robustness for IH: Change in flow rate

Condition	Tailing of standard solution	Theoretical Plate Count of 6 injections of standard solution	Precision of 6 injections of standard solution (%RSD)	Mean assay of 6 samples (%)	Different in assay (%)
A1	1.6	6676.8	0.02	99.72	-
B	1.5	6186.9	0.07	99.82	(-)0.10
C	1.6	7319.9	0.07	99.87	(-)0.15
Acceptance criteria	≤ 2	> 2000	≤ 2.0	Different in assay compare against Condition A1 $\pm 2\%$	

Table 23: IH standard and sample use in robustness (mobile phase composition and pH)

Imipramine Hydrochloride	
Weight of standard	30.23 mg
Concentration of standard	0.3023 mg/mL
Weight of sample (mg)	168.70mg,168.20mg,168.66mg,168.21mg, 168.23mg, 16825mg

Table 24: Results of robustness for IH: Change in mobile phase composition and pH

Condition	Tailing of standard solution	Theoretical	Precision of 6	Mean assay	
		Plate Count of 6 injections of standard solution	injections of standard solution (%RSD)	of 6 samples (%)	Different in assay (%)
A2	1.6	6251.9	0.28	100.66	-
D	1.6	6287.9	0.46	100.63	(+)0.03
E	1.6	6668.8	0.54	100.66	0.00
F	1.6	6341.1	0.38	100.96	(-)0.30
G	1.6	6312.1	0.45	100.43	(-)0.23
Acceptance criteria	≤ 2	> 2000	≤ 2.0	Different in assay compare against Condition A2 $\pm 2\%$	

Table 25: Acceptance Criteria for Robustness

Parameters	Acceptance criteria	Results
System suitability for 6 conditions is met if:		
a) %RSD for standard peak area	$\leq 2\%$	Complies
b) Tailing factor of standard solution	≤ 2	Complies
c) Theoretical plate count	> 2000	Complies
Difference of mean values of assay results for 6 conditions compare to original conditions	not more than $\pm 2\%$	Complies
% RSD of standard retention time for 6 conditions	$\leq 1\%$	Complies
% RSD of sample assay for 6	$\leq 2\%$	Complies

4.2.7 Solution Stability

Solution stability of the drug substance or drug product after preparation should be evaluated according to the test method. Most laboratories utilized auto samplers with overnight runs and the sample will be in solution for hours in the laboratory environment before the test procedure is completed. This is concern especially for drugs that can undergo degradation by hydrolysis, photolysis or adhesion to glassware. The solution stability test has been performed in order to support the solution stability data of the sample under normal laboratory conditions. ^[6] Hence, the sample preparation had prepared at initial stage (Day 1) and the same sample has been re-injected after 24 hours- final stage (Day 2). However, the standard preparation for IH has been prepared freshly for each preparation. Table 26 and 27;

and Table 28 and 29 show the results for initial stage (Day 1) and final stage (day 2), respectively.

Table 26: IH Standard use in solution stability (Day 1)

Imipramine Hydrochloride	
Weight of standard	30.71 mg
Concentration of standard	0.3071 mg/mL
Weight of 20 Imipress Tablets 25 mg	2798.36 mg
Purity of IH	100.00%

Table 27: Results for solution stability (Day 1)

No	Retention	Retention		W	Assay	%Assay	
	Time	Area	Time				Area
	Standard (Day 1)	Standard	Sample (Day 1)				Sample
1	7.85	7144443.2	7.78	6949239.2	168.35	24.82	99.26
2	7.82	7147948.4	7.80	6990946.0	168.50	24.94	99.77
3	7.80	7148073.9	7.81	6958568.6	168.54	24.82	99.28
4	7.79	7148737.2	7.83	6992220.7	168.26	24.98	99.93
5	7.78	7148939.2	7.84	7000723.6	168.25	25.01	100.06
6	7.76	7146315.3	7.84	6997234.1	168.25	25.00	100.01
Mean	7.80	7147409.5	7.82	6981488.7	168.36	24.93	99.72
SD	0.032	1722.255	0.024	21853.497	0.131	0.090	0.358
%RSD	0.4	0.02	0.3	0.3	0.1	0.4	0.4

Table 28: IH Standard use in solution stability (Day 2)

Imipramine Hydrochloride	
Weight of standard	30.86 mg
Concentration of standard	0.3086 mg/mL
Weight of 20 Imipress Tablets 25 mg	2798.36 mg
Purity of IH	100.00%

Table 29: Results for solution stability (Day 2)

No	Retention		Retention		W	Assay	%Assay
	Time	Area	Time	Area			
	Standard (Day 2)	Standard	Sample (Day 1)	Sample			
1	8.11	7134729.5	8.70	7106053.5	168.35	25.25	100.98
2	8.11	7282552.2	8.24	7087123.4	168.50	25.16	100.62
3	8.12	7227620.4	8.20	7219952.4	168.54	25.62	102.49
4	8.15	7230680.7	8.19	7069441.1	168.26	25.13	100.52
5	8.16	7220042.4	8.15	7074559.1	168.25	25.15	100.60
6	8.16	7220295.0	8.12	7075949.4	168.25	25.15	100.62
Mean	8.13	7219320.0	8.27	7105513.1	168.36	25.24	100.97
SD	0.022	43499.614	0.197	52548.673	0.131	0.190	0.760
%RSD	0.3	0.6	2.4	0.7	0.1	0.8	0.8

Table 30: Different in sample value

Active	Day 1	Day 2	% Difference
IH	99.72	100.97	(-) 1.2%

$$\begin{aligned} \text{Difference of peak response} &= \frac{7219320.0}{7147409.5} \times 100\% \\ &= 101.0\% \end{aligned}$$

Table 31: Difference of peak response

Active	Initial	24 hours	% Difference
IH	7219320.0	7147591.9	101.0

Table 32: Acceptance Criteria for Solution Stability

Parameters	Acceptance criteria	Results
Difference in sample value with Day 1	$\leq 2\%$	(-)1.2%
Difference of peak response for freshly prepared sample solution and sample solution kept for specific hours	98% to 102%.	101.0%

The stability of the solution conforms to the acceptance criteria. The solution is stable up to 24 hours storage in HPLC auto sampler at room temperature.

CHAPTER 5

CONCLUSION

The analytical method developed and validated for assay by HPLC is suitable for the accurate and precise determination of Imipramine Hydrochloride content in Imipress Tablet 2 mg. This method has been shown to be linear, accurate, precise, rugged and robust. The HPLC method is therefore suitable for use as a stability indicating method for determination of Imipramine Hydrochloride content in Imipress Tablet 2 mg.

REFERENCES

1. Daily Made Current Medical Information. Tofranil (Imipramine Hydrochloride) Tablet, <http://dailymed.nlm.nih.gov/dailymed/lookup.cfm?setid=1827a5aa-733a-49d9-89d9-48ea0367b230> (accessed April 28, 2014).
2. International Conference on Harmonization, ICH Topic Q2 (R1) Validation of Analytical Procedure: Text and Methodology, Step 5: Note for Guidance on validation of analytical procedure: Text and Methodology (CPMP/ICH/381/95), European Medicines Agency.
3. Robert A. Nash and Alfred H. Wachter. Pharmaceutical Process Validation. An International Third Edition, Revised and Expanded, Drug and the Pharmaceutical Sciences, Volume 129. Chapter 15: Validation of Analytical Methods and Processes, Ludwig Huber, Agilent Technologies GmbH, Waldbronn, Germany. Page 507 to 522.
4. Kim Hoynh-Ba. Handbook of Stability Testing in Pharmaceutical Development. Regulations and Methodologies, Springer Science + Business Media, LCC 2009. Chapter 7: Development of Stability Indicating Method, Anne-Francoise Anbry, Peter Tattersall and Joan Ruan. Page: 139-146.
5. Waters 996 Photodiode Detector: Peak Purity I, Performance Perspectives, Waters Cooperations, Wildford, MA 01757, 1998.
6. Center for Drug Evaluation and Research (CDER). Reviewer Guidance. Validation of Chromatographic methods. November 1994, CMC3. H: Sample Solution Stability. Page 17.

7. Onna Mcpollin. Validation of analytical methods for pharmaceutical analysis. Mourne Training Services, MTS, 2009. Validation in the pharmaceutical industry, page: 1.
8. Onna Mcpollin. Validation of analytical methods for pharmaceutical analysis. Mourne Training Services, MTS, 2009. Purpose of analytical methods, page: 3.
9. Major Depressive Disorder. http://en.wikipedia.org/wiki/Major_depressive_disorder (accessed May 13, 2014).
10. Enuresis. <http://en.wikipedia.org/wiki/Enuresis> (accessed May 13, 2014)
11. Imipramine Hydrochloride. <http://en.wikipedia.org/wiki/Imipramine>. (accessed May 13, 2014)
12. Delini-Stula A, Mikkelsen H, Angst J (October 1995). "Therapeutic efficacy of antidepressants in agitated anxious depression--a meta-analysis of moclobemide studies". *J Affect Disord* 35 (1–2): 21–30
13. Skidmore-Roth, L.(ed.). (2010). *Mosby's Nursing Drug Reference* (23rd ed.). St. Louis, MO: Mosby Elsevier.
14. K. Susmitha, M. Thirumalachary, T. Vinod Kumar and G. Venkateshwarlu (2013). Spectrophotometric determination of imipramine HCl in pure and pharmaceutical forms. *Scholars Research Library Der Pharma Chemica*, 5(3):271-279.
15. Introduction and importance to stability indicating analytical method development. Chapter 1, stability requirement during development of drug, 1.5.2: Chromatographic. http://shodhganga.inflibnet.ac.in/bitstream/10603/8382/9/09_chapter%201.pdf. (accessed May 13, 2014)

APPENDICES

**Operating Procedure for Analytical Method Validation
by HPLC and UPLC (Assay)**

1.0 Purpose

1.1 To define the procedure to perform the analytical method validation by HPLC and UPLC.

2.0 Scope

2.1 This SOP covers the validation for assay analysis in pharmaceutical products by HPLC and UPLC method.

2.2 The method could be compendial method, non-compendial method or in-house method.

3.0 Responsibility

3.1.1 Validation team

4.0 Frequency

4.1 Upon development of new or upon modification of existing HPLC and UPLC methods for assay analysis

5.0 Procedures

5.1 **The typical validation characteristics considered for Assay Analysis are:**

- i. Specificity
- ii. Linearity and Range
- iii. Accuracy
- iv. Repeatability
- v. Intermediate Precision
- vi. Robustness (optional)
- vii. Solution stability (optional)
- viii. System suitability
- ix. Limit of Quantification (LOQ)
- x. Limit of Detection (LOD)

5.2 **Assay Method Validation**

5.2.1 **Specificity**

Specificity is the ability to measure accurately and specifically the analyte of interest in the presence of other components that may be expected to be present in the sample matrix. Typically these might include impurities, degradants, matrix, etc.

5.2.1.1 **Specificity determination (Placebo analysis):**

- a) The analysis of a placebo (sample matrix without the analyte) is analyzed and the resulting system response is examined for

the presence of response which interferes or overlap with that of analyte of interest.

- b) In this case, prepare the placebo as in the manufacture formula.
- c) Dilute the placebo as in the Assay preparation and inject it into the chromatogram.

5.2.1.2 Specificity determination (Force degradation):

- a) For full validation, force degradation study will be carried out for placebo, standard and sample. For verification of compendia method, force degradation study will be carried out for sample only.
- b) The force degradation study is complete when the standard and sample are degraded at least 20% by one of the following degradation method ie: acid hydrolysis, basic hydrolysis, aqueous degradation, oxidation degradation, photolysis or thermolysis.

5.2.1.3 Specificity determination (Peak purity assessment using a diode array detector):

- a) The peak purity test is to show that the analyte chromatographic peak is not attributable to more than one component.

5.2.2 Linearity and Range

- a) The linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration of analyte in the sample.
- b) The range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.
- c) Linearity and Range determination:
 - i. Prepare minimum five (5) standard solutions with concentration from at least 50% to 150% of the working concentration. The targeted concentration of the analyte as described in the method must fall between this range
 - ii. If assay and impurity are performed together as one test and only a 100% standard is used, linearity should cover the range from the reporting level of the impurities (LOQ) to 120% of the assay specification (reference to ICH Q2 R1 guidelines).
 - iii. For each of the minimum five (5) concentrations prepared, inject three (3) times for each of the solutions onto HPLC / UPLC system.
 - iv. The result of the mean peak area obtained for each solution is plotted against its corresponding theoretical concentration,

and a linear regression analysis is performed on the minimum five (5) coordinates.

5.2.3 Accuracy

- a) The accuracy is the measure of exactness of an analytical method, or the closeness agreement between the measured value and the value that is accepted either as a conventional, true value or an accepted reference value.
- b) Accuracy determination:
 1. There are minimum three (3) methods to assess accuracy:
 - i. Prepare a known standard stock solution and spike into the placebo at 3 levels of analyte concentrations, ie: 80%, 100% and 120%. The spiked solutions are analyzed for the content of analyte against a Standard calibration curve obtained from Linearity and Range.
 - ii. Weigh and add the known amount of analyte into placebo at 3 levels of analyte concentrations, ie: 80%, 100% and 120%. The weighed analytes is analyzed against a Standard calibration curve obtained from Linearity and Range.
 - iii. For standard additional method, spike in the known amount of analyte into finished product at 3 levels of analyte concentrations, ie: 80%, 100% and 120%. The accuracy of spiked analyte is calculated based on the

found concentration of analyte against theoretical concentration of analyte.

2. The 3 levels of analyte concentrations, ie. 80%, 100% and 120% can be changed wherever necessary, but it should be at least cover 80% to 120% of the working concentration of analyte.
3. If the method to assess the accuracy of the analytical method is different from the three (3) methods as stated in Section 5.3.3.1(1), this method must be described clearly in the individual protocol before execution.
4. For method 5.3.3.1(1a) and 5.3.3.1(1b), the amount of analyte recovered is calculated from the peak response of each test solution obtained:

$$\% \text{ accuracy} = \frac{(\text{Peak response} - \text{intercept})}{\text{Slope}} \times 100\%$$

$$\frac{\text{Peak response} - \text{intercept}}{\text{Slope}}$$

Theoretical concentration

5. For method 5.3.3.1(1c), the amount of analyte recovered is calculated based on the formula below:

$$\% \text{ accuracy} = \frac{W_2}{W_1} \times 100\%$$

W_1

which, W_2 = Found concentration of analyte

W_1 = Theoretical concentration of analyte

5.2.4 Precision

- a) The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed condition.
- b) Two (2) levels : Repeatability and Intermediate precision

5.2.4.1 Repeatability:

- a) Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.
- b) Repeatability determination:
- i) Determine the precision of the method by preparing six (6) samples from the same lot of product. Prepare test solutions according to the method under validation.

- ii) Calculate the %RSD of the six results for each analyte with respect to retention time and peak responses.

5.2.4.2 Intermediate precision

- a) Intermediate precision refers to the results from within-lab variations due to random events within laboratories' variations. This is to test on ruggedness for the same homogenous lot of sample (finish product or simulated product) by two different analysts which the testing is carried out on:
 - i) Different days
 - ii) Different standard preparation
 - iii) Different sample preparation
 - iv) Different instrument/column
- b) Intermediate precision determination:
 - i) One (1) standard solution and six (6) sample solutions are prepared by analyst 1 on the first day and analyzed according to the method under validation.
 - ii) One (1) standard solution and six (6) sample solutions are prepared by analyst two on different day using the same/different instrument according to the method under validation.

5.2.5 Robustness (Optional)

- a) The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate, variations in method parameters and provides an indication of its reliability during normal usage. It should be evaluated during the development phase.
- b) Perform at least two (2) variations such as different brand of column, temperature, pH of buffer, ratio of mobile phase, flow rate, gradient curve etc.
- c) Robustness determination:
 - i) Prepare one (1) standard solution and six (6) replicates of sample solutions under the condition as described in the assay method.

5.2.6 Solution Stability (Optional)

- a) The solution stability test is to check the stability of standard and sample solutions for specific hours at room temperature/fridge.
- b) All the test solutions from the Precision test are injected after being kept for specific hours at room temperature/fridge and calculated against freshly prepared standard.
- c) A new standard solution is prepared and compared to the standard solution that kept for specific hours at room temperature/fridge.
- d) If the solutions are not stable for specific hours, then the time period for which solutions are stable should be determined and the test procedure should contain a statement to this effect.

- e) Solution stability study is not listed as the compulsory test parameters for analytical test method validation. However, it should be considered at an appropriate stage in the development of the analytical procedure.

5.2.7 System Suitability

- a) Performance of a system is checked by performing system suitability tests that are designed to evaluate the performance of the entire system.
- b) The system suitability tests are established during method development and validation phases to check on the performance of the system.
- c) The performance is evaluated in terms of the following parameters:

- i) System precision:

- Six replicates of the working concentration (100%) are injected into the chromatograph.

- ii) Theoretical Plate Count (N):

- The efficiency is defined in terms of the number of theoretical plates (N) per column and is calculated based on equation below:

$$N = 5.54 (V_R^2 / W_{h2})$$

- where,

- V_R = The distance along the baseline between the point of injection and a perpendicular dropped from the maximum of the peak of interest.

W_h = The width of the peak of interest at half peak height, measured in the same units as V_R .

iii) Tailing factor

The accuracy of quantitation decreases with increase in peak tailing because of the difficulties encountered by the integrator in determining where / when the peak ends and hence the calculation of the area under the peak. The symmetry factor is an indicator of peak skewness and is calculated using the equation.

$$f = W_x / 2d$$

where,

W_x = Peak width at 5% of peak height.

D = Distance between the perpendicular dropped from the peak maximum and the leading edge of the peak at 5% of the peak height.

iv) Capacity factor (k') (if applicable):

The capacity factor is a measure of where the peak of interest is located with respect to the void volume, ie: the elution time of the non-retained components (0.01% Uracil in diluents) or column void volume. It is calculated using the equation:

$$k' = \frac{T_R - T_0}{T_0}$$

where,

T_R = Elution time of the analyte.

T_O = Elution time of the void volume or non-retained components.

Normally for T_O , the retention time of a 0.01% of Uracil in the diluent, is used.

v) Resolution (if applicable):

The resolution is to measure the quality of separations of the adjacent peaks.

6.0 Acceptance Criteria

Analytical Test Method	Analytical Performance Characteristics	Acceptance criteria
Assay	Specificity (Placebo Analysis)	No peak/s greater than noise at the $\pm 5\%$ retention time window of the principal analyte peak.
	Specificity (Force degradation)	i. Analyte peak must be well resolved from the other peaks (Resolution > 1.5 unless otherwise specified in the individual monograph).

	Specificity (Peak purity assessment using a diode array detector)	i. The peak purity index as obtained is greater than 0.95.
	Linearity and Range	i. $r^2 > 0.999$ ii. %RSD of peak response $\leq 2\%$ iii. Magnitude of intercept against 100% working standard $\pm 2\%$
	Accuracy	i. Overall mean accuracy within $100\% \pm 2\%$ for ethical finished products and $100\% \pm 5\%$ for OTC finished products or unless otherwise specified in the individual protocol. ii. Confidence limit at 95%, μ is $100\% \pm 2\%$ for ethical finished product and $100\% \pm 5\%$ for OTC finished products or unless otherwise specified in the individual protocol. iii. % RSD for the measurement precision $\leq 2\%$ for ethical finished products and $\leq 5\%$ for OTC finished products or unless otherwise specified in the individual protocol
	Precision: Repeatability	i. % RSD of the retention time for standard $\leq 1\%$ ii. %RSD of the standard peak area $\leq 1\%$ unless

		<p>otherwise specified in the individual monograph.</p> <p>iii. %RSD of the samples results $\leq 2\%$ for ethical finished products and $\leq 5\%$ for OTC finished products or unless otherwise specified in the individual protocol.</p>
	<p>Precision: Intermediate precision</p>	<p>i. % RSD of the retention time for standard $\leq 1\%$</p> <p>ii. %RSD of the standard peak area $\leq 1\%$ unless otherwise specified in the individual monograph.</p> <p>iii. %RSD of sample results $\leq 2\%$ for ethical finished products and $\leq 5\%$ for OTC finished products or unless otherwise specified in the individual protocol.</p> <p>iv. %RSD of combined results $\leq 2\%$ for ethical finished product and $\leq 5\%$ for OTC finished products or unless otherwise specified in the individual protocol.</p> <p>v. Difference of mean value of results between analysts within $\pm 2\%$ for ethical finished products and $\pm 5\%$ for OTC finished products or unless otherwise specified in the individual protocol.</p>

	Robustness	<ul style="list-style-type: none"> i. % RSD of the retention time for standard $\leq 1\%$ ii. %RSD of sample assay $\leq 2\%$ iii. Difference in assay results not more than $\pm 2\%$. iv. Achieve the system suitability requirements.
	Solution stability	<ul style="list-style-type: none"> i. Difference in assay results $\pm 2\%$ for ethical finished products and $\pm 5\%$ for OTC finished products ii. Difference in peak response of freshly prepared standard solution and standard solution kept for specific hours $\pm 1\%$ or unless otherwise specified in the individual protocol.
	System suitability a) System precision	<ul style="list-style-type: none"> i. %RSD of standard retention time $\leq 1\%$. ii. %RSD of standard peak area $\leq 1\%$.
	System suitability b) Theoretical Plate Count (N)	The column efficiency is generally recommended to be more than 2000 unless otherwise specified in the individual monograph.
	System suitability c) Tailing factor	<ul style="list-style-type: none"> i. Tailing factor is generally ≤ 2 unless otherwise specified in the individual protocol. ii. Tailing factor is 0.8 to 1.5 for BP monograph method

	System suitability d) Capacity factor	$k' > 2$ unless otherwise specified in the individual monograph.
	System suitability e) Resolution	Resolution > 2 unless otherwise specified in the individual monograph.

Operating Procedure for the Shimadzu Prominence High Performance Liquid Chromatography

1. Purpose

To guide the user to operate the instrument accordingly

2. Scope

The Work Instruction applies to the description and usage of:

- a. The degasser; Model: Shimadzu DGU-20A₃ Degasser
- b. The pump; Model: Shimadzu LC-20AT Prominence LC
- c. The detector; Model: Shimadzu SPD-20A Prominence uv/vis Detector
- d. The auto sampler; Model: Shimadzu SIL-20A Prominence
- e. Column oven; Model Shimadzu CTO – 10AS VP
- f. The computer; Model: DELL

3. Responsibility

3.1 Lab Technician

- Responsible to strictly adhere to this work instruction.

3.2 Lab supervisor/ Executive

- Responsible to ensure strict adherence to this work instruction.

4. Procedures

4.1 Turn ON the power of the pump A, detector, auto sampler, oven, pump B and computer.

4.2 Logging into LC solution.

4.2.1 Double-click on **Analysis 1** (Instrument 1) from main menu.

4.3 **Setting up run parameters.**

4.3.1 Select **New** or **Open** from **File > New Method File / Open Method File.**

4.3.2 Select **Method > Instrument Parameters.**

- First select the **Advanced > Pump**, then select the **Isocratic Flow / Binary Gradient** mode.
- Set the flow rate, upper and lower pressure limit for pump A and pump B pressure limit at 40.0 MPa.
- Set the temperature required and the T. Max. at 85°C.
- Set the time expected for the actual analysis in **LC Stop Time** at **Data acquisition** text box > **Apply to All acquisition time.**
- When using the **isocratic flow** enter a stop time for the time program under the **Time Program** table and if using the **Binary gradient** set the time program under the **Time program** table.
- For a **Detector A**, select the **D2 lamp** ratio and designate the required wavelength.
- Click on the **Download** button to send the setting to the system and save the method.
- Press the **Instrument On/Off** button on the Direct Control toolbar to activate the system.

- Open the drain valve and press the **Purge sampler** button to begin the purge cycle of the auto sampler syringe; wait until finished.
- Close the drain valve and press the **pump On/Off** button.
- From the **File** menu, select **Personal name folder > Method file > Product Tested and** save under the created method and save the file.
- When the signal stabilizes, press Zero Detector A button in the Direct Control toolbar to the zero signal. Wait for the baseline to stabilize.

4.4 Sample Injection:

4.4.1 Single sample injection

- Click the **Single Start** button.
- Enter the settings required for analysis and press **OK**.
- Save the **Method file** and **Data File** under the same folder.
- Once the actual retention time get set the **LC Stop Time > Apply to All acquisition time**.

4.4.2 Batch processing injection

- **Open the new Batch Processing table**
- From the **File** menu, click the **New Batch File**.
- Click the **Wizard** button and enter the **start vial** and **injection volume**.
- Press **Next** and select the **standard location in the sequence**.
- Press **Next** again and enter the **sample name** and **sample ID**.
- To automatically increment the Data file name, use parentheses around the starting number, i.e. **Sample 001.lcd**.

- Click the **Data File** and select in the same folder with **Method file**.
- Change the number of **standard sample vial per level** and **repetition run**.
- Select the **Print report** and change the **Report format file** in the same folder with **Method file** and **Data file**.
- Click **Next** and enter the **Sample name** and **Sample ID**.
- Select the **Data File** in the same folder with **Method File** and **Data file**.
- Change the number of **unknown sample vials in each group** and **Repetition per Run**.
- Select the **Print report** and change the **Report format file** in the same folder with **Method file** and **Data file**.
- **Click Next > Finish**.
- Ensure all the information appears on the **Batch table**.
- Once the Batch processing is complete, click **File > Save Batch File As > person name > Method File > Product tested > File Name > Save**.
(* Save the Batch Files As in the same folder with method File and Data File)
- Click **Batch Start > Yes**.

4.5 Print a Report

4.5.1 Data Report

- Click **LC Postrun Analysis** at the LC Solution menu.
- Double click **File > Open > Data file** and double click the required data to open the Chromatogram.

- Click **Wizard** and set the **minimum area/height** and click **Next > select the peak > Next > Next** (change the name peak) > **Finish**.
- Click **View** button on the **Compound table view**.
- Click Data report.
- Open any report format from other file and drag it to the chromatogram.
- **Adjust the User Information;**
 - a. Double click at **user information**.
 - b. Click at **Sample information > default** and change the **acquired by** (user name) > **apply** and **OK**.
- **Adjust the chromatogram scale.**
 - a. Double click at **chromatogram**.
 - b. Change the **type** of chromatogram, range for **X schale (time)** and **Left Y schale (Inten.)**
 - c. Change to **User Defined** in Left Y schale (Inten.) and the limitation from **-ve integer – +ve integer**.
 - d. Click **OK**.
- **Adjust the other Item (eg. Theoretical Plate, Tailing Factor, Resolution, etc.)**
 - a. Double click at **chromatogram table**.
 - b. Insert the column by click at any table.
 - c. Choose the item need and click **Apply > OK**.
- Once the actual **Data Report** get, save into **File > Save Report Format file As > Method File > Product tested > Report Format > Save**.

- Then click **Return > Apply to the method > Method File > product tested > Save.**
- Finally click **File > Save data > Yes > OK.**

4.6 Cleaning of the Injection

- a. After all the analyses have been completed, remove the vials from the rack.
Press the rinse sampler button on the Direct Control toolbar to begin the rinse cycle of the auto sampler syringe.

4.7 Cleaning the HPLC

- a. Change the filtered water each time using of HPLC
- b. Change 50% Acetonitrile: Water and 100% Acetonitrile when the volume of this solution is less than 500ml.
- c. Remove all the Mobile Phase Bottle, flushing and storing reagent bottle from HPLC after using.

5 Maintenance

External cleaning of the Instrument:

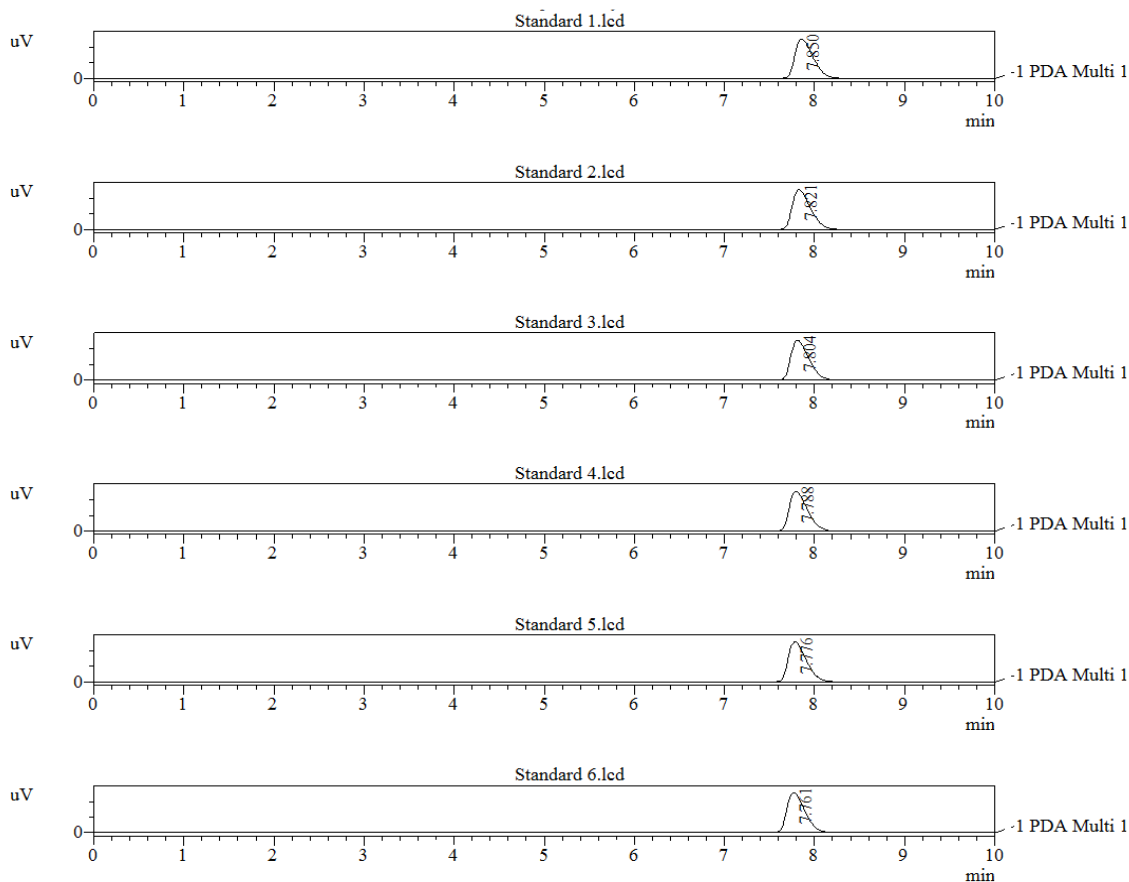
- a. Dust the external surface of the instrument and the surrounding area where the instrument is placed.
- b. The surrounding area is also cleaned with wet cloth.
- c. The external cleaning are done once in two weeks and monitored by Validation Chemists.

Chromatogram 1: Chromatogram of Imipramine Hydrochloride Working Standard

==== Shimadzu LcSolution Analysis Report ====

Acquired by : Radiatul Nadiah Razali
 Sample Name : Imipress Tablet (Assay)
 Sample ID : Method Validation
 Mobile phase : 0.06M Sodium Perchlorate: Acetonitrile: Triethylamine (625 : 375: 1)
 Column : Phenomenex C18, Gemini, 5 um, 150 mm, 4.6 mm
 Flow rate : 1.0 min/ mL

<Chromatogram>

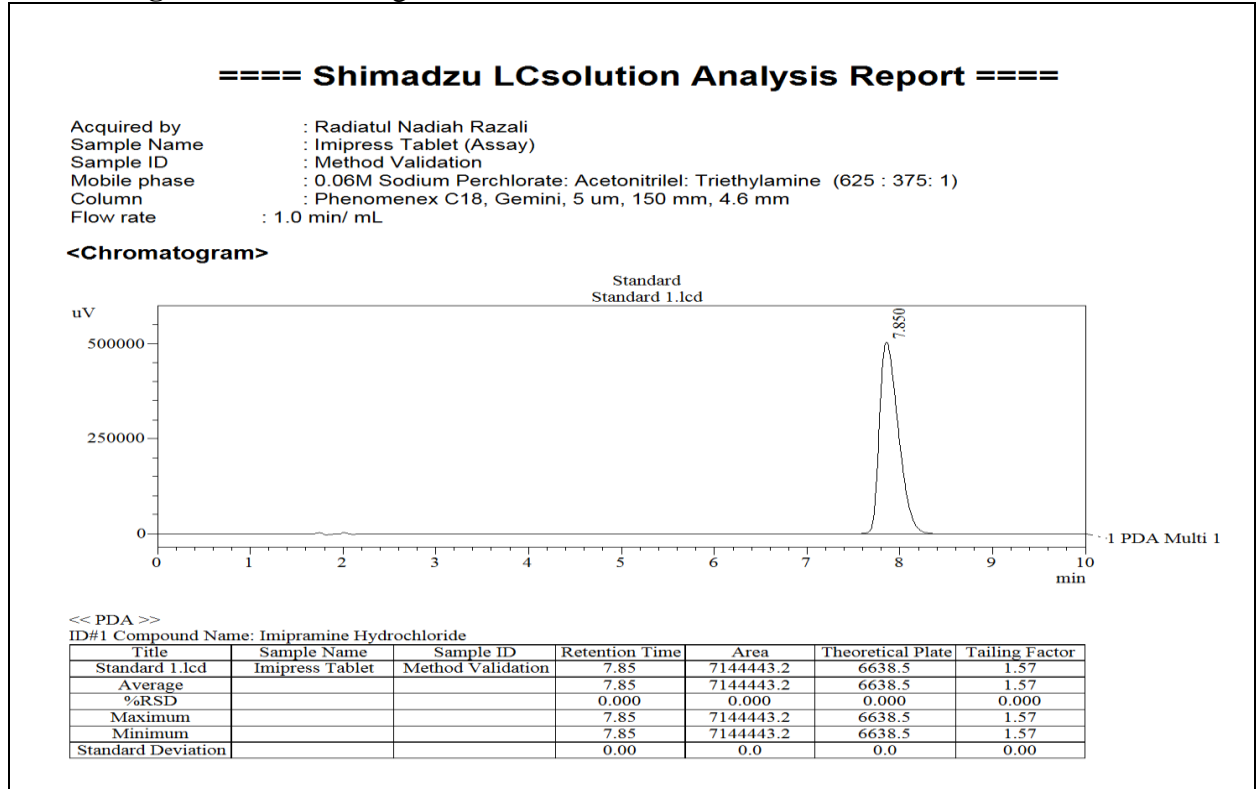


<< PDA >>

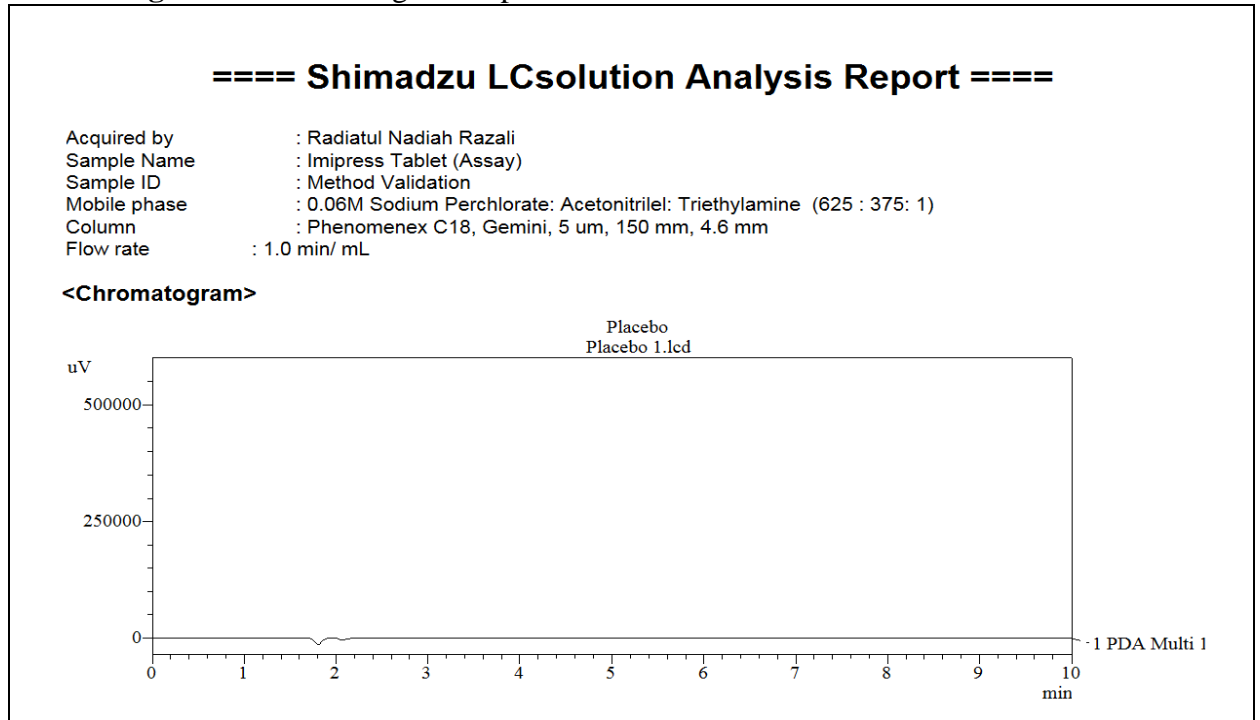
ID#1 Compound Name: Imipramine Hydrochloride

Title	Sample Name	Sample ID	Retention Time	Area	Theoretical Plate	Tailing Factor	Resolution
Standard 1.lcd	Imipress Tablet	Method Validation	7.85	7144443.2	6638.5	1.57	0.00
Standard 2.lcd	Imipress Tablet	Method Validation	7.82	7147948.4	6655.5	1.56	0.00
Standard 3.lcd	Imipress Tablet	Method Validation	7.80	7148073.9	6683.0	1.57	0.00
Standard 4.lcd	Imipress Tablet	Method Validation	7.79	7148737.2	6688.4	1.56	0.00
Standard 5.lcd	Imipress Tablet	Method Validation	7.78	7148939.2	6686.1	1.56	0.00
Standard 6.lcd	Imipress Tablet	Method Validation	7.76	7146315.3	6709.5	1.56	0.00
Average			7.80	7147409.5	6676.8	1.56	0.00
%RSD			0.405	0.024	0.382	0.330	0.000
Maximum			7.85	7148939.2	6709.5	1.57	0.00
Minimum			7.76	7144443.2	6638.5	1.56	0.00
Standard Deviation			0.03	1722.3	25.5	0.01	0.00

Chromatogram 2: Chromatogram of standard



Chromatogram 3: Chromatogram of placebo

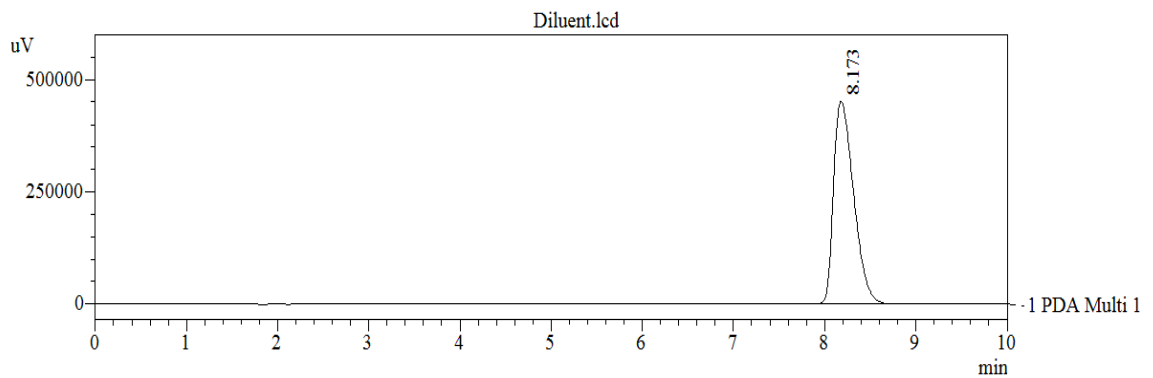
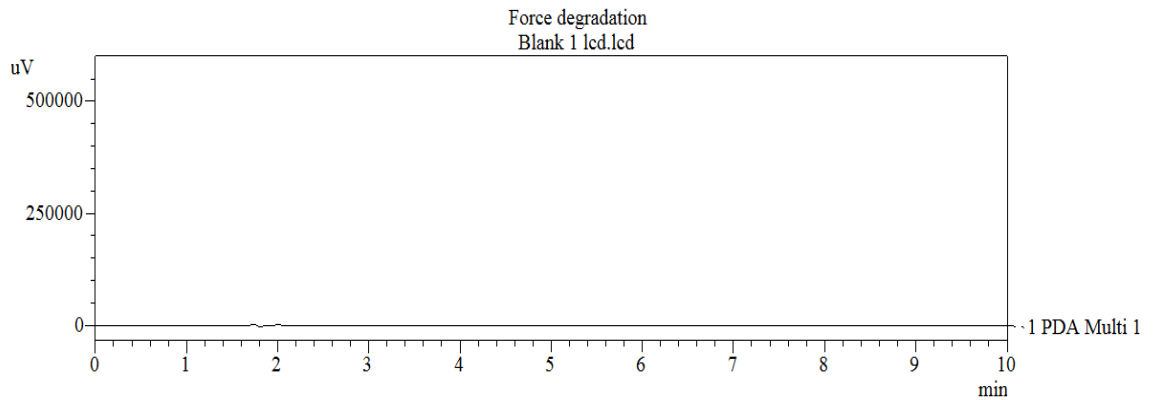


Chromatogram 4: Chromatogram of blank (diluent) versus sample solution in diluent.

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

Acquired by : Radiatul Nadiah Razali
 Sample Name : Imipress Tablet (Assay)
 Sample ID : Method Validation
 Mobile phase : 0.06M Sodium Perchlorate: Acetonitrile: Triethylamine (625 : 375: 1)
 Column : Phenomenex C18, Gemini, 5 um, 150 mm, 4.6 mm
 Flow rate : 1.0 min/ mL

<Chromatogram>



<< PDA >>

ID#1

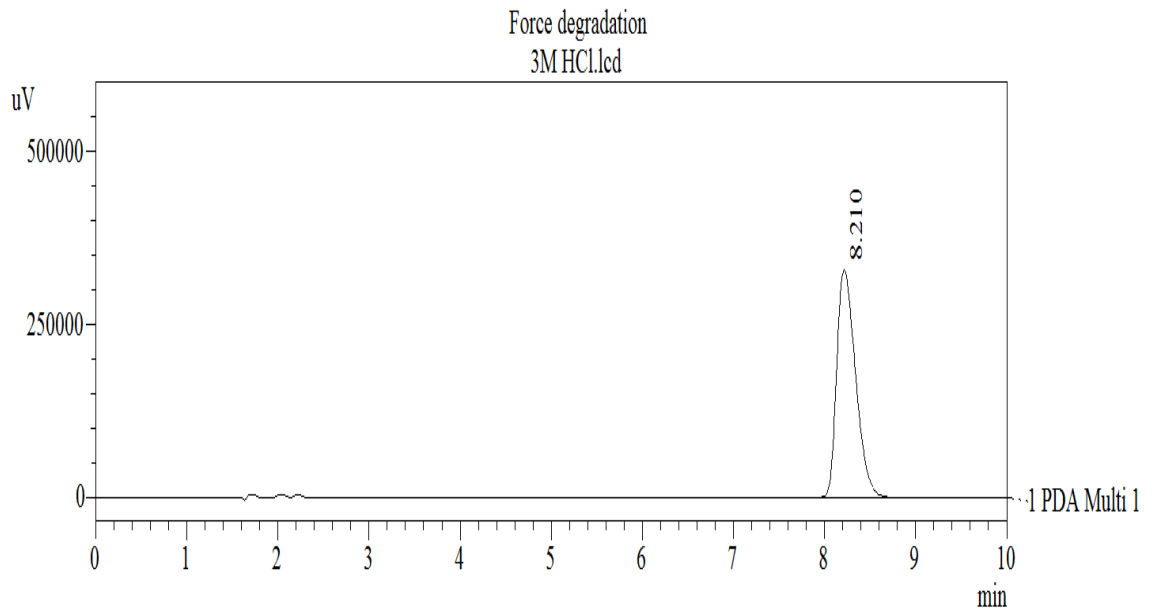
Title	Sample Name	Sample ID	Retention Time	Area	Theoretical Plate	Tailing Factor
Blank 1 lcd.lcd	Imipress Tablet	Method Validation				
Diluent.lcd	Imipress Tablet	Method Validation	8.17	6935146.1	6259.1	1.58
Average			8.17	6935146.1	6259.1	1.58
%RSD			0.000	0.000	0.000	0.000
Maximum			8.17	6935146.1	6259.1	1.58
Minimum			8.17	6935146.1	6259.1	1.58
Standard Deviation			0.00	0.0	0.0	0.00

Chromatogram 5: Chromatogram of sample solution in 3M HCl / 70°C/ 2 hours

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

Acquired by : Radiatul Nadiah Razali
 Sample Name : Imipress Tablet (Assay)
 Sample ID : Method Validation
 Mobile phase : 0.06M Sodium Perchlorate: Acetonitrile: Triethylamine (625 : 375: 1)
 Column : Phenomenex C18, Gemini, 5 um, 150 mm, 4.6 mm
 Flow rate : 1.0 min/ mL

<Chromatogram>



<<PDA>>

ID#1 Compound Name: Imipramine Hydrochloride

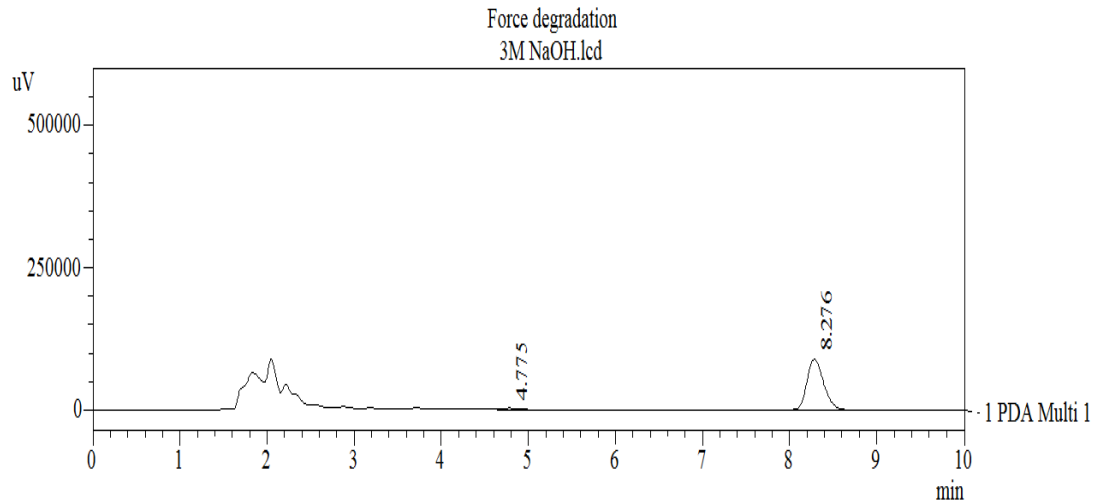
Title	Sample Name	Sample ID	Retention Time	Area	Theoretical Plate	Tailing Factor
3M HCl.lcd	Imipress Tablet	Method Validation	8.21	4752195.9	7106.5	1.47
Average			8.21	4752195.9	7106.5	1.47
%RSD			0.000	0.000	0.000	0.000
Maximum			8.21	4752195.9	7106.5	1.47
Minimum			8.21	4752195.9	7106.5	1.47
Standard Deviation			0.00	0.0	0.0	0.00

Chromatogram 6: Chromatogram of sample solution in 3M NaOH / 70°C/ 2 hours

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

Acquired by : Radiatul Nadiah Razali
 Sample Name : Imipress Tablet (Assay)
 Sample ID : Method Validation
 Mobile phase : 0.06M Sodium Perchlorate: Acetonitrile: Triethylamine (625 : 375: 1)
 Column : Phenomenex C18, Gemini, 5 um, 150 mm, 4.6 mm
 Flow rate : 1.0 min/ mL

<Chromatogram>



<< PDA >>

ID#1 Compound Name: RT4.775

Title	Sample Name	Sample ID	Retention Time	Area	Theoretical Plate	Tailing Factor	Resolution
3M NaOH.lcd	Imipress Tablet	Method Validation	4.77	21410.7	4846.7	1.28	0.00
Average			4.77	21410.7	4846.7	1.28	0.00
%RSD			0.000	0.000	0.000	0.000	0.000
Maximum			4.77	21410.7	4846.7	1.28	0.00
Minimum			4.77	21410.7	4846.7	1.28	0.00
Standard Deviation			0.00	0.0	0.0	0.00	0.00

ID#2 Compound Name: Imipramine Hydrochloride

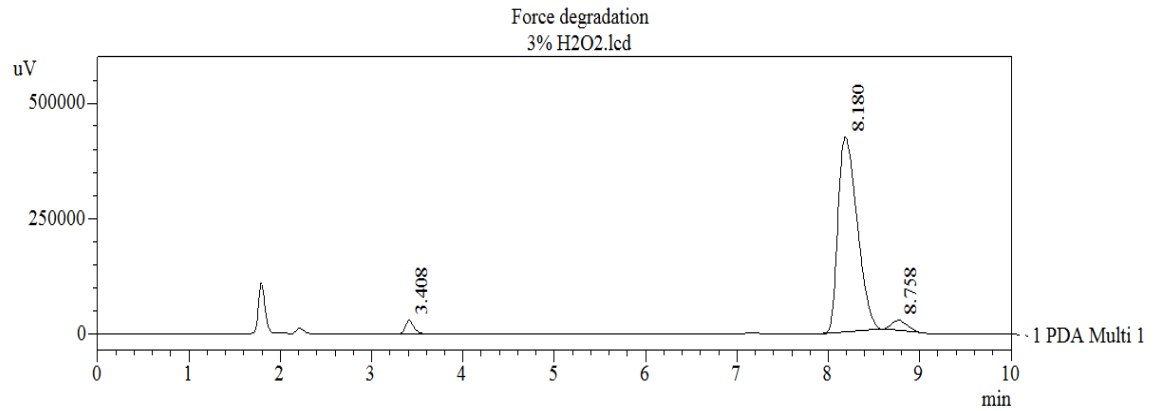
Title	Sample Name	Sample ID	Retention Time	Area	Theoretical Plate	Tailing Factor	Resolution
3M NaOH.lcd	Imipress Tablet	Method Validation	8.28	1218052.5	8314.6	1.26	10.99
Average			8.28	1218052.5	8314.6	1.26	10.99
%RSD			0.000	0.000	0.000	0.000	0.000
Maximum			8.28	1218052.5	8314.6	1.26	10.99
Minimum			8.28	1218052.5	8314.6	1.26	10.99
Standard Deviation			0.00	0.0	0.0	0.00	0.00

Chromatogram 7: Chromatogram of sample solution in 3% H₂O₂ / 70°C/ 2 hours

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

Acquired by : Radiatul Nadiah Razali
 Sample Name : Imipress Tablet (Assay)
 Sample ID : Method Validation
 Mobile phase : 0.06M Sodium Perchlorate: Acetonitrile: Triethylamine (625 : 375: 1)
 Column : Phenomenex C18, Gemini, 5 um, 150 mm, 4.6 mm
 Flow rate : 1.0 min/ mL

<Chromatogram>



<< PDA >>

ID#1 Compound Name: RT3.408

Title	Sample Name	Sample ID	Retention Time	Area	Theoretical Plate	Tailing Factor	Resolution
3% H2O2.lcd	Imipress Tablet	Method Validation	3.41	179799.4	5622.7	1.25	0.00
Average			3.41	179799.4	5622.7	1.25	0.00
%RSD			0.000	0.000	0.000	0.000	0.000
Maximum			3.41	179799.4	5622.7	1.25	0.00
Minimum			3.41	179799.4	5622.7	1.25	0.00
Standard Deviation			0.00	0.0	0.0	0.00	0.00

ID#2 Compound Name: Imipramine Hydrochloride

Title	Sample Name	Sample ID	Retention Time	Area	Theoretical Plate	Tailing Factor	Resolution
3% H2O2.lcd	Imipress Tablet	Method Validation	8.18	6174956.5	6677.3	1.50	16.39
Average			8.18	6174956.5	6677.3	1.50	16.39
%RSD			0.000	0.000	0.000	0.000	0.000
Maximum			8.18	6174956.5	6677.3	1.50	16.39
Minimum			8.18	6174956.5	6677.3	1.50	16.39
Standard Deviation			0.00	0.0	0.0	0.00	0.00

ID#3 Compound Name: RT8.758

Title	Sample Name	Sample ID	Retention Time	Area	Theoretical Plate	Tailing Factor	Resolution
3% H2O2.lcd	Imipress Tablet	Method Validation	8.76	240054.3	11424.4	1.31	1.59
Average			8.76	240054.3	11424.4	1.31	1.59
%RSD			0.000	0.000	0.000	0.000	0.000
Maximum			8.76	240054.3	11424.4	1.31	1.59
Minimum			8.76	240054.3	11424.4	1.31	1.59
Standard Deviation			0.00	0.0	0.0	0.00	0.00

Figure 6: Peak purity of Sample in diluent.

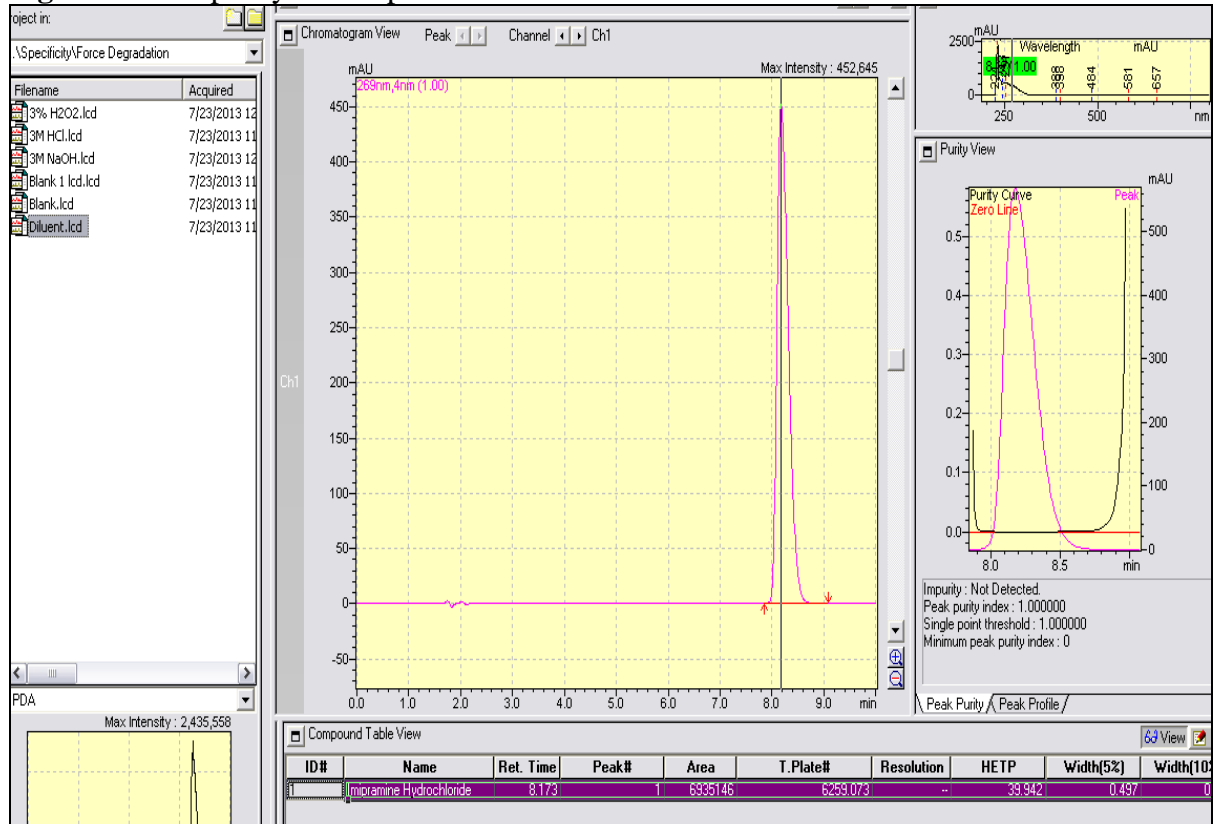


Figure 7: Peak purity of Sample in 3M HCl / 70°C/ 2 hours

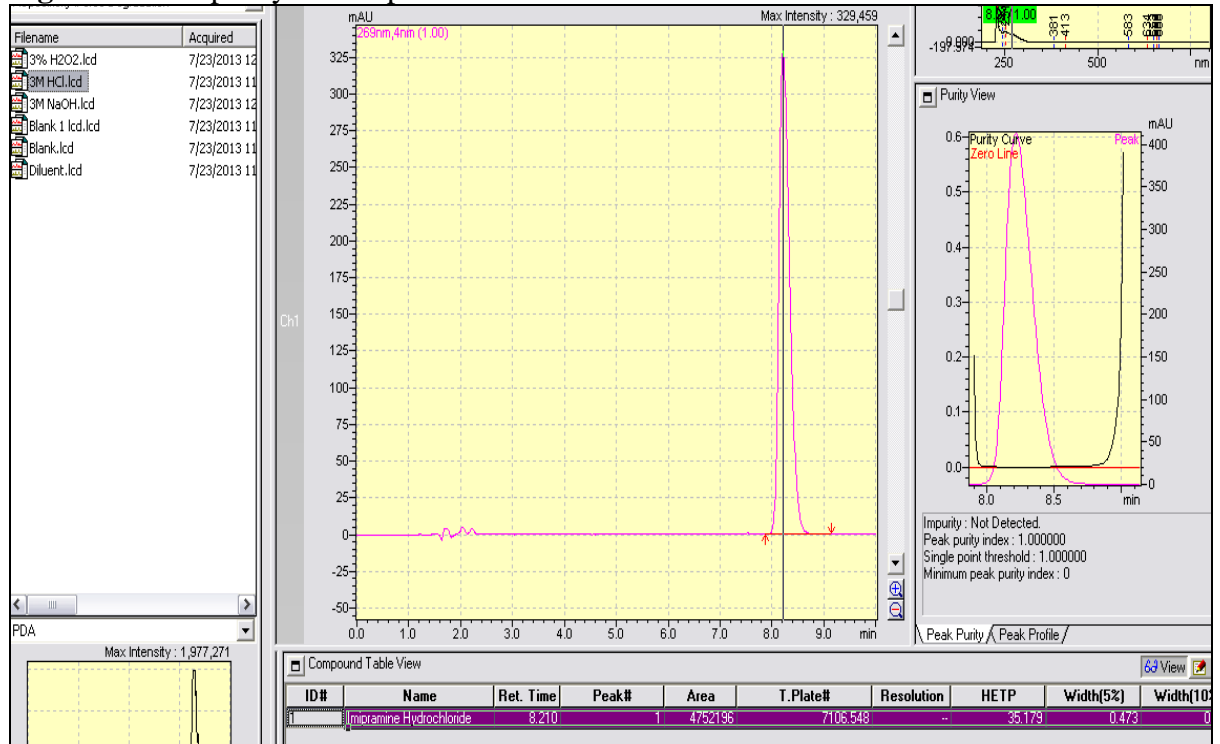


Figure 8: Peak purity of Sample in 3M NaOH / 70°C/ 2 hours

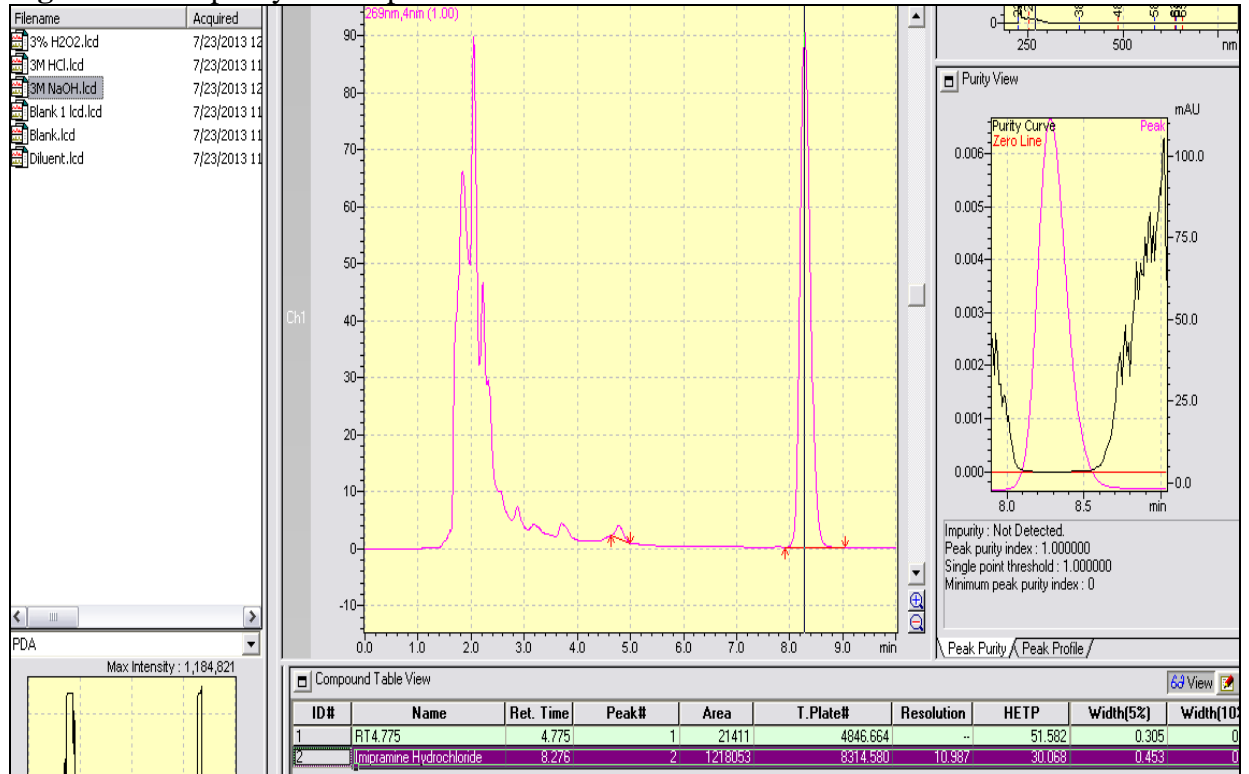
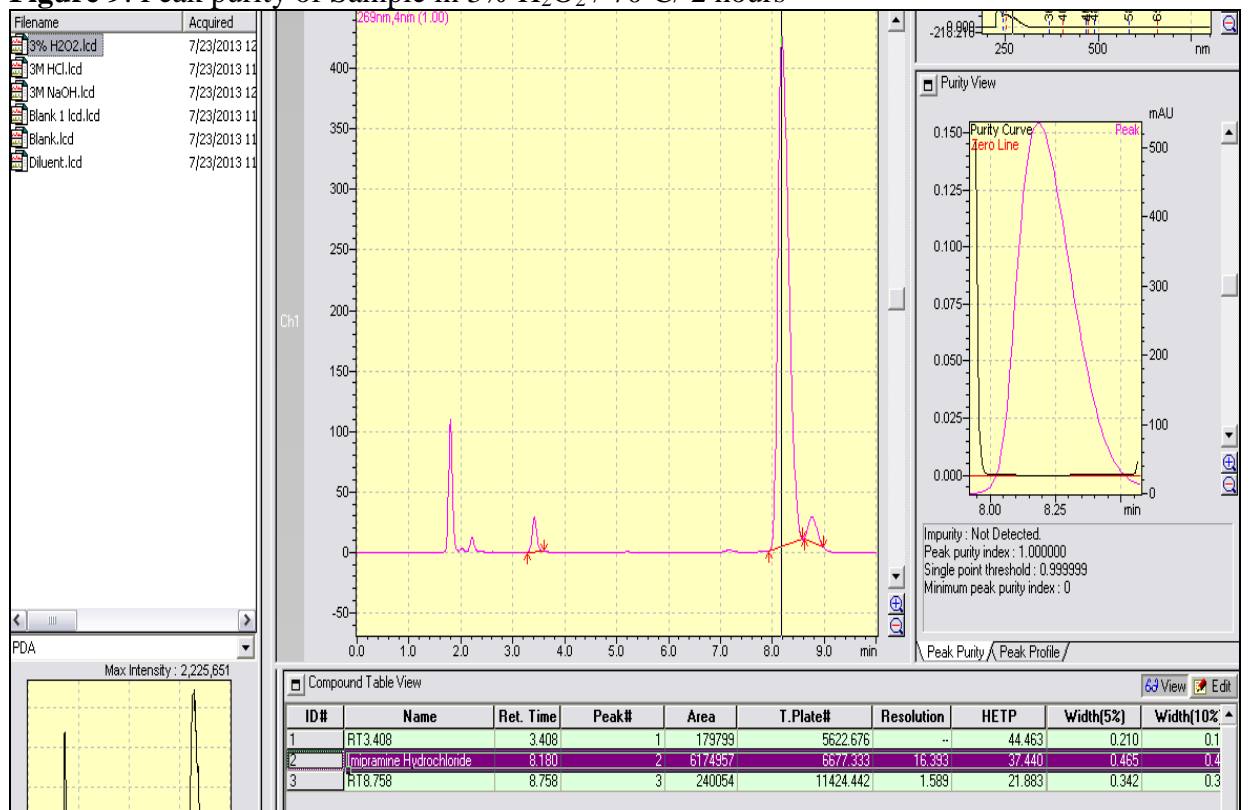


Figure 9: Peak purity of Sample in 3% H₂O₂ / 70°C/ 2 hours

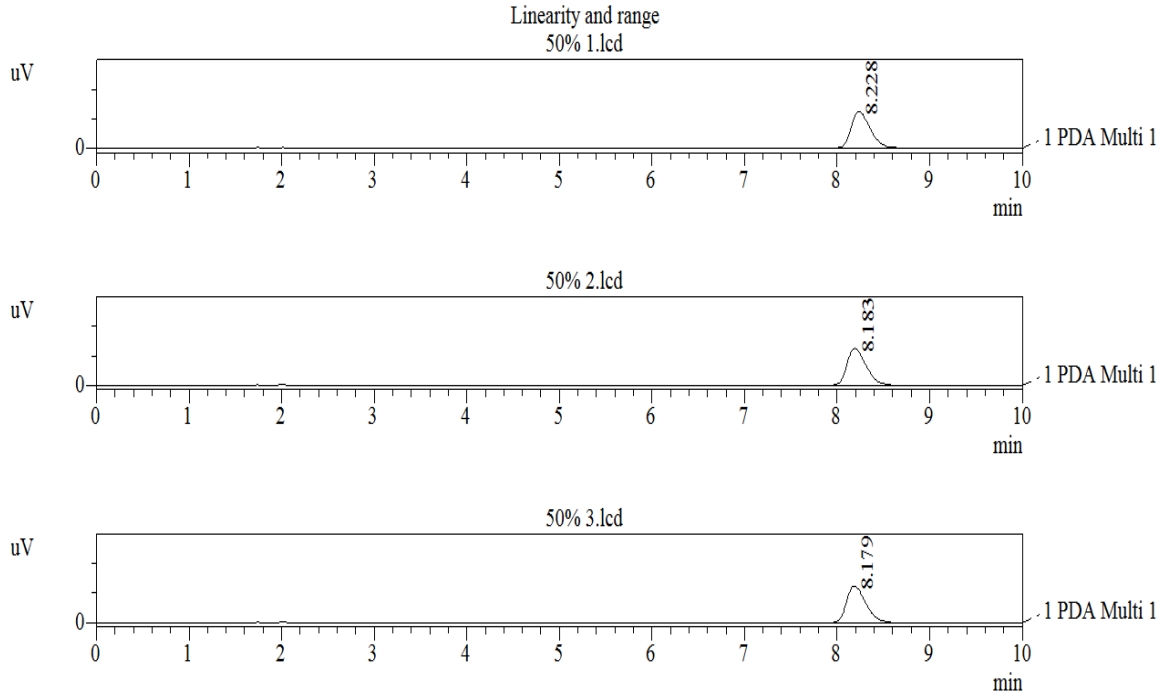


Chromatogram 8: Chromatogram for at 50% of target concentration.

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

Acquired by : Radiatul Nadiah Razali
 Sample Name : Imipress Tablet (Assay)
 Sample ID : Method Validation
 Mobile phase : 0.06M Sodium Perchlorate: Acetonitrile: Triethylamine (625 : 375: 1)
 Column : Phenomenex C18, Gemini, 5 um, 150 mm, 4.6 mm
 Flow rate : 1.0 min/ mL

<Chromatogram>



<< PDA >>

ID#1 Compound Name: Imipramine Hydrochloride

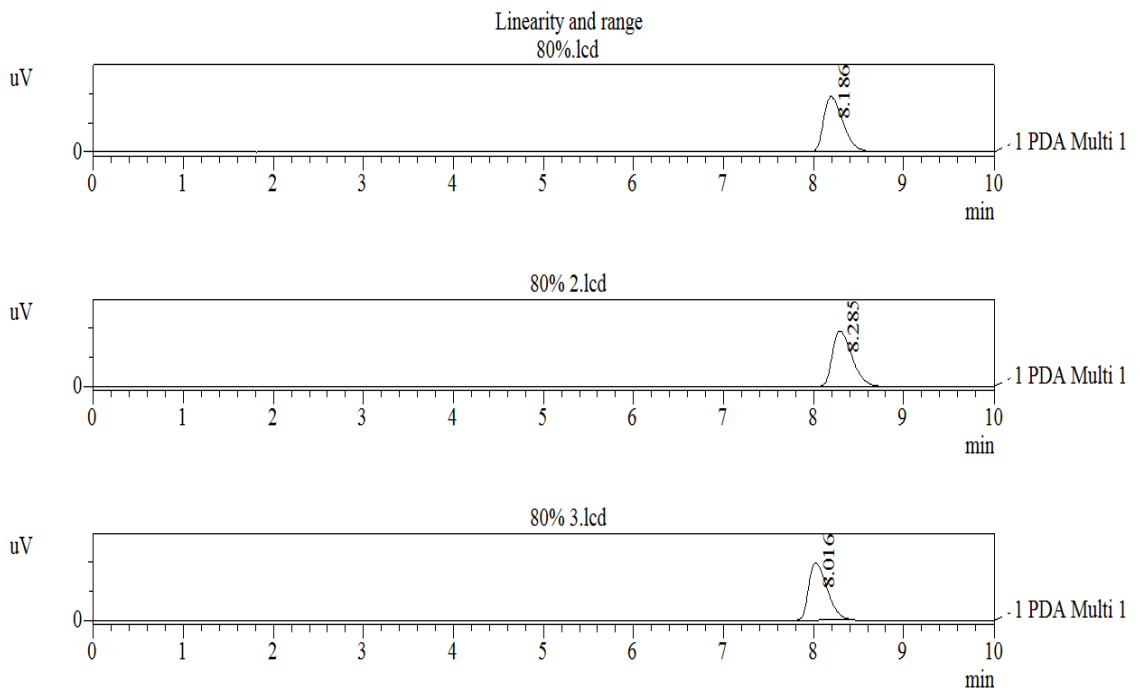
Title	Sample Name	Sample ID	Retention Time	Area	Theoretical Plate	Tailing Factor	Resolution
50% 1.lcd	Imipress Tablet	Method Validation	8.23	3545303.4	7254.3	1.39	0.00
50% 2.lcd	Imipress Tablet	Method Validation	8.18	3574339.3	7247.6	1.39	0.00
50% 3.lcd	Imipress Tablet	Method Validation	8.18	3565414.3	7263.7	1.40	0.00
Average			8.20	3561685.7	7255.2	1.39	0.00
%RSD			0.352	0.418	0.111	0.414	0.000
Maximum			8.23	3574339.3	7263.7	1.40	0.00
Minimum			8.18	3545303.4	7247.6	1.39	0.00
Standard Deviation			0.03	14872.7	8.1	0.01	0.00

Chromatogram 9: Chromatogram for at 80% of target concentration.

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

Acquired by : Radiatul Nadiyah Razali
 Sample Name : Imipress Tablet (Assay)
 Sample ID : Method Validation
 Mobile phase : 0.06M Sodium Perchlorate: Acetonitrile: Triethylamine (625 : 375: 1)
 Column : Phenomenex C18, Gemini, 5 um, 150 mm, 4.6 mm
 Flow rate : 1.0 min/ mL

<Chromatogram>



<< PDA >>

ID#1 Compound Name: Imipramine Hydrochloride

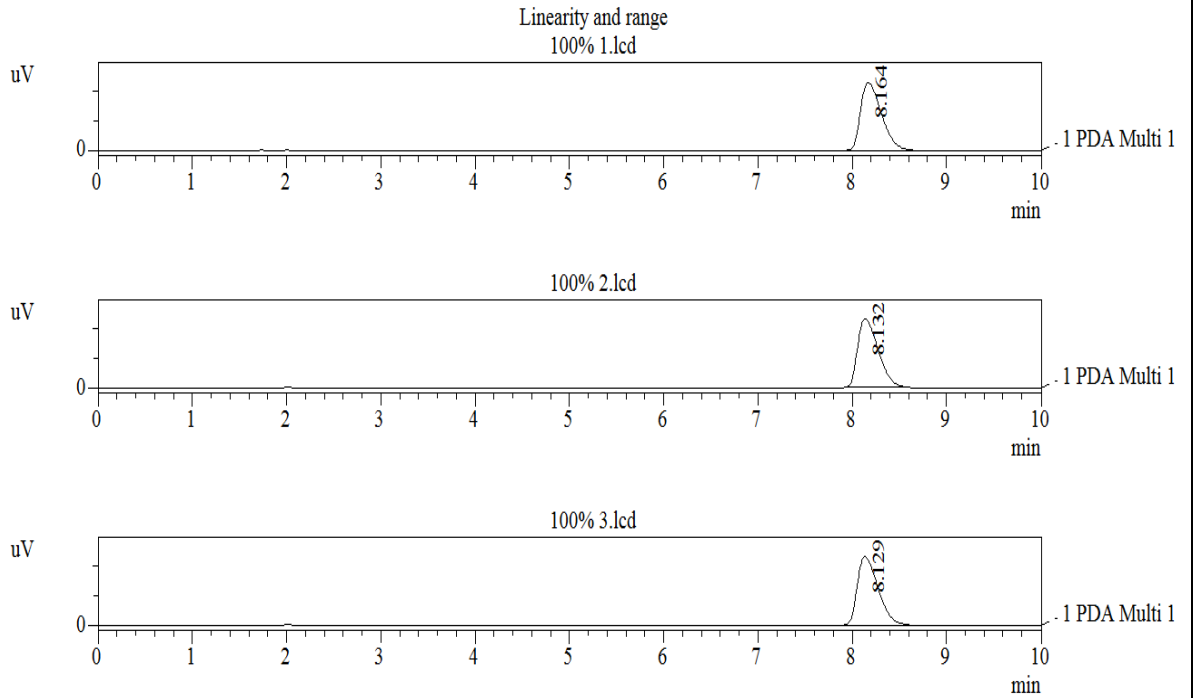
Title	Sample Name	Sample ID	Retention Time	Area	Theoretical Plate	Tailing Factor	Resolution
80%.lcd	Imipress Tablet	Method Validation	8.19	5676312.6	6617.7	1.51	0.00
80% 2.lcd	Imipress Tablet	Method Validation	8.29	5665818.4	6713.0	1.51	0.00
80% 3.lcd	Imipress Tablet	Method Validation	8.02	5671404.2	6851.5	1.52	0.00
Average			8.17	5671178.4	6727.4	1.51	0.00
%RSD			1.671	0.093	1.748	0.382	0.000
Maximum			8.29	5676312.6	6851.5	1.52	0.00
Minimum			8.02	5665818.4	6617.7	1.51	0.00
Standard Deviation			0.14	5250.7	117.6	0.01	0.00

Chromatogram 10: Chromatogram for at 100% of target concentration.

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

Acquired by : Radiatul Nadiah Razali
 Sample Name : Imipress Tablet (Assay)
 Sample ID : Method Validation
 Mobile phase : 0.06M Sodium Perchlorate: Acetonitrile: Triethylamine (625 : 375: 1)
 Column : Phenomenex C18, Gemini, 5 um, 150 mm, 4.6 mm
 Flow rate : 1.0 min/ mL

<Chromatogram>



<<PDA >>

ID#1 Compound Name: Imipramine Hydrochloride

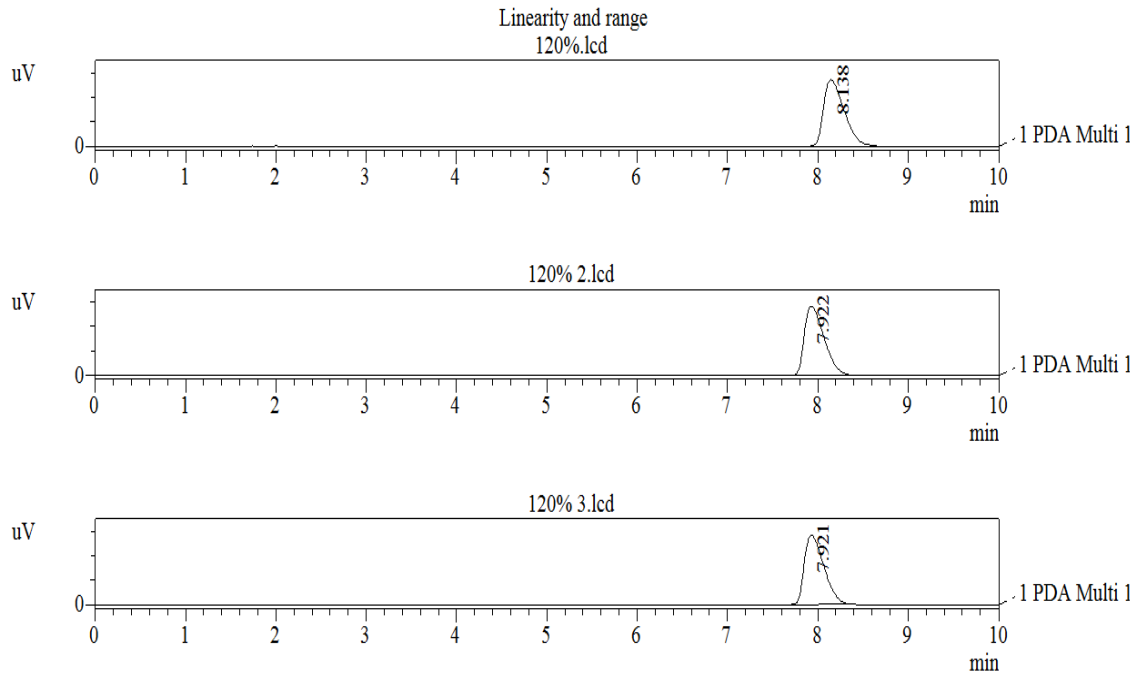
Title	Sample Name	Sample ID	Retention Time	Area	Theoretical Plate	Tailing Factor	Resolution
100% 1.lcd	Imipress Tablet	Method Validation	8.16	7099833.1	6216.7	1.58	0.00
100% 2.lcd	Imipress Tablet	Method Validation	8.13	7093459.0	6261.4	1.58	0.00
100% 3.lcd	Imipress Tablet	Method Validation	8.13	7099350.0	6249.6	1.57	0.00
Average			8.14	7097547.4	6242.6	1.58	0.00
%RSD			0.213	0.050	0.371	0.366	0.000
Maximum			8.16	7099833.1	6261.4	1.58	0.00
Minimum			8.13	7093459.0	6216.7	1.57	0.00
Standard Deviation			0.02	3548.9	23.2	0.01	0.00

Chromatogram 11: Chromatogram for at 120% of target concentration.

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

Acquired by : Radiatul Nadiah Razali
 Sample Name : Imipress Tablet (Assay)
 Sample ID : Method Validation
 Mobile phase : 0.06M Sodium Perchlorate: Acetonitrile: Triethylamine (625 : 375: 1)
 Column : Phenomenex C18, Gemini, 5 um, 150 mm, 4.6 mm
 Flow rate : 1.0 min/ mL

<Chromatogram>



<< PDA >>

ID#1 Compound Name: Imipramine Hydrochloride

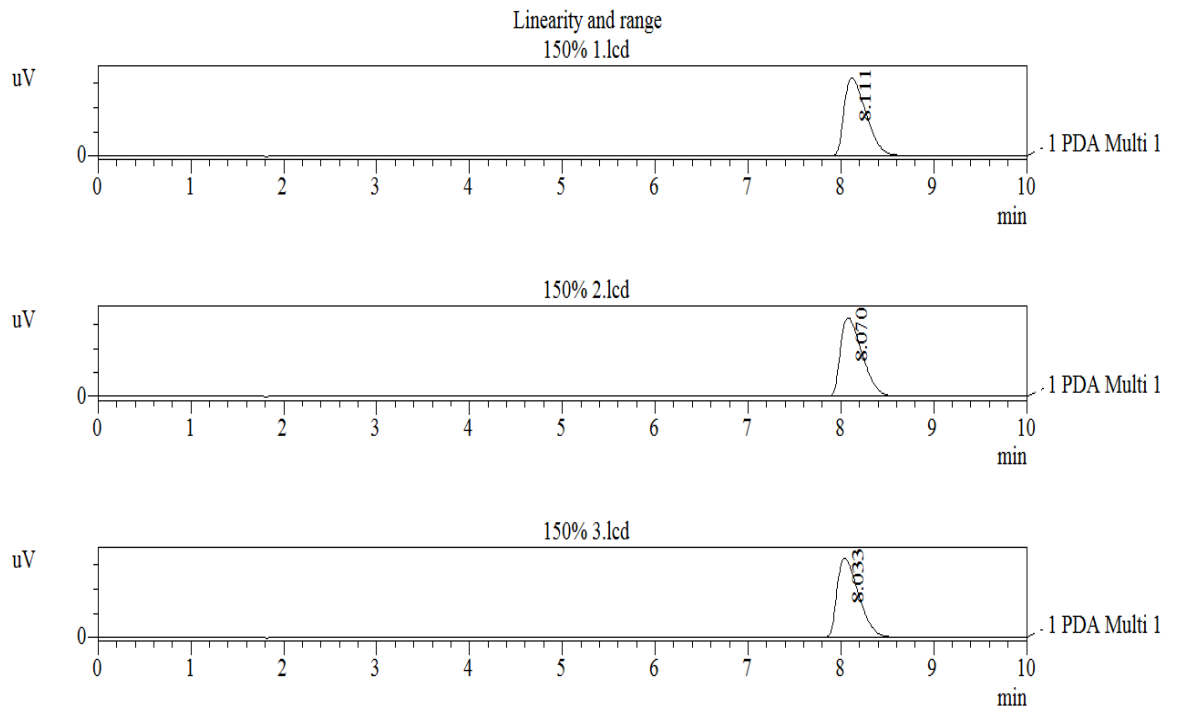
Title	Sample Name	Sample ID	Retention Time	Area	Theoretical Plate	Tailing Factor	Resolution
120%.lcd	Imipress Tablet	Method Validation	8.14	8510913.8	5856.6	1.64	0.00
120% 2.lcd	Imipress Tablet	Method Validation	7.92	8598557.2	5964.1	1.67	0.00
120% 3.lcd	Imipress Tablet	Method Validation	7.92	8573588.6	5991.3	1.66	0.00
Average			7.99	8561019.9	5937.3	1.66	0.00
%RSD			1.589	0.527	1.200	0.922	0.000
Maximum			8.14	8598557.2	5991.3	1.67	0.00
Minimum			7.92	8510913.8	5856.6	1.64	0.00
Standard Deviation			0.13	45153.3	71.2	0.02	0.00

Chromatogram 12: Chromatogram for at 150% of target concentration.

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

Acquired by : Radiatul Nadiah Razali
 Sample Name : Imipress Tablet (Assay)
 Sample ID : Method Validation
 Mobile phase : 0.06M Sodium Perchlorate: Acetonitrile: Triethylamine (625 : 375: 1)
 Column : Phenomenex C18, Gemini, 5 um, 150 mm, 4.6 mm
 Flow rate : 1.0 min/ mL

<Chromatogram>



<<PDA >>

ID#1 Compound Name: Imipramine Hydrochloride

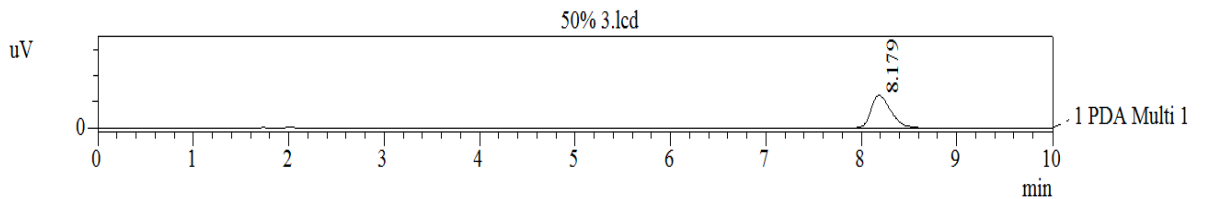
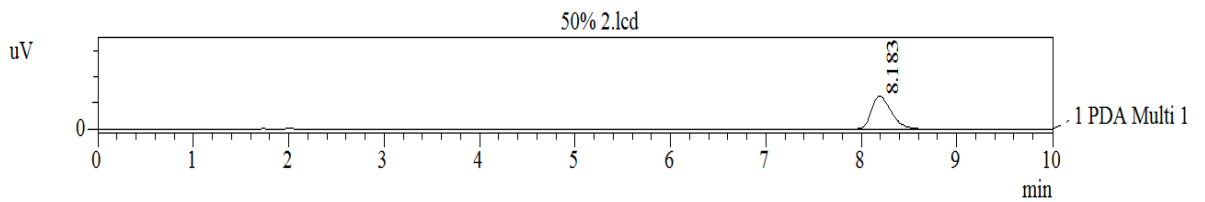
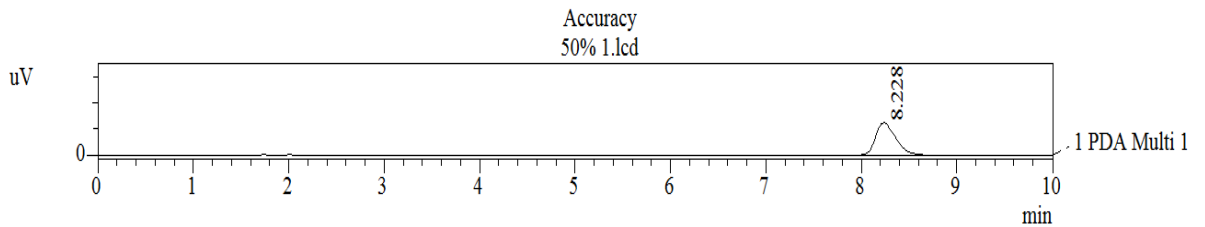
Title	Sample Name	Sample ID	Retention Time	Area	Theoretical Plate	Tailing Factor	Resolution
150% 1.lcd	Imipress Tablet	Method Validation	8.11	10658558.2	5366.3	1.72	0.00
150% 2.lcd	Imipress Tablet	Method Validation	8.07	10692079.1	5380.0	1.72	0.00
150% 3.lcd	Imipress Tablet	Method Validation	8.03	10626836.6	5407.9	1.72	0.00
Average			8.07	10659158.0	5384.7	1.72	0.00
%RSD			0.496	0.306	0.394	0.000	0.000
Maximum			8.11	10692079.1	5407.9	1.72	0.00
Minimum			8.03	10626836.6	5366.3	1.72	0.00
Standard Deviation			0.04	32625.4	21.2	0.00	0.00

Chromatogram 13: Chromatogram for 3 samples at 50% of target concentration.

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

Acquired by : Radiatul Nadiah Razali
 Sample Name : Imipress Tablet (Assay)
 Sample ID : Method Validation
 Mobile phase : 0.06M Sodium Perchlorate: Acetonitrile: Triethylamine (625 : 375: 1)
 Column : Phenomenex C18, Gemini, 5 um, 150 mm, 4.6 mm
 Flow rate : 1.0 min/ mL

<Chromatogram>



<< PDA >>

ID#1 Compound Name: Imipramine Hydrochloride

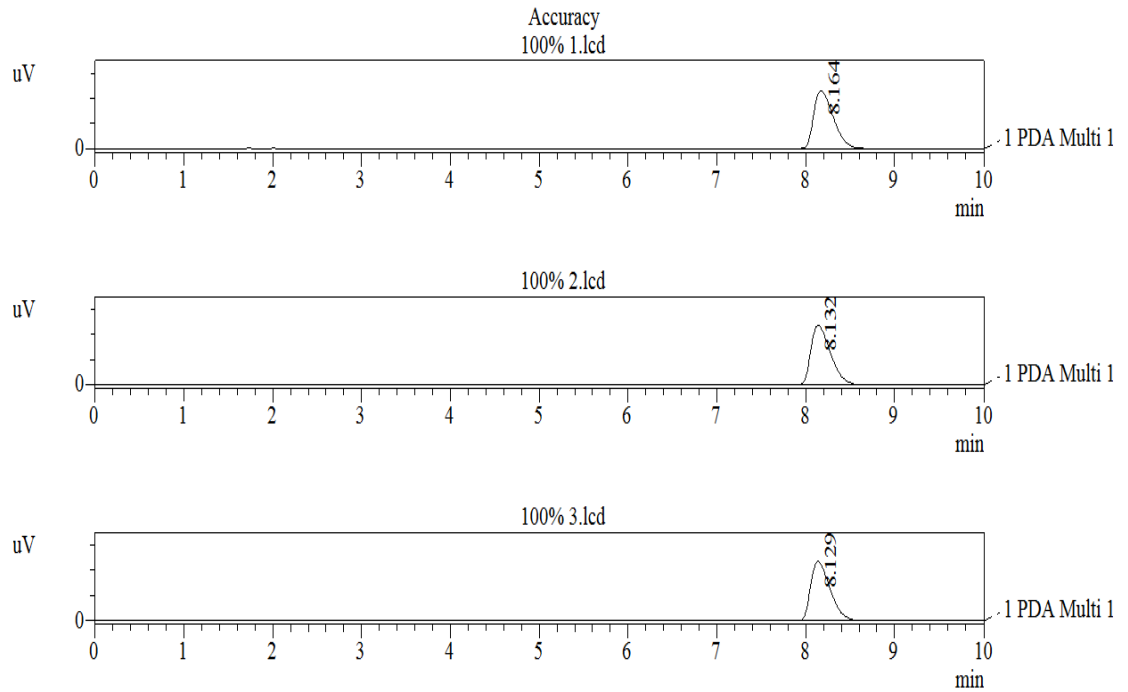
Title	Sample Name	Sample ID	Retention Time	Area	Theoretical Plate	Tailing Factor	Resolution
50% 1.lcd	Imipress Tablet	Method Validation	8.23	3545303.4	7254.3	1.39	0.00
50% 2.lcd	Imipress Tablet	Method Validation	8.18	3574339.3	7247.6	1.39	0.00
50% 3.lcd	Imipress Tablet	Method Validation	8.18	3565414.3	7263.7	1.40	0.00
Average			8.20	3561685.7	7255.2	1.39	0.00
%RSD			0.352	0.418	0.111	0.414	0.000
Maximum			8.23	3574339.3	7263.7	1.40	0.00
Minimum			8.18	3545303.4	7247.6	1.39	0.00
Standard Deviation			0.03	14872.7	8.1	0.01	0.00

Chromatogram 14: Chromatogram for 3 samples at 100% of the target concentration

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

Acquired by : Radiatul Nadiah Razali
 Sample Name : Imipress Tablet (Assay)
 Sample ID : Method Validation
 Mobile phase : 0.06M Sodium Perchlorate: Acetonitrile: Triethylamine (625 : 375: 1)
 Column : Phenomenex C18, Gemini, 5 um, 150 mm, 4.6 mm
 Flow rate : 1.0 min/ mL

<Chromatogram>



<< PDA >>

ID#1 Compound Name: Imipramine Hydrochloride

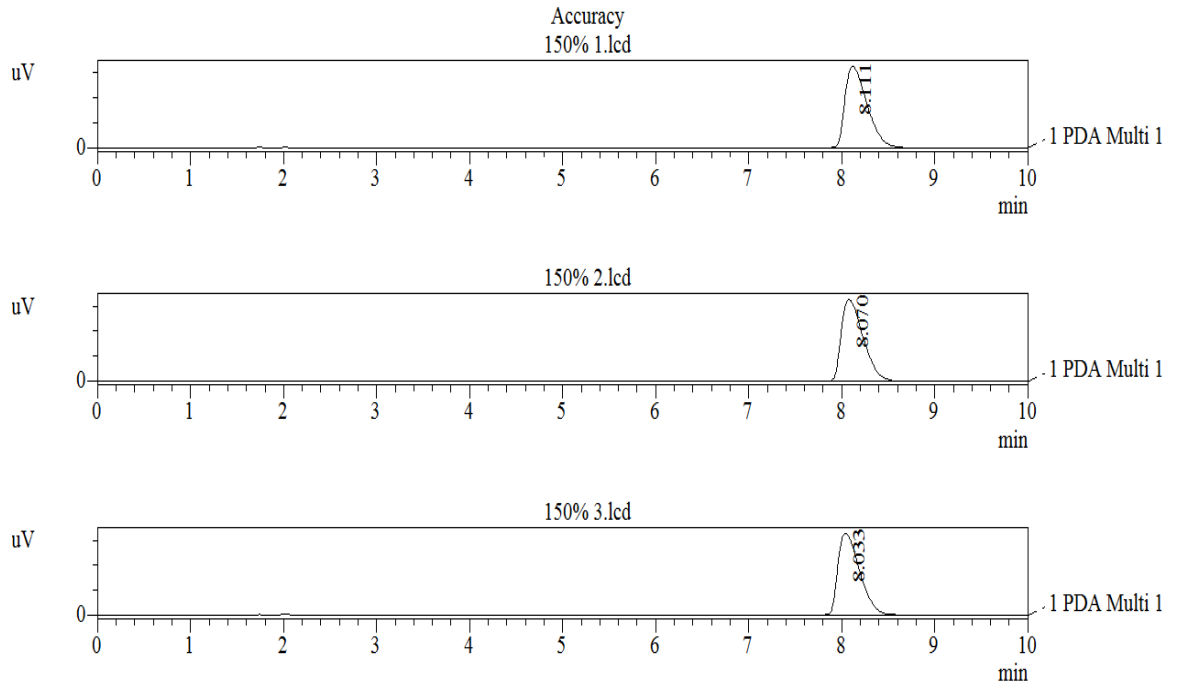
Title	Sample Name	Sample ID	Retention Time	Area	Theoretical Plate	Tailing Factor	Resolution
100% 1.lcd	Imipress Tablet	Method Validation	8.16	7099833.1	6216.7	1.58	0.00
100% 2.lcd	Imipress Tablet	Method Validation	8.13	7093459.0	6261.4	1.58	0.00
100% 3.lcd	Imipress Tablet	Method Validation	8.13	7099350.0	6249.6	1.57	0.00
Average			8.14	7097547.4	6242.6	1.58	0.00
%RSD			0.213	0.050	0.371	0.366	0.000
Maximum			8.16	7099833.1	6261.4	1.58	0.00
Minimum			8.13	7093459.0	6216.7	1.57	0.00
Standard Deviation			0.02	3548.9	23.2	0.01	0.00

Chromatogram 15: Chromatogram for 3 samples at 150% of the target concentration.

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

Acquired by : Radiatul Nadiah Razali
 Sample Name : Imipress Tablet (Assay)
 Sample ID : Method Validation
 Mobile phase : 0.06M Sodium Perchlorate: Acetonitrile: Triethylamine (625 : 375: 1)
 Column : Phenomenex C18, Gemini, 5 um, 150 mm, 4.6 mm
 Flow rate : 1.0 min/ mL

<Chromatogram>



<< PDA >>

ID#1 Compound Name: Imipramine Hydrochloride

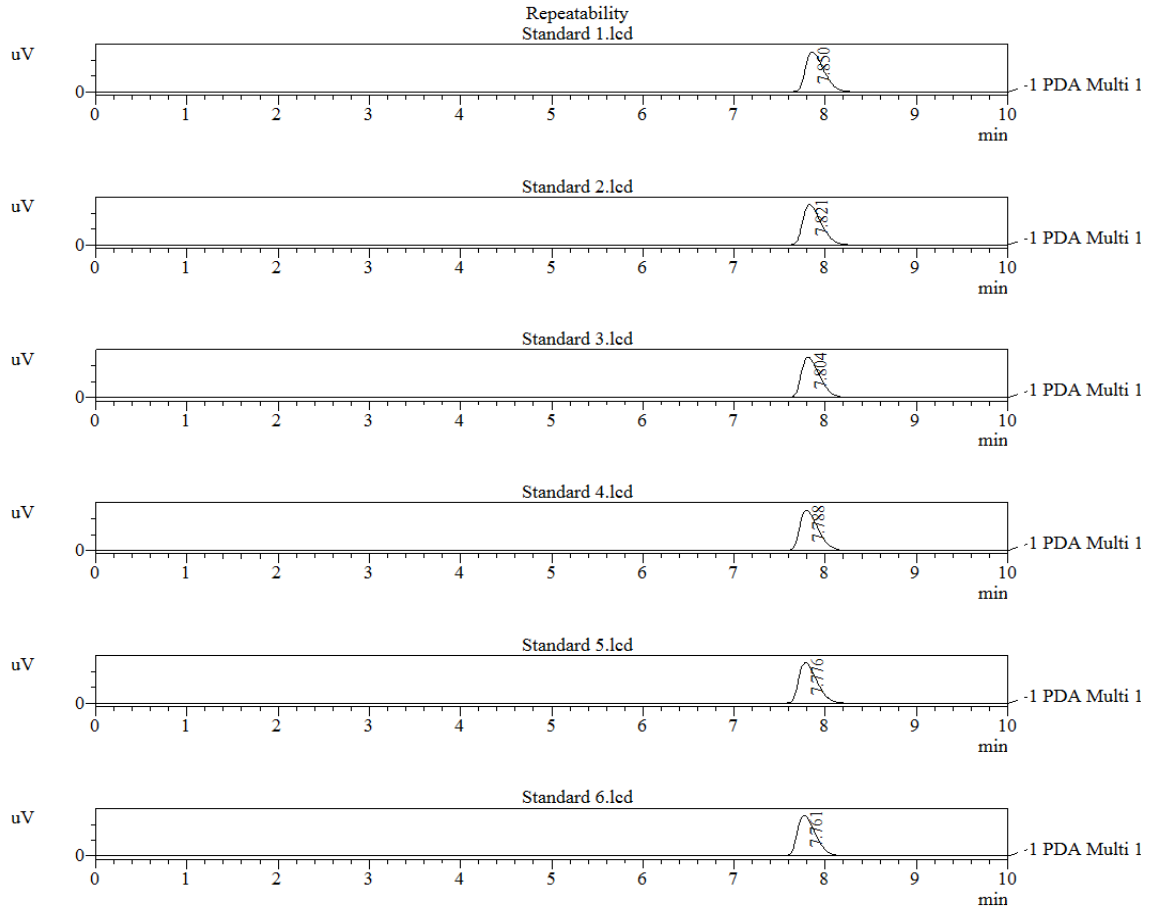
Title	Sample Name	Sample ID	Retention Time	Area	Theoretical Plate	Tailing Factor	Resolution
150% 1.lcd	Imipress Tablet	Method Validation	8.11	10658558.2	5366.3	1.72	0.00
150% 2.lcd	Imipress Tablet	Method Validation	8.07	10692079.1	5380.0	1.72	0.00
150% 3.lcd	Imipress Tablet	Method Validation	8.03	10626836.6	5407.9	1.72	0.00
Average			8.07	10659158.0	5384.7	1.72	0.00
%RSD			0.496	0.306	0.394	0.000	0.000
Maximum			8.11	10692079.1	5407.9	1.72	0.00
Minimum			8.03	10626836.6	5366.3	1.72	0.00
Standard Deviation			0.04	32625.4	21.2	0.00	0.00

Chromatogram 16: Chromatogram of standard

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

Acquired by : Radiatul Nadiah Razali
 Sample Name : Imipress Tablet (Assay)
 Sample ID : Method Validation
 Mobile phase : 0.06M Sodium Perchlorate: Acetonitrile: Triethylamine (625 : 375: 1)
 Column : Phenomenex C18, Gemini, 5 um, 150 mm, 4.6 mm
 Flow rate : 1.0 min/ mL

<Chromatogram>



<< PDA >>

ID#1 Compound Name: Imipramine Hydrochloride

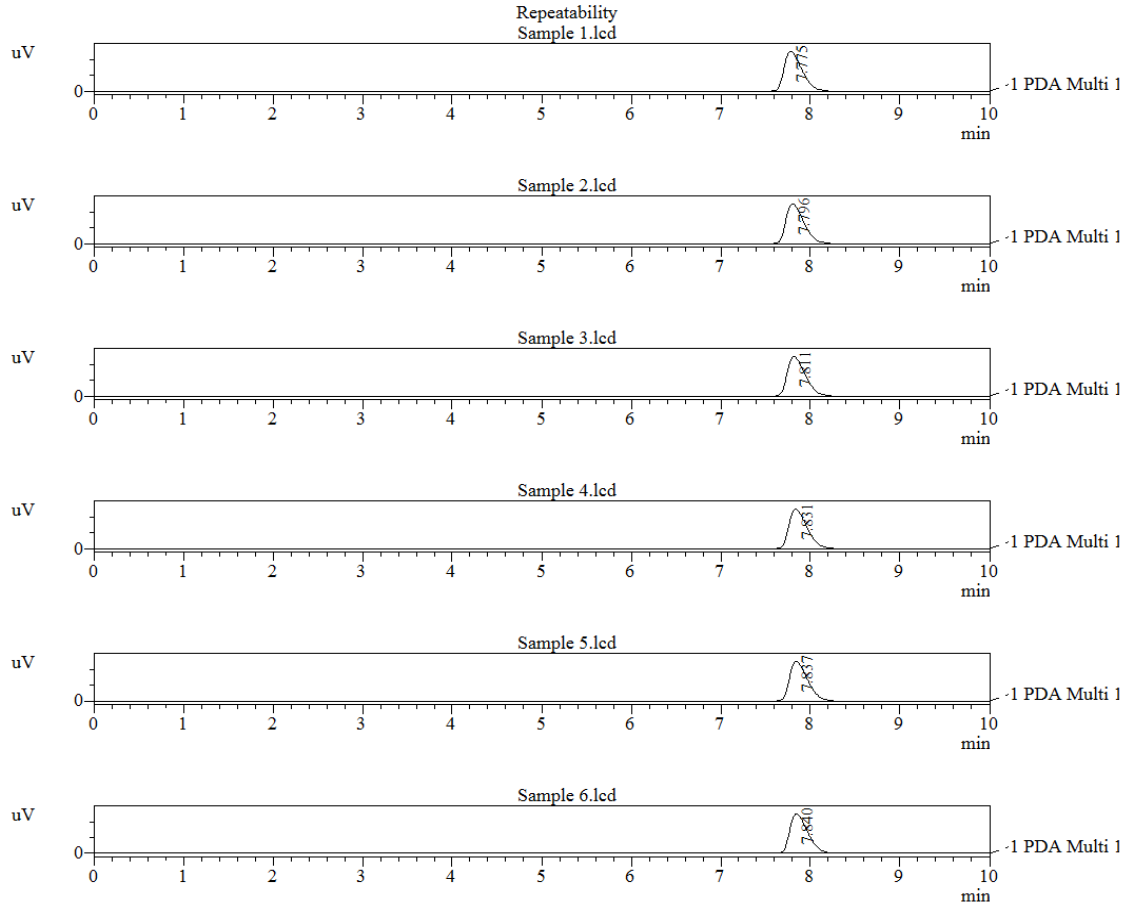
Title	Sample Name	Sample ID	Retention Time	Area	Theoretical Plate	Tailing Factor	Resolution
Standard 1.lcd	Imipress Tablet	Method Validation	7.85	7144443.2	6638.5	1.57	0.00
Standard 2.lcd	Imipress Tablet	Method Validation	7.82	7147948.4	6655.5	1.56	0.00
Standard 3.lcd	Imipress Tablet	Method Validation	7.80	7148073.9	6683.0	1.57	0.00
Standard 4.lcd	Imipress Tablet	Method Validation	7.79	7148737.2	6688.4	1.56	0.00
Standard 5.lcd	Imipress Tablet	Method Validation	7.78	7148939.2	6686.1	1.56	0.00
Standard 6.lcd	Imipress Tablet	Method Validation	7.76	7146315.3	6709.5	1.56	0.00
Average			7.80	7147409.5	6676.8	1.56	0.00
%RSD			0.405	0.024	0.382	0.330	0.000
Maximum			7.85	7148939.2	6709.5	1.57	0.00
Minimum			7.76	7144443.2	6638.5	1.56	0.00
Standard Deviation			0.03	1722.3	25.5	0.01	0.00

Chromatogram 17: Chromatogram of samples

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

Acquired by : Radiatul Nadiah Razali
 Sample Name : Imipress Tablet (Assay)
 Sample ID : Method Validation
 Mobile phase : 0.06M Sodium Perchlorate: Acetonitrile: Triethylamine (625 : 375: 1)
 Column : Phenomenex C18, Gemini, 5 um, 150 mm, 4.6 mm
 Flow rate : 1.0 min/ mL

<Chromatogram>



<< PDA >>

ID#1 Compound Name: Imipramine Hydrochloride

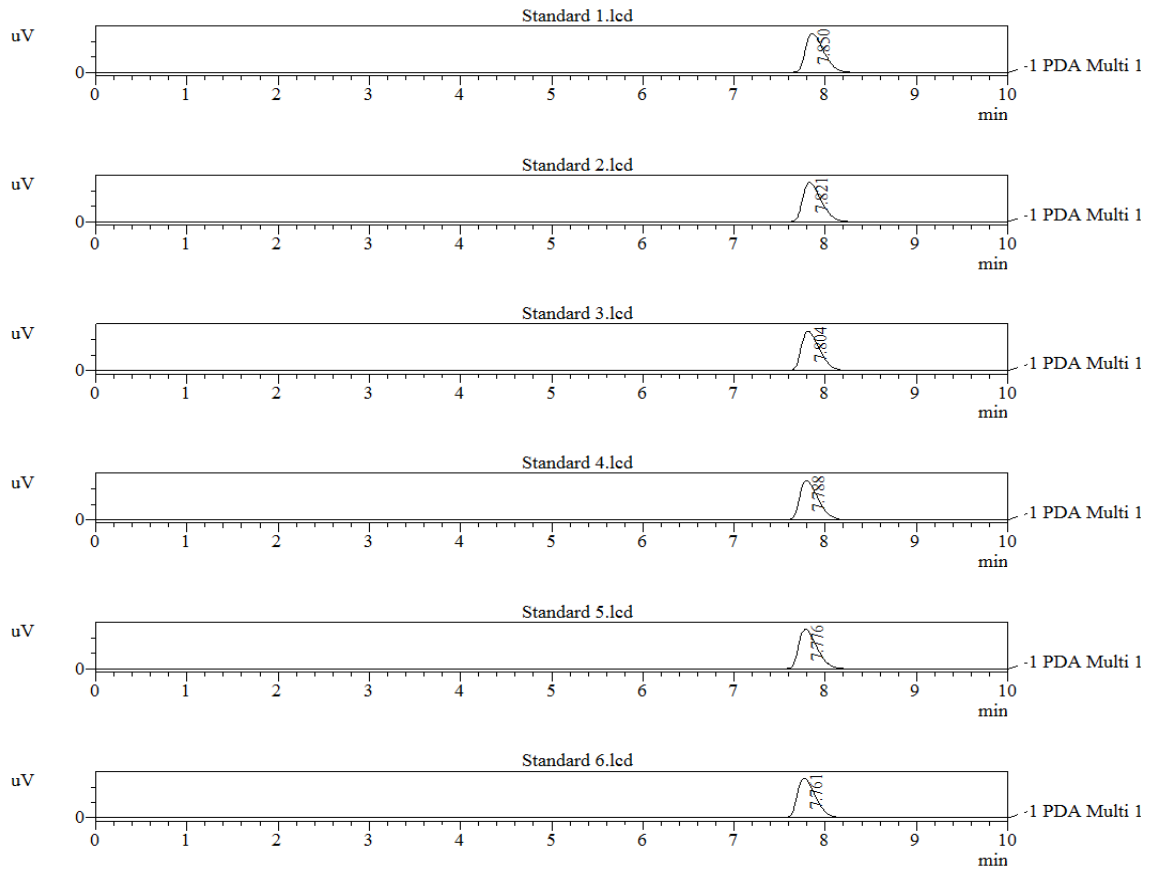
Title	Sample Name	Sample ID	Retention Time	Area	Theoretical Plate	Tailing Factor	Resolution
Sample 1.1cd	Imipress Tablet	Method Validation	7.78	6949239.2	6802.7	1.54	0.00
Sample 2.1cd	Imipress Tablet	Method Validation	7.80	6990946.0	6784.6	1.54	0.00
Sample 3.1cd	Imipress Tablet	Method Validation	7.81	6958568.6	6763.9	1.54	0.00
Sample 4.1cd	Imipress Tablet	Method Validation	7.83	6992220.7	6751.0	1.54	0.00
Sample 5.1cd	Imipress Tablet	Method Validation	7.84	7000723.6	6779.9	1.54	0.00
Sample 6.1cd	Imipress Tablet	Method Validation	7.84	6997234.1	6760.9	1.54	0.00
Average			7.82	6981488.7	6773.8	1.54	0.00
%RSD			0.310	0.313	0.278	0.000	0.000
Maximum			7.84	7000723.6	6802.7	1.54	0.00
Minimum			7.78	6949239.2	6751.0	1.54	0.00
Standard Deviation			0.02	21853.5	18.8	0.00	0.00

Chromatogram 18: Day 1, Analyst 1, Instrument 1 and Column 1_Chromatogram of 6 Standard Injections.

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

Acquired by : Radiatul Nadiah Razali
 Sample Name : Imipress Tablet (Assay)
 Sample ID : Method Validation
 Mobile phase : 0.06M Sodium Perchlorate: Acetonitrile: Triethylamine (625 : 375: 1)
 Column : Phenomenex C18, Gemini, 5 um, 150 mm, 4.6 mm
 Flow rate : 1.0 min/ mL

<Chromatogram>



<< PDA >>

ID#1 Compound Name: Imipramine Hydrochloride

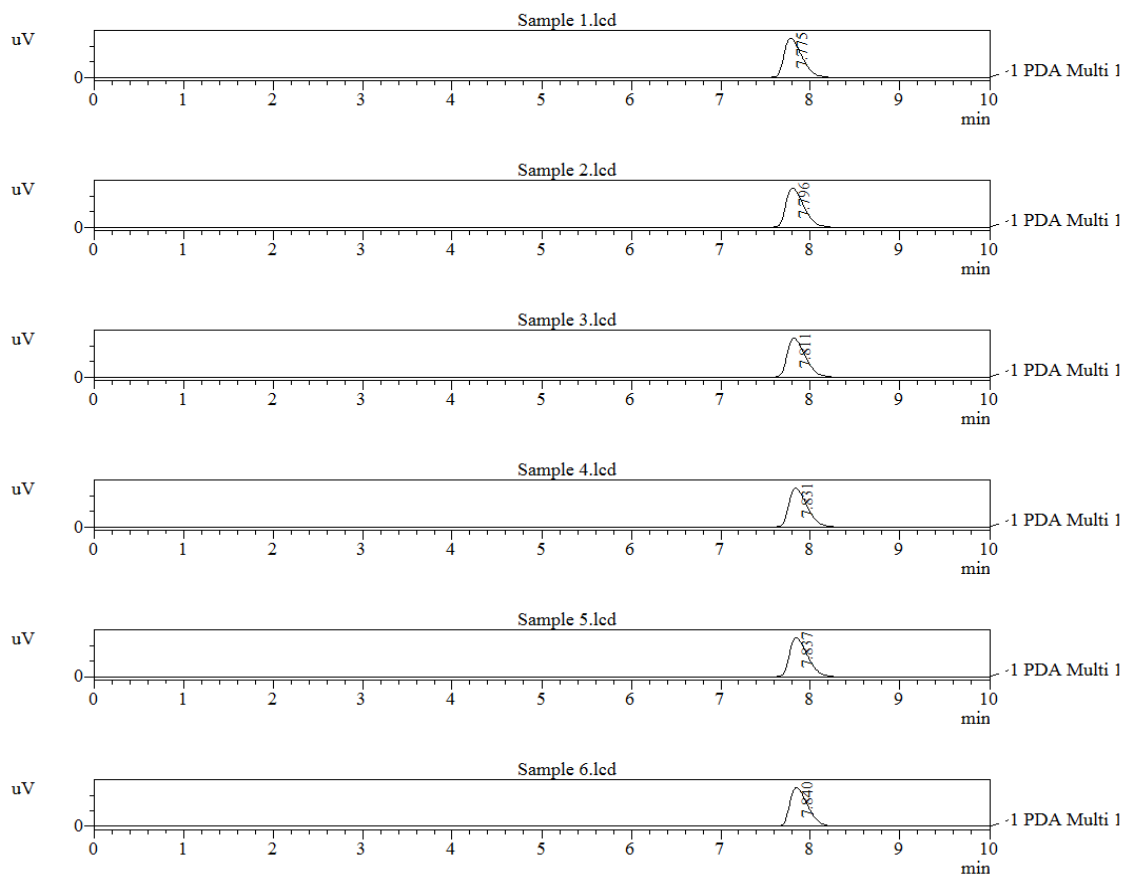
Title	Sample Name	Sample ID	Retention Time	Area	Theoretical Plate	Tailing Factor	Resolution
Standard 1.lcd	Imipress Tablet	Method Validation	7.85	7144443.2	6638.5	1.57	0.00
Standard 2.lcd	Imipress Tablet	Method Validation	7.82	7147948.4	6655.5	1.56	0.00
Standard 3.lcd	Imipress Tablet	Method Validation	7.80	7148073.9	6683.0	1.57	0.00
Standard 4.lcd	Imipress Tablet	Method Validation	7.79	7148737.2	6688.4	1.56	0.00
Standard 5.lcd	Imipress Tablet	Method Validation	7.78	7148939.2	6686.1	1.56	0.00
Standard 6.lcd	Imipress Tablet	Method Validation	7.76	7146315.3	6709.5	1.56	0.00
Average			7.80	7147409.5	6676.8	1.56	0.00
%RSD			0.405	0.024	0.382	0.330	0.000
Maximum			7.85	7148939.2	6709.5	1.57	0.00
Minimum			7.76	7144443.2	6638.5	1.56	0.00
Standard Deviation			0.03	1722.3	25.5	0.01	0.00

Chromatogram 19: Day 1, Analyst 1, Instrument 1 and Column 1_Chromatogram of 6 replicates sample Injections.

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

Acquired by : Radiatul Nadiah Razali
 Sample Name : Imipress Tablet (Assay)
 Sample ID : Method Validation
 Mobile phase : 0.06M Sodium Perchlorate: Acetonitrile: Triethylamine (625 : 375: 1)
 Column : Phenomenex C18, Gemini, 5 um, 150 mm, 4.6 mm
 Flow rate : 1.0 min/ mL

<Chromatogram>



<< PDA >>

ID#1 Compound Name: Imipramine Hydrochloride

Title	Sample Name	Sample ID	Retention Time	Area	Theoretical Plate	Tailing Factor	Resolution
Sample 1.lcd	Imipress Tablet	Method Validation	7.78	6949239.2	6802.7	1.54	0.00
Sample 2.lcd	Imipress Tablet	Method Validation	7.80	6990946.0	6784.6	1.54	0.00
Sample 3.lcd	Imipress Tablet	Method Validation	7.81	6958568.6	6763.9	1.54	0.00
Sample 4.lcd	Imipress Tablet	Method Validation	7.83	6992220.7	6751.0	1.54	0.00
Sample 5.lcd	Imipress Tablet	Method Validation	7.84	7000723.6	6779.9	1.54	0.00
Sample 6.lcd	Imipress Tablet	Method Validation	7.84	6997234.1	6760.9	1.54	0.00
Average			7.82	6981488.7	6773.8	1.54	0.00
%RSD			0.310	0.313	0.278	0.000	0.000
Maximum			7.84	7000723.6	6802.7	1.54	0.00
Minimum			7.78	6949239.2	6751.0	1.54	0.00
Standard Deviation			0.02	21853.5	18.8	0.00	0.00

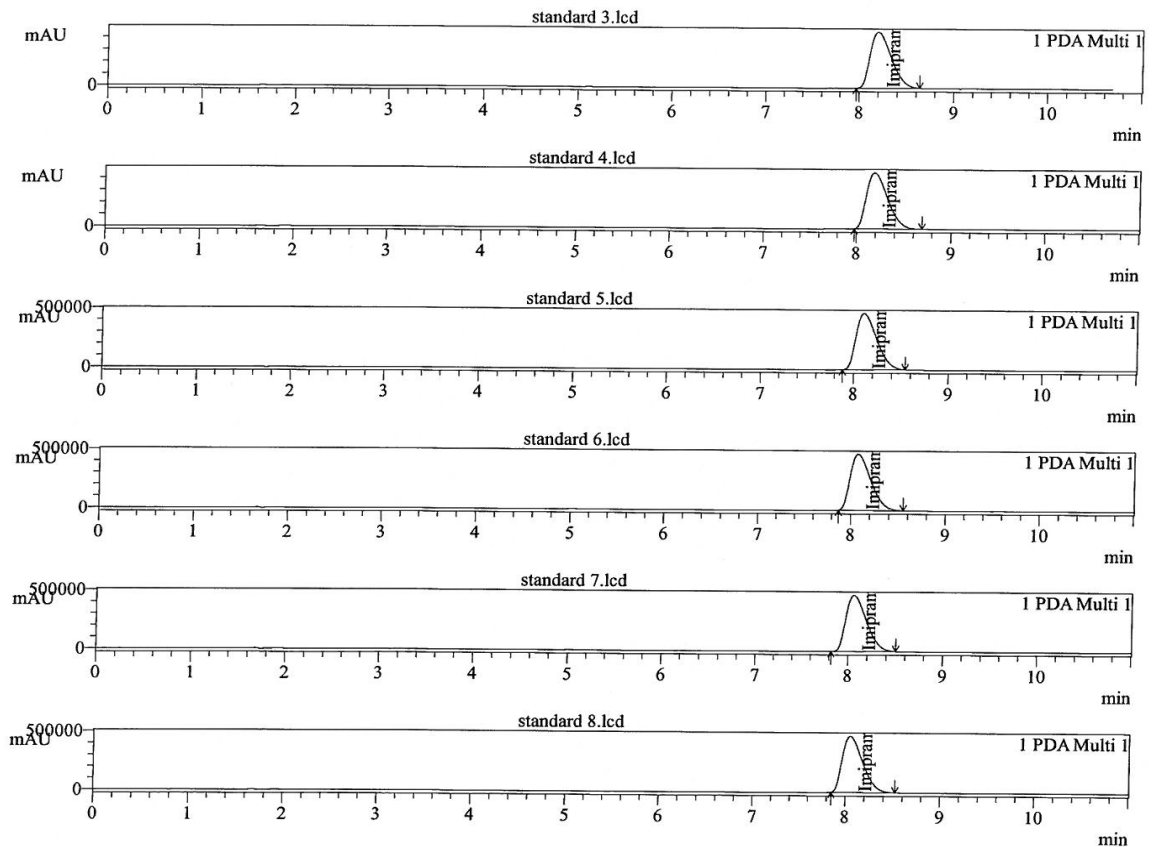
Chromatogram 20: Day 2, Analyst 2, Instrument 2 and Column 2_Chromatogram of 6 Standard Injections.

SHIMADZU LabSolutions Analysis Report

<Sample Information>

Sample Name : Imipramine Tablet
 Sample ID : MV
 Injection Volume : 20 uL
 Date Acquired : 30/7/2013 5:01:14 PM
 Date Processed : 31/7/2013 8:15:39 AM
 Acquired by : Paremala

<Chromatogram>



<< PDA >>

ID#1 Compound Name: Imipramine HCl

Title	Sample Name	Sample ID	Ret. Time	Area	Theoretical Plate(USP)	Tailing Factor
standard 3.lcd	Imipramine Tablet	MV	8.20	7164573.2	6110.4	1.50
standard 4.lcd	Imipramine Tablet	MV	8.19	7166265.2	6071.3	1.50
standard 5.lcd	Imipramine Tablet	MV	8.10	7170866.3	6184.4	1.51
standard 6.lcd	Imipramine Tablet	MV	8.07	7158157.1	6209.6	1.52
standard 7.lcd	Imipramine Tablet	MV	8.05	7194894.2	6240.0	1.51
standard 8.lcd	Imipramine Tablet	MV	8.05	7143083.2	6304.5	1.52
Average			8.11	7166306.5	6186.7	1.51
%RSD			0.81	0.24	1.38	0.66
Standard Deviatc			0.07	17010.5	85.3	0.01

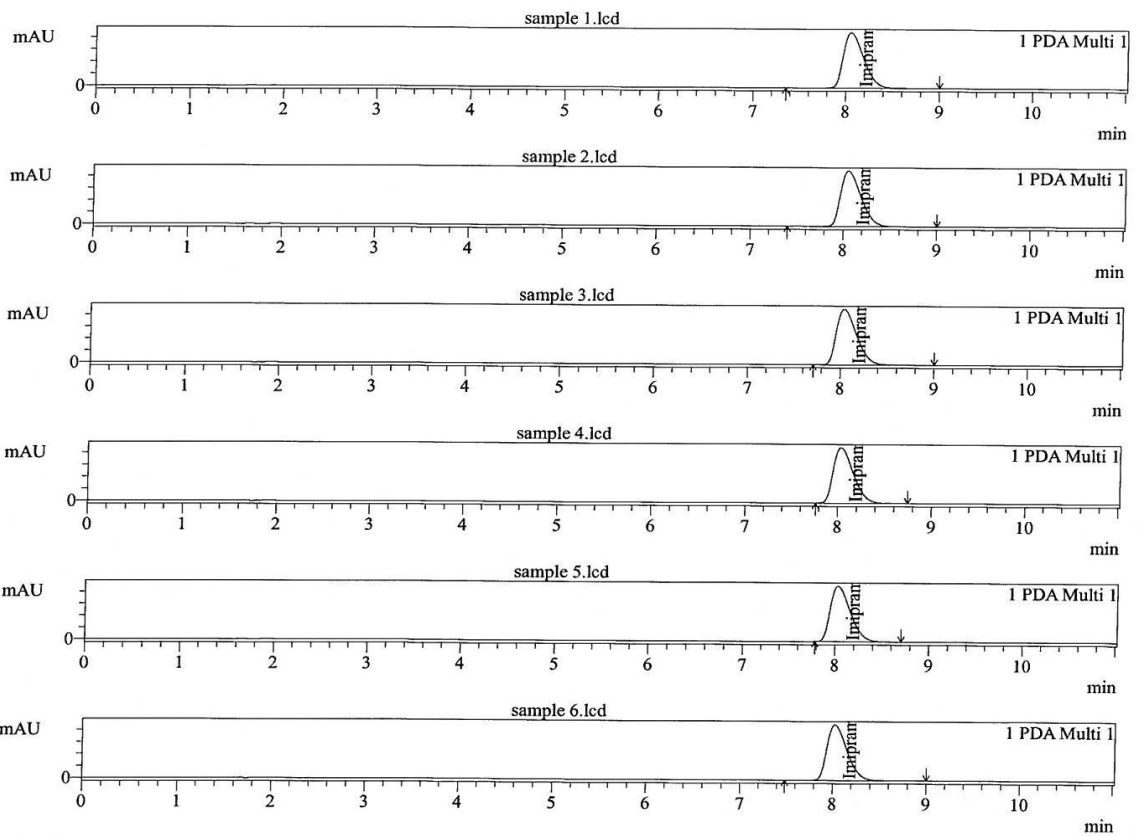
Chromatogram 20: Day 2, Analyst 2, Instrument 2 and Column 2_Chromatogram of 6 Replicates Sample Injection.

SHIMADZU LabSolutions Analysis Report

<Sample Information>

Sample Name : Imipramine Tablet
 Sample ID : MV
 Injection Volume : 20 uL
 Date Acquired : 30/7/2013 5:58:24 PM
 Date Processed : 30/7/2013 6:19:15 PM
 Acquired by : Paremala

<Chromatogram>



<< PDA >>

ID#1 Compound Name: Imipramine HCl

Title	Sample Name	Sample ID	Ret. Time	Area	Theoretical Plate(USP)	Tailing Factor
sample 1.lcd	Imipramine Tablet	MV	8.05	6846088.0	6342.5	1.50
sample 2.lcd	Imipramine Tablet	MV	8.05	6878338.3	6322.6	1.50
sample 3.lcd	Imipramine Tablet	MV	8.04	6901685.0	6346.8	1.50
sample 4.lcd	Imipramine Tablet	MV	8.03	6849749.3	6377.8	1.50
sample 5.lcd	Imipramine Tablet	MV	8.03	6932935.9	6370.2	1.50
sample 6.lcd	Imipramine Tablet	MV	8.02	6893588.3	6355.8	1.49
Average			8.04	6883730.8	6352.6	1.50
%RSD			0.18	0.48	0.31	0.16
Standard Deviativ			0.01	32989.3	20.0	--

Chromatogram 21: Chromatogram of 6 Injections of Standard at Condition A1

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

Acquired by : Radiatul Nadiah Razali
 Sample Name : Imipress Tablet (Assay)
 Sample ID : Method Validation
 Mobile phase : 0.06M Sodium Perchlorate: Acetonitrile: Triethylamine (625 : 375: 1)
 Column : Phenomenex C18, Gemini, 5 um, 150 mm, 4.6 mm
 Flow rate : 1.0 min/ mL

<Chromatogram>



<< PDA >>

ID#1 Compound Name: Imipramine Hydrochloride

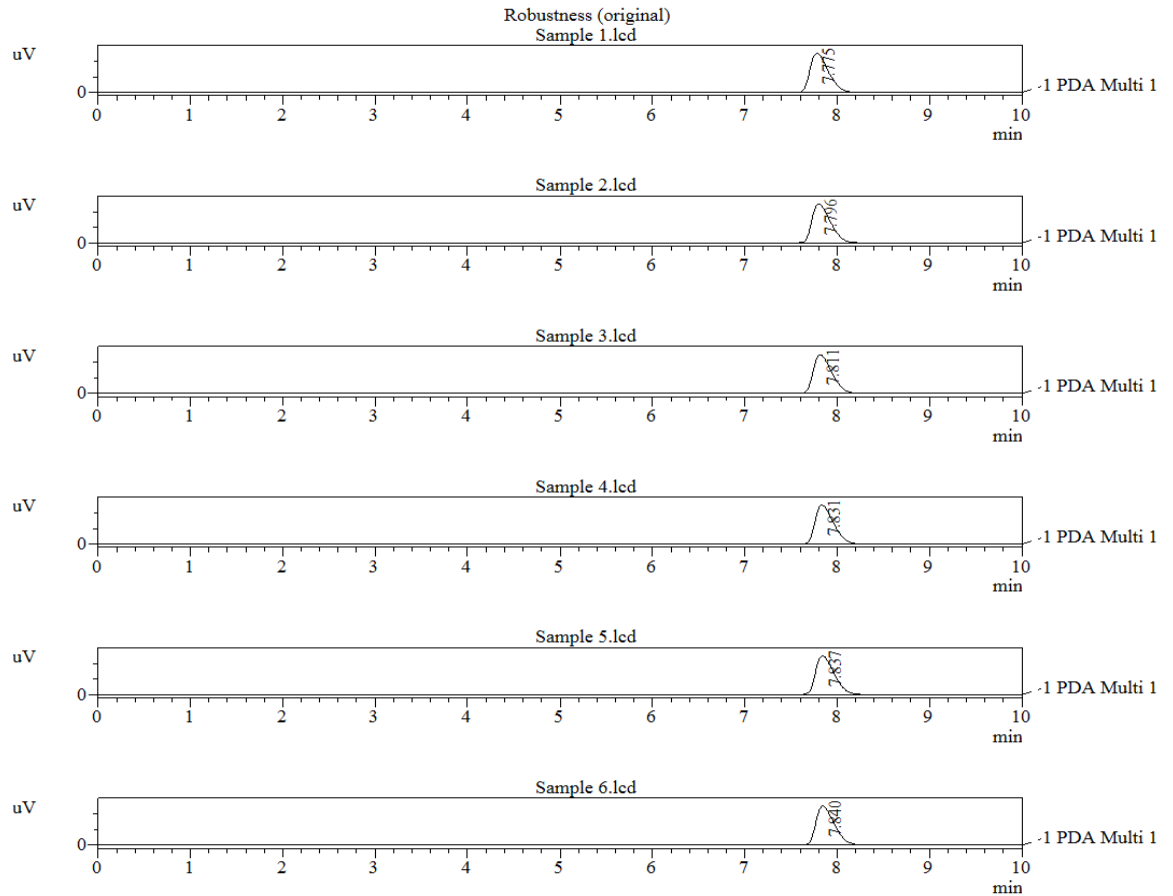
Title	Sample Name	Sample ID	Retention Time	Area	Theoretical Plate	Tailing Factor	Resolution
Standard 1.lcd	Imipress Tablet	Method Validation	7.85	7144443.2	6638.5	1.57	0.00
Standard 2.lcd	Imipress Tablet	Method Validation	7.82	7147948.4	6655.5	1.56	0.00
Standard 3.lcd	Imipress Tablet	Method Validation	7.80	7148073.9	6683.0	1.57	0.00
Standard 4.lcd	Imipress Tablet	Method Validation	7.79	7148737.2	6688.4	1.56	0.00
Standard 5.lcd	Imipress Tablet	Method Validation	7.78	7148939.2	6686.1	1.56	0.00
Standard 6.lcd	Imipress Tablet	Method Validation	7.76	7146315.3	6709.5	1.56	0.00
Average			7.80	7147409.5	6676.8	1.56	0.00
%RSD			0.405	0.024	0.382	0.330	0.000
Maximum			7.85	7148939.2	6709.5	1.57	0.00
Minimum			7.76	7144443.2	6638.5	1.56	0.00
Standard Deviation			0.03	1722.3	25.5	0.01	0.00

Chromatogram 22: Chromatogram of 6 Samples Injection at Condition A1

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

Acquired by : Radiatul Nadiah Razali
 Sample Name : Imipress Tablet (Assay)
 Sample ID : Method Validation
 Mobile phase : 0.06M Sodium Perchlorate: Acetonitrile: Triethylamine (625 : 375: 1)
 Column : Phenomenex C18, Gemini, 5 um, 150 mm, 4.6 mm
 Flow rate : 1.0 min/ mL

<Chromatogram>



<<PDA >>

ID#1 Compound Name: Imipramine Hydrochloride

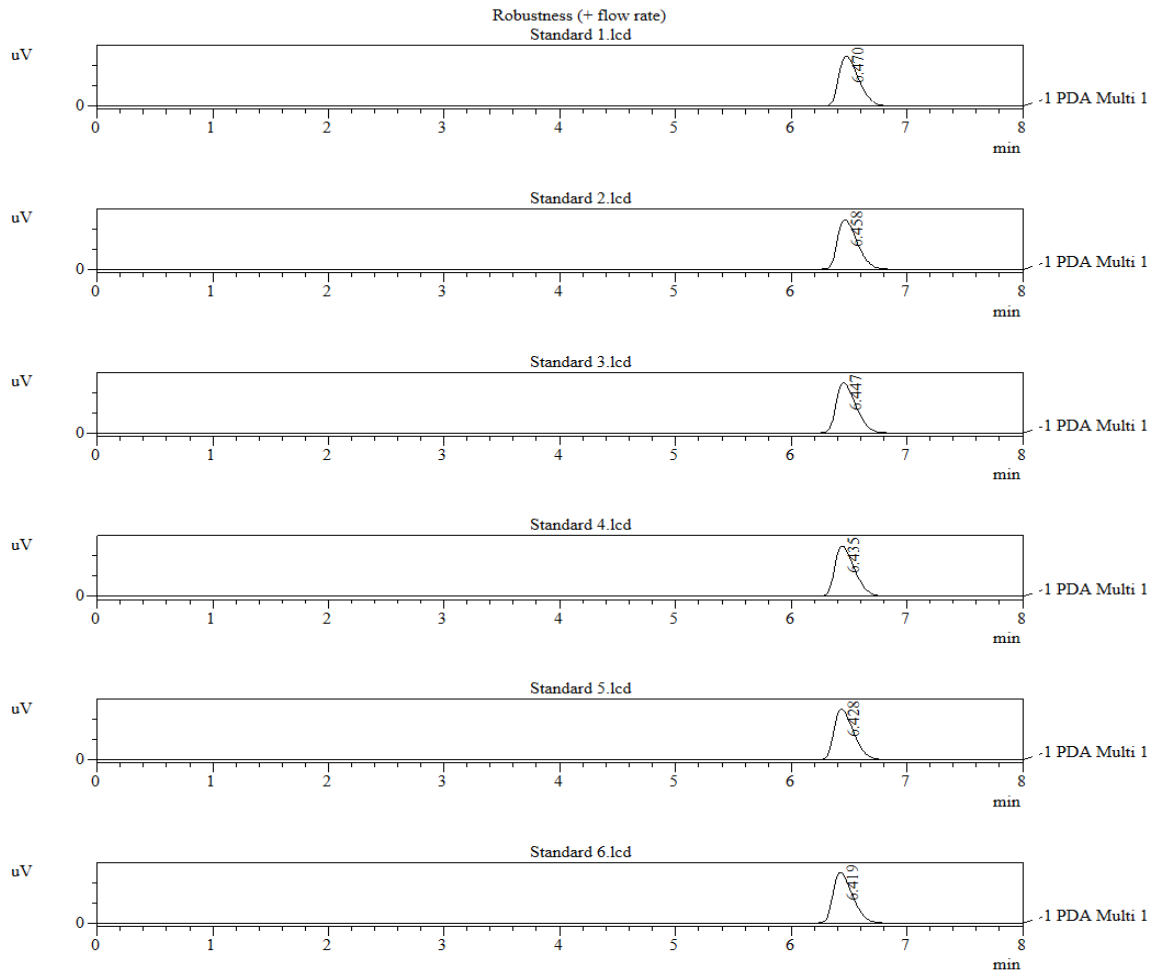
Title	Sample Name	Sample ID	Retention Time	Area	Theoretical Plate	Tailing Factor	Resolution
Sample 1.lcd	Imipress Tablet	Method Validation	7.78	6949239.2	6802.7	1.54	0.00
Sample 2.lcd	Imipress Tablet	Method Validation	7.80	6990946.0	6784.6	1.54	0.00
Sample 3.lcd	Imipress Tablet	Method Validation	7.81	6958568.6	6763.9	1.54	0.00
Sample 4.lcd	Imipress Tablet	Method Validation	7.83	6992220.7	6751.0	1.54	0.00
Sample 5.lcd	Imipress Tablet	Method Validation	7.84	7000723.6	6779.9	1.54	0.00
Sample 6.lcd	Imipress Tablet	Method Validation	7.84	6997234.1	6760.9	1.54	0.00
Average			7.82	6981488.7	6773.8	1.54	0.00
%RSD			0.310	0.313	0.278	0.000	0.000
Maximum			7.84	7000723.6	6802.7	1.54	0.00
Minimum			7.78	6949239.2	6751.0	1.54	0.00
Standard Deviation			0.02	21853.5	18.8	0.00	0.00

Chromatogram 23: Chromatogram of 6 Injections of Standard at Condition B

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

Acquired by : Radiatul Nadiah Razali
 Sample Name : Imipress Tablet (Assay)
 Sample ID : Method Validation
 Mobile phase : 0.06M Sodium Perchlorate: Acetonitrile: Triethylamine (625 : 375: 1)
 Column : Phenomenex C18, Gemini, 5 um, 150 mm, 4,6 mm
 Flow rate : 1.0 min/ mL

<Chromatogram>



<< PDA >>

ID#1 Compound Name: Imipramine Hydrochloride

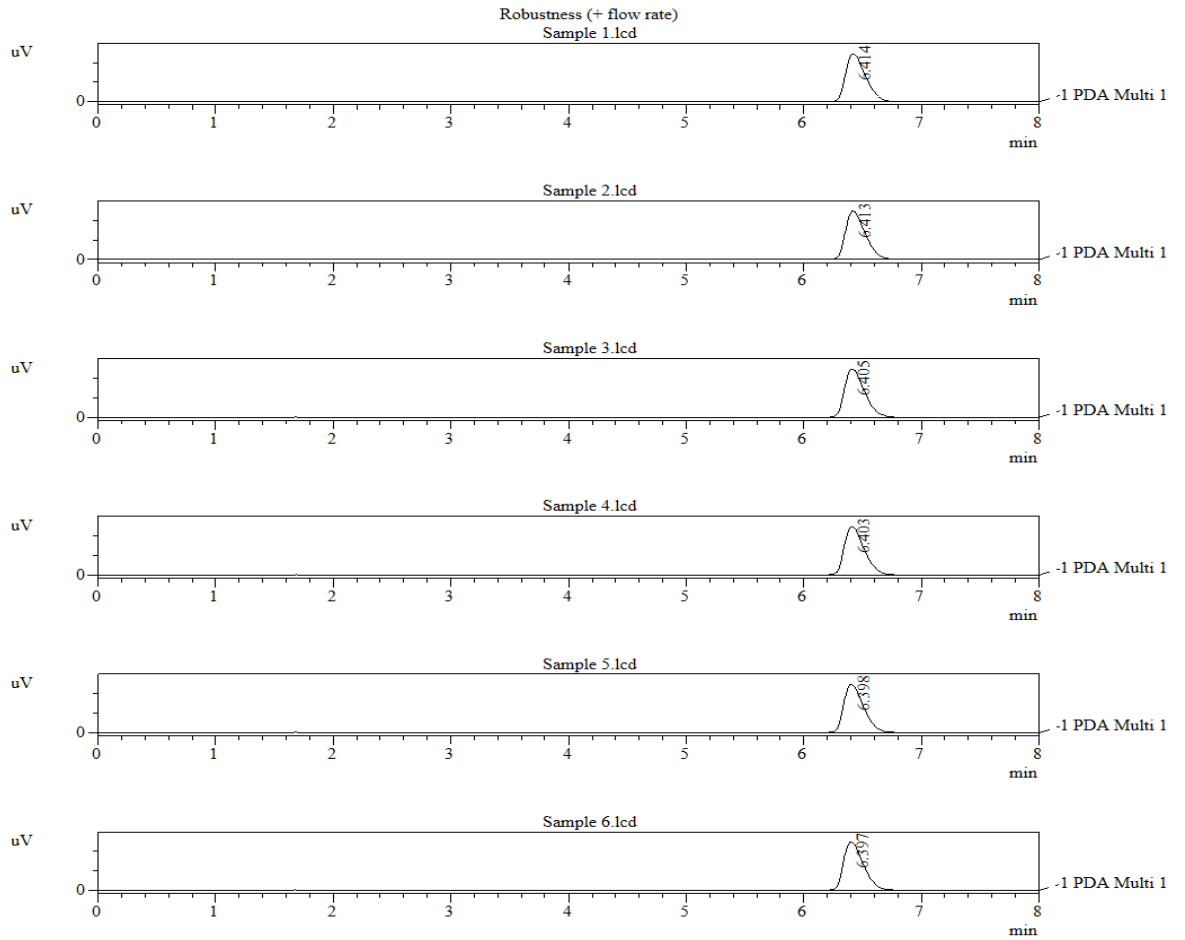
Title	Sample Name	Sample ID	Retention Time	Area	Theoretical Plate	Tailing Factor	Resolution
Standard 1.lcd	Imipress Tablet	Method Validation	6.47	5966482.4	6160.3	1.52	0.00
Standard 2.lcd	Imipress Tablet	Method Validation	6.46	5968438.4	6165.2	1.52	0.00
Standard 3.lcd	Imipress Tablet	Method Validation	6.45	5971429.3	6159.8	1.52	0.00
Standard 4.lcd	Imipress Tablet	Method Validation	6.44	5973406.3	6173.9	1.52	0.00
Standard 5.lcd	Imipress Tablet	Method Validation	6.43	5973596.6	6222.4	1.53	0.00
Standard 6.lcd	Imipress Tablet	Method Validation	6.42	5977428.4	6240.1	1.53	0.00
Average			6.45	5971796.9	6186.9	1.52	0.00
%RSD			0.290	0.066	0.568	0.339	0.000
Maximum			6.47	5977428.4	6240.1	1.53	0.00
Minimum			6.42	5966482.4	6159.8	1.52	0.00
Standard Deviation			0.02	3929.4	35.1	0.01	0.00

Chromatogram 24: Chromatogram of 6 Samples Injection at Condition B

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

Acquired by : Radiatul Nadiah Razali
 Sample Name : Imipress Tablet (Assay)
 Sample ID : Method Validation
 Mobile phase : 0.06M Sodium Perchlorate: Acetonitrile: Triethylamine (625 : 375: 1)
 Column : Phenomenex C-18, Gemini, 5 um, 150 mm, 4.6 mm
 Flow rate : 1.0 min/ mL

<Chromatogram>



<< PDA >>

ID#1 Compound Name: Imipramine Hydrochloride

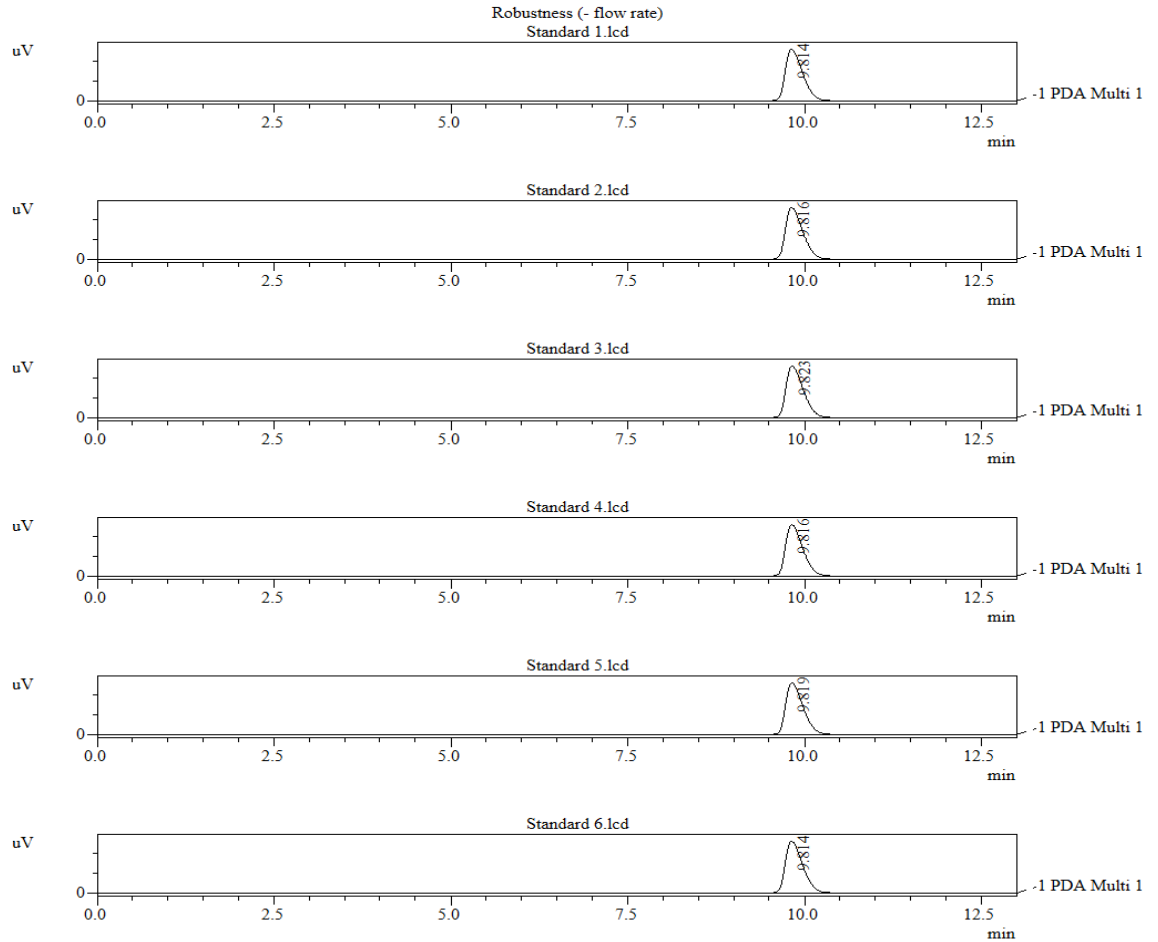
Title	Sample Name	Sample ID	Retention Time	Area	Theoretical Plate	Tailing Factor	Resolution
Sample 1.lcd	Imipress Tablet	Method Validation	6.41	5808047.2	6309.4	1.52	0.00
Sample 2.lcd	Imipress Tablet	Method Validation	6.41	5843357.8	6322.5	1.52	0.00
Sample 3.lcd	Imipress Tablet	Method Validation	6.41	5838782.2	6308.3	1.52	0.00
Sample 4.lcd	Imipress Tablet	Method Validation	6.40	5852115.7	6299.1	1.52	0.00
Sample 5.lcd	Imipress Tablet	Method Validation	6.40	5846993.1	6298.9	1.52	0.00
Sample 6.lcd	Imipress Tablet	Method Validation	6.40	5846747.0	6302.3	1.52	0.00
Average			6.41	5839340.5	6306.8	1.52	0.00
%RSD			0.086	0.273	0.141	0.000	0.000
Maximum			6.41	5852115.7	6322.5	1.52	0.00
Minimum			6.40	5808047.2	6298.9	1.52	0.00
Standard Deviation			0.01	15952.1	8.9	0.00	0.00

Chromatogram 25: Chromatogram of 6 Injections of Standard at Condition C

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

Acquired by : Radiatul Nadiah Razali
 Sample Name : Imipress Tablet (Assay)
 Sample ID : Method Validation
 Mobile phase : 0.06M Sodium Perchlorate: Acetonitrile: Triethylamine (625 : 375: 1)
 Column : Phenomenex C18, Gemini, 5 um, 150 mm, 4.6 mm
 Flow rate : 1.0 min/ mL

<Chromatogram>



<< PDA >>

ID#1 Compound Name: Imipramine Hydrochloride

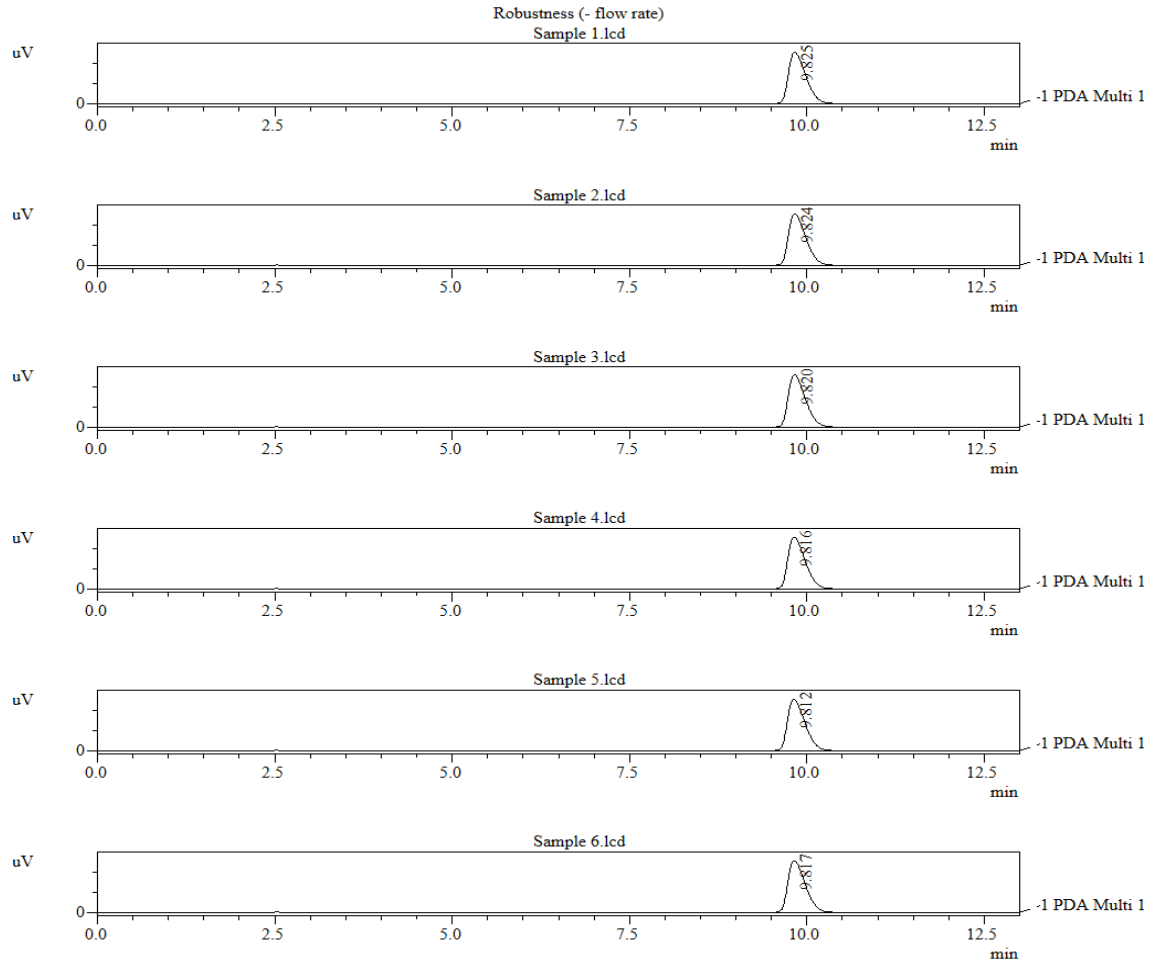
Title	Sample Name	Sample ID	Retention Time	Area	Theoretical Plate	Tailing Factor	Resolution
Standard 1.lcd	Imipress Tablet	Method Validation	9.81	8966914.4	7318.9	1.57	0.00
Standard 2.lcd	Imipress Tablet	Method Validation	9.82	8972005.8	7322.8	1.57	0.00
Standard 3.lcd	Imipress Tablet	Method Validation	9.82	8976265.3	7337.7	1.57	0.00
Standard 4.lcd	Imipress Tablet	Method Validation	9.82	8974441.4	7312.9	1.57	0.00
Standard 5.lcd	Imipress Tablet	Method Validation	9.82	8981504.5	7319.8	1.57	0.00
Standard 6.lcd	Imipress Tablet	Method Validation	9.81	8982488.7	7307.0	1.57	0.00
Average			9.82	8975603.4	7319.9	1.57	0.00
%RSD			0.053	0.065	0.142	0.000	0.000
Maximum			9.82	8982488.7	7337.7	1.57	0.00
Minimum			9.81	8966914.4	7307.0	1.57	0.00
Standard Deviation			0.01	5873.0	10.4	0.00	0.00

Chromatogram 26: Chromatogram of 6 Samples Injection at Condition C

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

Acquired by : Radiatul Nadiah Razali
 Sample Name : Imipress Tablet (Assay)
 Sample ID : Method Validation
 Mobile phase : 0.06M Sodium Perchlorate: Acetonitrile: Triethylamine (625 : 375: 1)
 Column : Phenomenex C18, Gemini, 5 um, 150 mm, 4.6 mm
 Flow rate : 1.0 min/ mL

<Chromatogram>



<< PDA >>

ID#1 Compound Name: Imipramine Hydrochloride

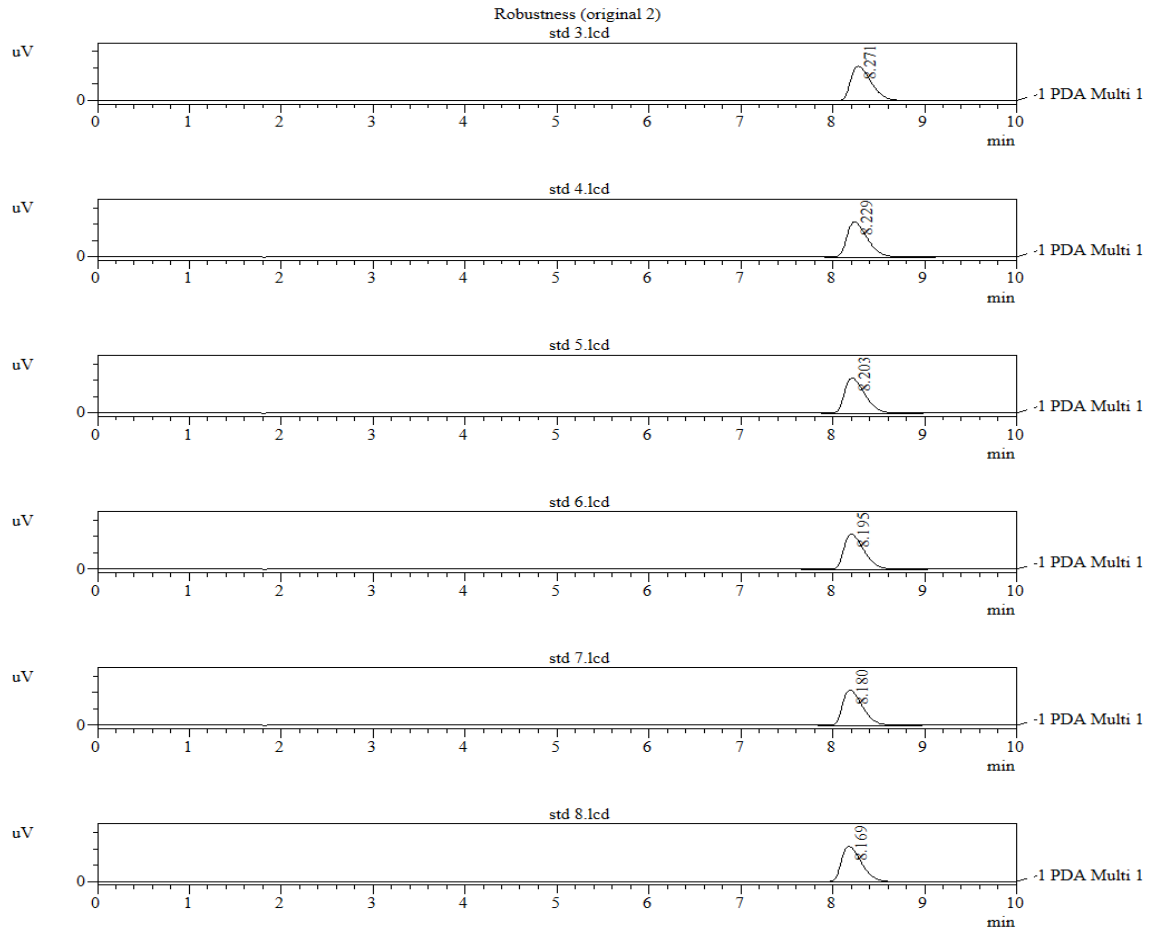
Title	Sample Name	Sample ID	Retention Time	Area	Theoretical Plate	Tailing Factor	Resolution
Sample 1.lcd	Imipress Tablet	Method Validation	9.82	8719796.4	7438.6	1.56	0.00
Sample 2.lcd	Imipress Tablet	Method Validation	9.82	8774879.9	7398.2	1.56	0.00
Sample 3.lcd	Imipress Tablet	Method Validation	9.82	8842131.4	7399.4	1.57	0.00
Sample 4.lcd	Imipress Tablet	Method Validation	9.82	8787107.8	7384.2	1.56	0.00
Sample 5.lcd	Imipress Tablet	Method Validation	9.81	8779535.3	7390.6	1.56	0.00
Sample 6.lcd	Imipress Tablet	Method Validation	9.82	8778388.4	7392.9	1.56	0.00
Average			9.82	8780306.5	7400.7	1.56	0.00
%RSD			0.042	0.443	0.262	0.261	0.000
Maximum			9.82	8842131.4	7438.6	1.57	0.00
Minimum			9.81	8719796.4	7384.2	1.56	0.00
Standard Deviation			0.00	38894.1	19.4	0.00	0.00

Chromatogram 27: Chromatogram of 6 Injections of Standard at Condition A2

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

Acquired by : Radiatul Nadiah Razali
 Sample Name : Imipress Tablet (Assay)
 Sample ID : Method Validation
 Mobile phase : 0.06M Sodium Perchlorate: Acetonitrile: Triethylamine (625 : 375: 1)
 Column : Phenomenex C18, Gemini, 5 um, 150 mm, 4.6 mm
 Flow rate : 1.0 min/ mL

<Chromatogram>



<< PDA >>

ID#1 Compound Name: Imipramine Hydrochloride

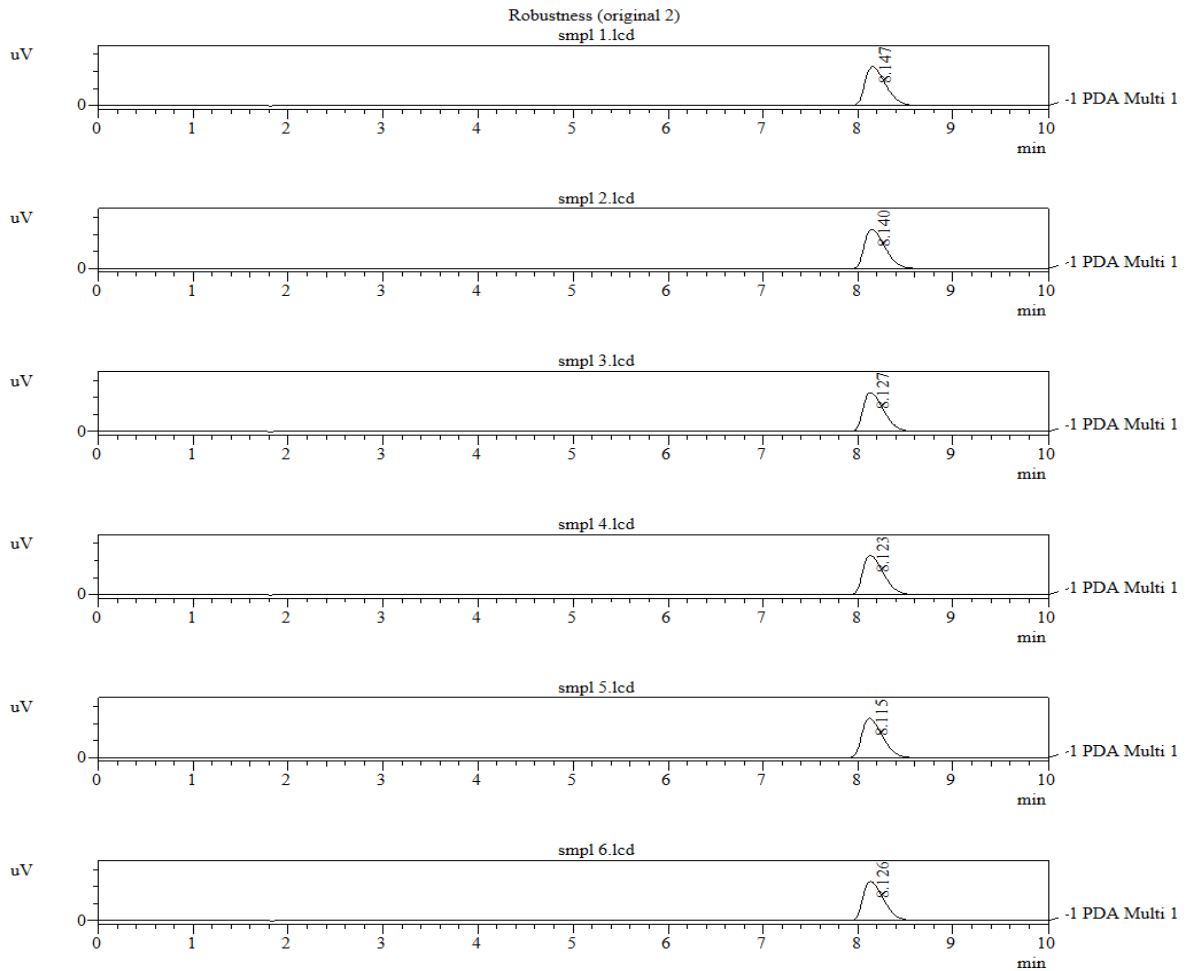
Title	Sample Name	Sample ID	Retention Time	Area	Theoretical Plate	Tailing Factor	Resolution
std 3.lcd	Imipress Tablet	Method Validation	8.27	6849308.5	6230.2	1.58	0.00
std 4.lcd	Imipress Tablet	Method Validation	8.23	6823512.5	6238.4	1.59	0.00
std 5.lcd	Imipress Tablet	Method Validation	8.20	6807574.3	6234.7	1.59	0.00
std 6.lcd	Imipress Tablet	Method Validation	8.20	6832235.5	6278.5	1.59	0.00
std 7.lcd	Imipress Tablet	Method Validation	8.18	6816424.4	6261.0	1.58	0.00
std 8.lcd	Imipress Tablet	Method Validation	8.17	6856990.3	6268.5	1.59	0.00
Average			8.21	6831007.6	6251.9	1.59	0.00
%RSD			0.445	0.280	0.321	0.325	0.000
Maximum			8.27	6856990.3	6278.5	1.59	0.00
Minimum			8.17	6807574.3	6230.2	1.58	0.00
Standard Deviation			0.04	19128.5	20.1	0.01	0.00

Chromatogram 28: Chromatogram of 6 Samples Injection at Condition A2

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

Acquired by : Radiatul Nadiah Razali
 Sample Name : Imipress Tablet (Assay)
 Sample ID : Method Validation
 Mobile phase : 0.06M Sodium Perchlorate: Acetonitrile: Triethylamine (625 : 375: 1)
 Column : Phenomenex C18, Gemini, 5 um, 150 mm, 4.6 mm
 Flow rate : 1.0 min/ mL

<Chromatogram>



<< PDA >>

ID#1 Compound Name: Imipramine Hydrochloride

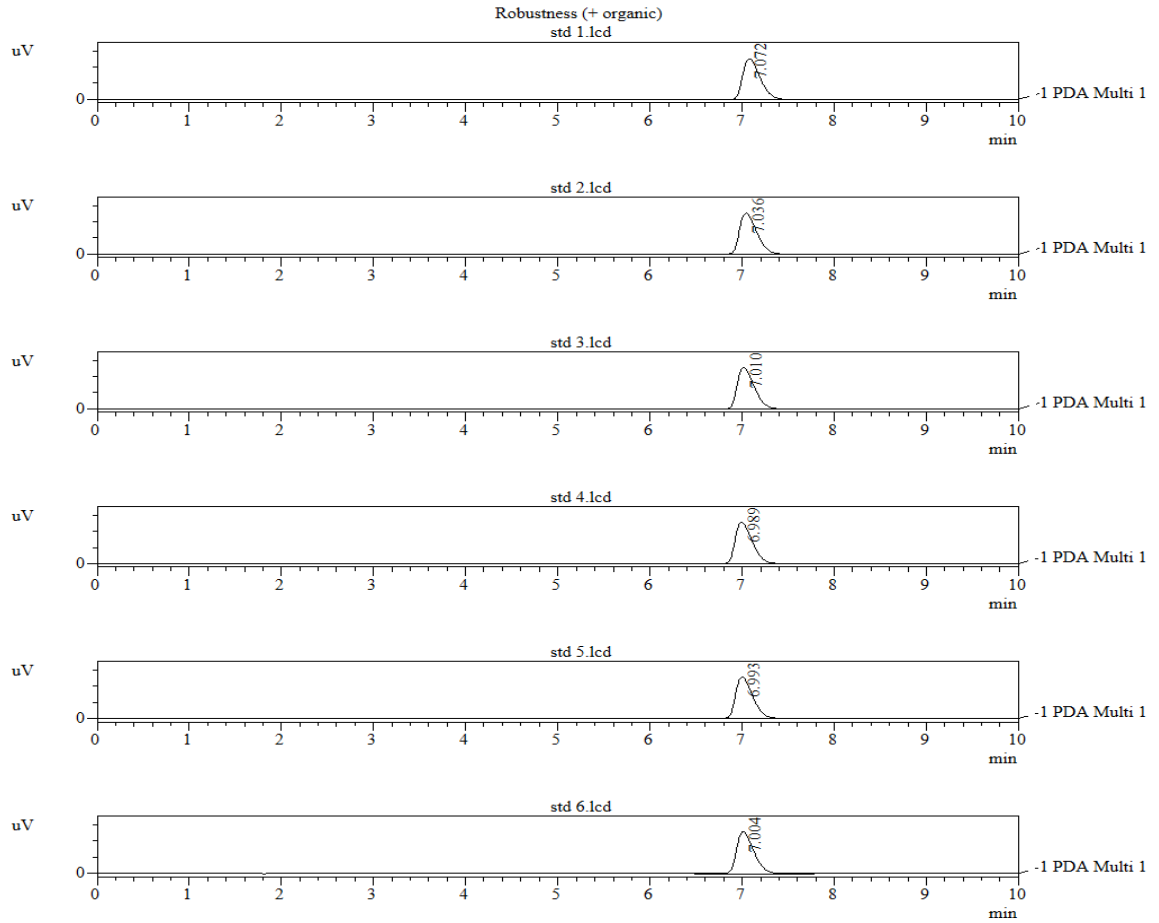
Title	Sample Name	Sample ID	Retention Time	Area	Theoretical Plate	Tailing Factor	Resolution
smpl 1.lcd	Imipress Tablet	Method Validation	8.15	6854533.4	6288.2	1.59	0.00
smpl 2.lcd	Imipress Tablet	Method Validation	8.14	6835482.7	6340.0	1.59	0.00
smpl 3.lcd	Imipress Tablet	Method Validation	8.13	6827754.4	6334.5	1.60	0.00
smpl 4.lcd	Imipress Tablet	Method Validation	8.12	6872376.5	6335.1	1.58	0.00
smpl 5.lcd	Imipress Tablet	Method Validation	8.11	6834644.2	6341.3	1.58	0.00
smpl 6.lcd	Imipress Tablet	Method Validation	8.13	6833606.1	6367.3	1.56	0.00
Average			8.13	6843066.2	6334.4	1.58	0.00
%RSD			0.174	0.248	0.405	0.863	0.000
Maximum			8.15	6872376.5	6367.3	1.60	0.00
Minimum			8.11	6827754.4	6288.2	1.56	0.00
Standard Deviation			0.01	16988.1	25.7	0.01	0.00

Chromatogram 29: Chromatogram of 6 Injections of Standard at Condition D

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

Acquired by : Radiatul Nadiah Razali
 Sample Name : Imipress Tablet (Assay)
 Sample ID : Method Validation
 Mobile phase : 0.06M Sodium Perchlorate: Acetonitrile: Triethylamine (625 : 375: 1)
 Column : Phenomenex C18, Gemini, 5 um, 150 mm, 4.6 mm
 Flow rate : 1.0 min/ mL

<Chromatogram>



<< PDA >>

ID#1 Compound Name: Imipramine Hydrochloride

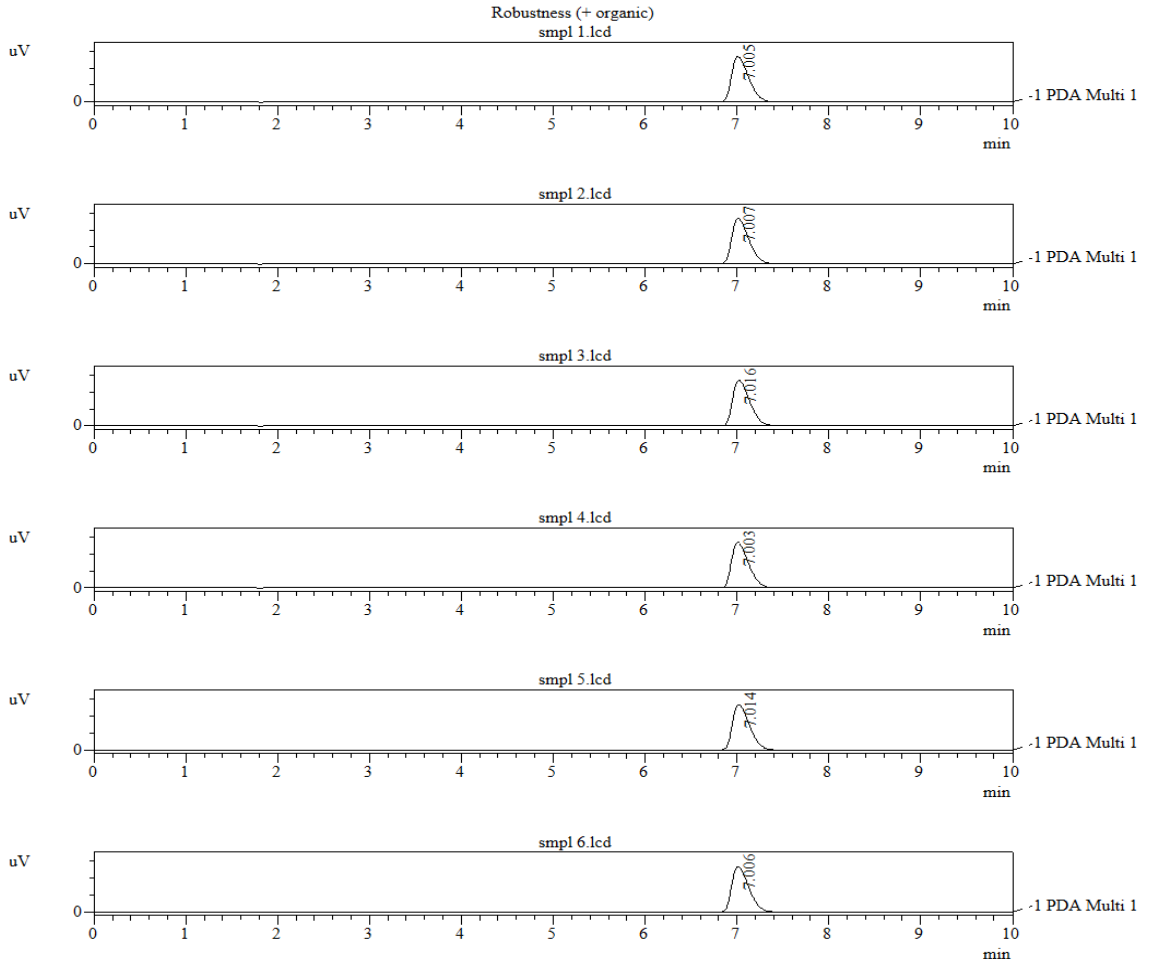
Title	Sample Name	Sample ID	Retention Time	Area	Theoretical Plate	Tailing Factor	Resolution
std 1.lcd	Imipress Tablet	Method Validation	7.07	6868260.8	6251.3	1.55	0.00
std 2.lcd	Imipress Tablet	Method Validation	7.04	6894866.5	6257.0	1.55	0.00
std 3.lcd	Imipress Tablet	Method Validation	7.01	6878825.6	6316.1	1.55	0.00
std 4.lcd	Imipress Tablet	Method Validation	6.99	6861065.6	6320.1	1.55	0.00
std 5.lcd	Imipress Tablet	Method Validation	6.99	6802130.5	6296.0	1.54	0.00
std 6.lcd	Imipress Tablet	Method Validation	7.00	6860724.9	6286.7	1.54	0.00
Average			7.02	6860979.0	6287.9	1.55	0.00
%RSD			0.457	0.460	0.461	0.334	0.000
Maximum			7.07	6894866.5	6320.1	1.55	0.00
Minimum			6.99	6802130.5	6251.3	1.54	0.00
Standard Deviation			0.03	31569.3	29.0	0.01	0.00

Chromatogram 30: Chromatogram of 6 Samples Injection at Condition D

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

Acquired by : Radiatul Nadiah Razali
 Sample Name : Imipress Tablet (Assay)
 Sample ID : Method Validation
 Mobile phase : 0.06M Sodium Perchlorate: Acetonitrile: Triethylamine (625 : 375: 1)
 Column : Phenomenex C18, Gemini, 5 um, 150 mm, 4.6 mm
 Flow rate : 1.0 min/ mL

<Chromatogram>



<< PDA >>

ID#1 Compound Name: Imipramine Hydrochloride

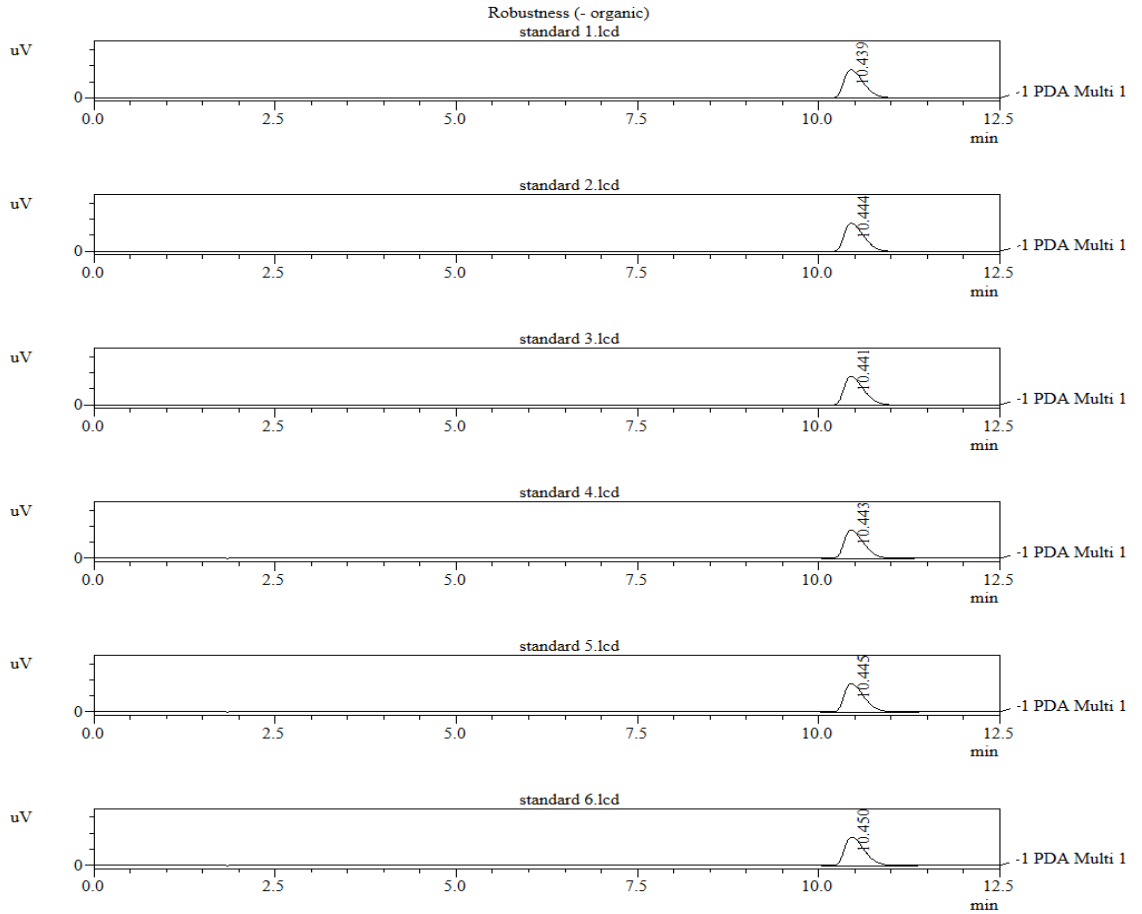
Title	Sample Name	Sample ID	Retention Time	Area	Theoretical Plate	Tailing Factor	Resolution
smpl 1.lcd	Imipress Tablet	Method Validation	7.00	6819946.5	6327.2	1.56	0.00
smpl 2.lcd	Imipress Tablet	Method Validation	7.01	6876803.6	6310.7	1.56	0.00
smpl 3.lcd	Imipress Tablet	Method Validation	7.02	6872977.7	6330.0	1.57	0.00
smpl 4.lcd	Imipress Tablet	Method Validation	7.00	6883733.8	6296.1	1.57	0.00
smpl 5.lcd	Imipress Tablet	Method Validation	7.01	6881862.6	6312.2	1.57	0.00
smpl 6.lcd	Imipress Tablet	Method Validation	7.01	6892259.7	6301.3	1.56	0.00
Average			7.01	6871264.0	6312.9	1.57	0.00
%RSD			0.107	0.378	0.215	0.350	0.000
Maximum			7.02	6892259.7	6330.0	1.57	0.00
Minimum			7.00	6819946.5	6296.1	1.56	0.00
Standard Deviation			0.01	25983.7	13.6	0.01	0.00

Chromatogram 31: Chromatogram of 6 Injections of Standard at Condition E

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

Acquired by : Radiatul Nadiah Razali
 Sample Name : Imipress Tablet (Assay)
 Sample ID : Method Validation
 Mobile phase : 0.06M Sodium Perchlorate: Acetonitrile: Triethylamine (625 : 375: 1)
 Column : Phenomenex C18, Gemini, 5 um, 150 mm, 4.6 mm
 Flow rate : 1.0 min/ mL

<Chromatogram>



<< PDA >>

ID#1 Compound Name: Imipramine Hydrochloride

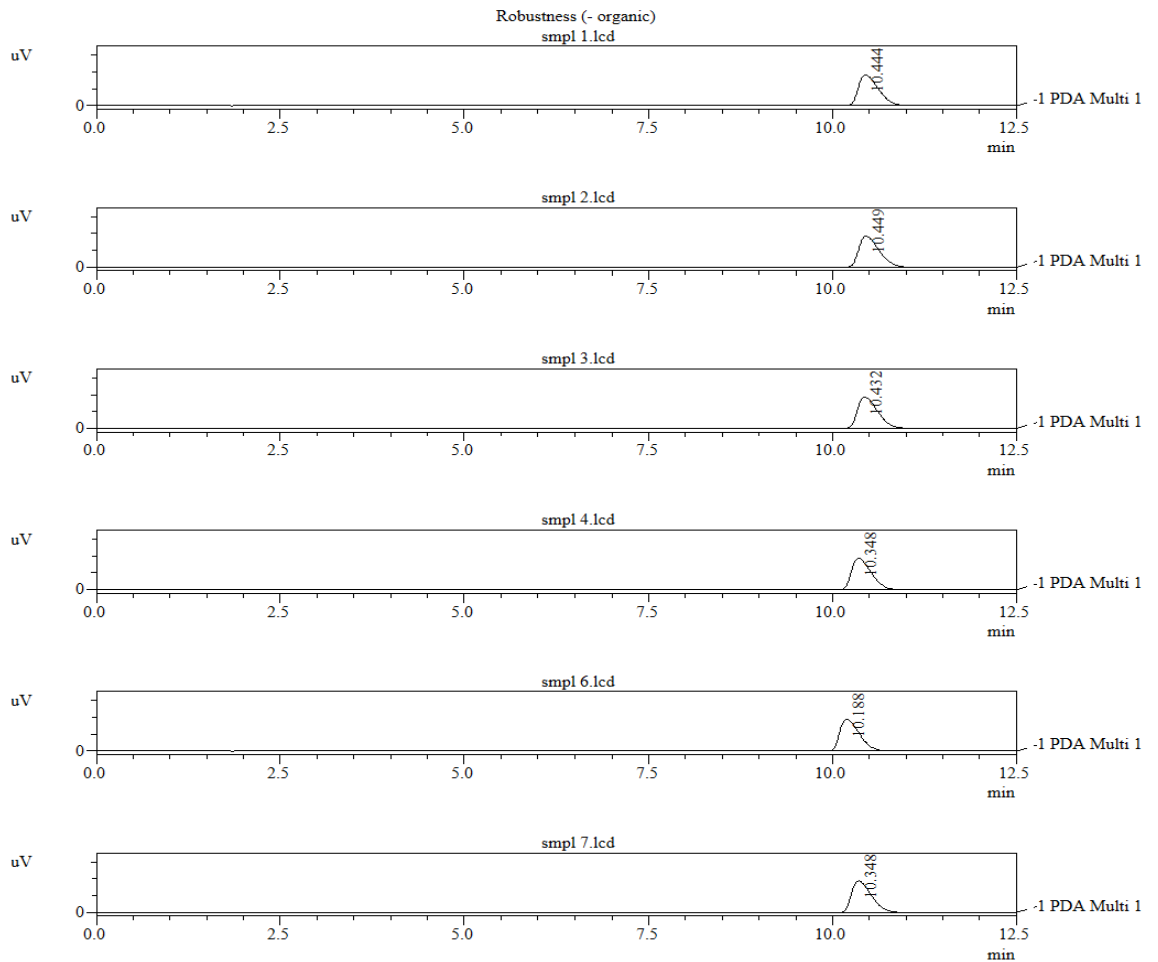
Title	Sample Name	Sample ID	Retention Time	Area	Theoretical Plate	Tailing Factor	Resolution
standard 1.lcd	Imipress Tablet	Method Validation	10.44	6869747.9	6658.3	1.61	0.00
standard 2.lcd	Imipress Tablet	Method Validation	10.44	6890216.7	6649.4	1.60	0.00
standard 3.lcd	Imipress Tablet	Method Validation	10.44	6863618.7	6667.7	1.61	0.00
standard 4.lcd	Imipress Tablet	Method Validation	10.44	6803104.3	6686.9	1.60	0.00
standard 5.lcd	Imipress Tablet	Method Validation	10.45	6858703.4	6660.2	1.60	0.00
standard 6.lcd	Imipress Tablet	Method Validation	10.45	6801929.7	6690.3	1.60	0.00
Average			10.44	6847886.8	6668.8	1.60	0.00
%RSD			0.049	0.537	0.247	0.322	0.000
Maximum			10.45	6890216.7	6690.3	1.61	0.00
Minimum			10.44	6801929.7	6649.4	1.60	0.00
Standard Deviation			0.01	36747.1	16.4	0.01	0.00

Chromatogram 32: Chromatogram of 6 Samples Injection at Condition E

==== Shimadzu LcSolution Analysis Report ====

Acquired by : Radiatul Nadiah Razali
 Sample Name : Imipress Tablet (Assay)
 Sample ID : Method Validation
 Mobile phase : 0.06M Sodium Perchlorate: Acetonitrile: Triethylamine (625 : 375: 1)
 Column : Phenomenex C18, Gemini, 5 um, 150 mm, 4.6 mm
 Flow rate : 1.0 min/ mL

<Chromatogram>



<< PDA >>

ID#1 Compound Name: Imipramine Hydrochloride

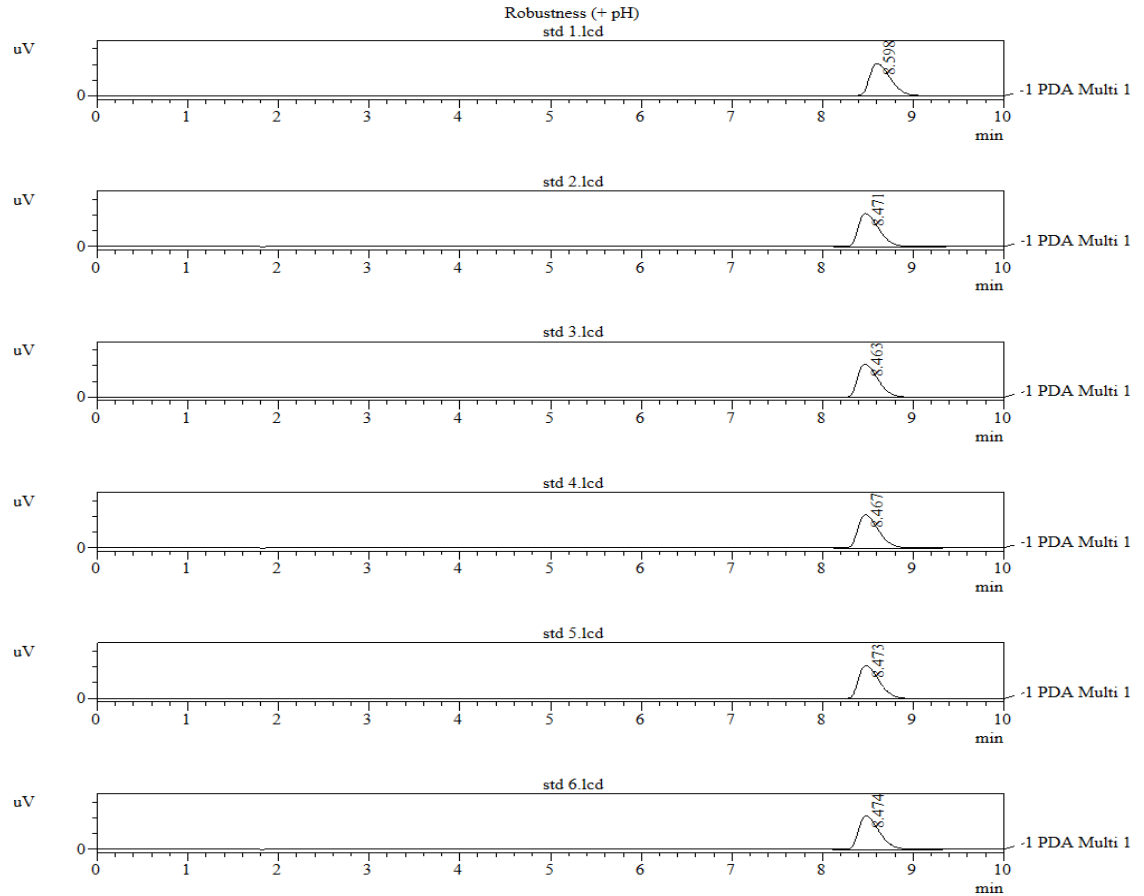
Title	Sample Name	Sample ID	Retention Time	Area	Theoretical Plate	Tailing Factor	Resolution
smpl 1.lcd	Imipress Tablet	Method Validation	10.44	6894165.2	6675.6	1.61	0.00
smpl 2.lcd	Imipress Tablet	Method Validation	10.45	6863857.5	6700.2	1.60	0.00
smpl 3.lcd	Imipress Tablet	Method Validation	10.43	6876264.2	6740.5	1.60	0.00
smpl 4.lcd	Imipress Tablet	Method Validation	10.35	6852063.9	6779.4	1.62	0.00
smpl 6.lcd	Imipress Tablet	Method Validation	10.19	6832563.6	6847.2	1.63	0.00
smpl 7.lcd	Imipress Tablet	Method Validation	10.35	6839931.4	6792.9	1.59	0.00
Average			10.37	6859807.6	6756.0	1.61	0.00
%RSD			0.946	0.337	0.937	0.915	0.000
Maximum			10.45	6894165.2	6847.2	1.63	0.00
Minimum			10.19	6832563.6	6675.6	1.59	0.00
Standard Deviation			0.10	23086.5	63.3	0.01	0.00

Chromatogram 33: Chromatogram of 6 Injections of Standard at Condition F

==== Shimadzu LcSolution Analysis Report ====

Acquired by : Radiatul Nadiah Razali
 Sample Name : Impress Tablet (Assay)
 Sample ID : Method Validation
 Mobile phase : 0.06M Sodium Perchlorate: Acetonitrile: Triethylamine (625 : 375: 1)
 Column : Phenomenex C18, Gemini, 5 um, 150 mm, 4.6 mm
 Flow rate : 1.0 min/ mL

<Chromatogram>



<< PDA >>

ID#1 Compound Name: Imipramine Hydrochloride

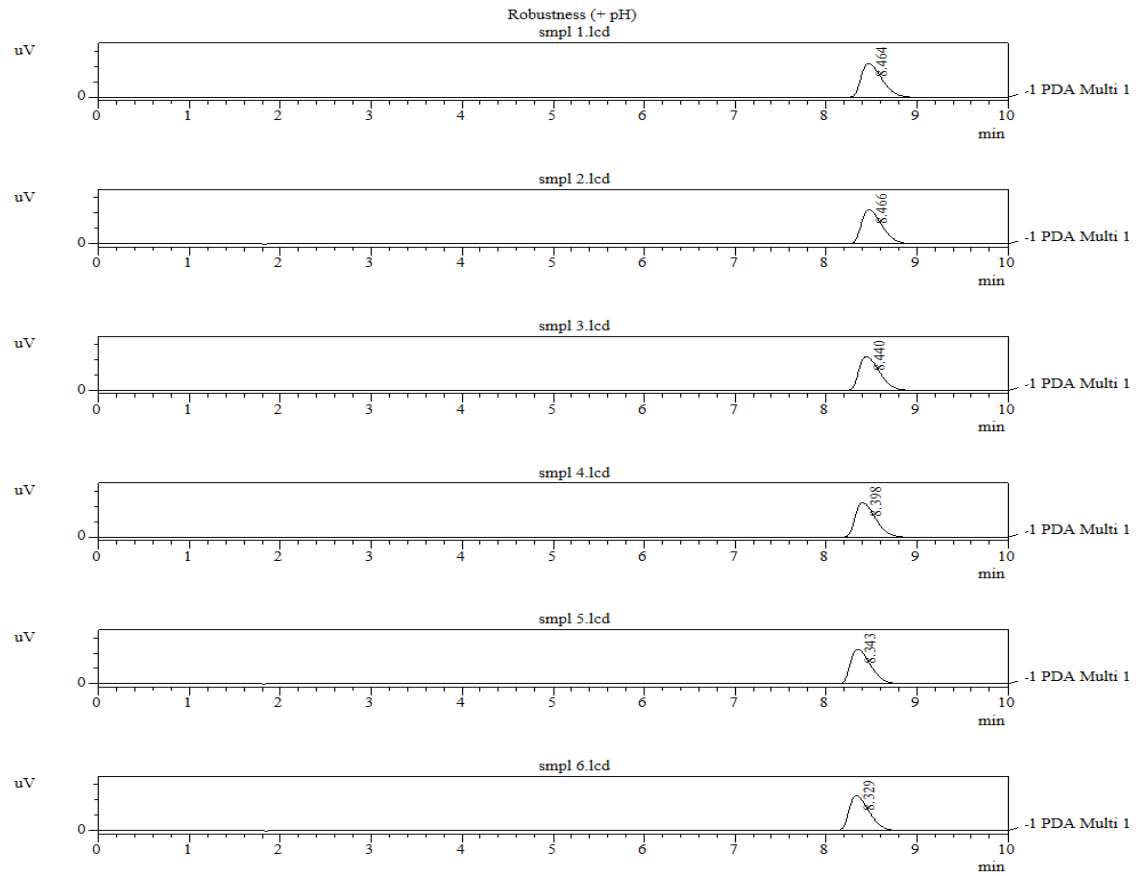
Title	Sample Name	Sample ID	Retention Time	Area	Theoretical Plate	Tailing Factor	Resolution
std 1.lcd	Impress Tablet	Method Validation	8.60	6886048.1	6280.9	1.60	0.00
std 2.lcd	Impress Tablet	Method Validation	8.47	6828717.9	6356.7	1.61	0.00
std 3.lcd	Impress Tablet	Method Validation	8.46	6855558.9	6326.1	1.60	0.00
std 4.lcd	Impress Tablet	Method Validation	8.47	6830122.7	6355.4	1.61	0.00
std 5.lcd	Impress Tablet	Method Validation	8.47	6815014.6	6364.7	1.60	0.00
std 6.lcd	Impress Tablet	Method Validation	8.47	6826584.5	6362.6	1.60	0.00
Average			8.49	6840341.1	6341.1	1.60	0.00
%RSD			0.636	0.381	0.514	0.322	0.000
Maximum			8.60	6886048.1	6364.7	1.61	0.00
Minimum			8.46	6815014.6	6280.9	1.60	0.00
Standard Deviation			0.05	26041.9	32.6	0.01	0.00

Chromatogram 34: Chromatogram of 6 Samples Injection at Condition F

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

Acquired by : Radiatul Nadiah Razali
 Sample Name : Imipress Tablet (Assay)
 Sample ID : Method Validation
 Mobile phase : 0.06M Sodium Perchlorate: Acetonitrile: Triethylamine (625 : 375: 1)
 Column : Phenomenex C18, Gemini, 5 um, 150 mm, 4.6 mm
 Flow rate : 1.0 min/ mL

<Chromatogram>



<< PDA >>

ID#1 Compound Name: Imipramine Hydrochloride

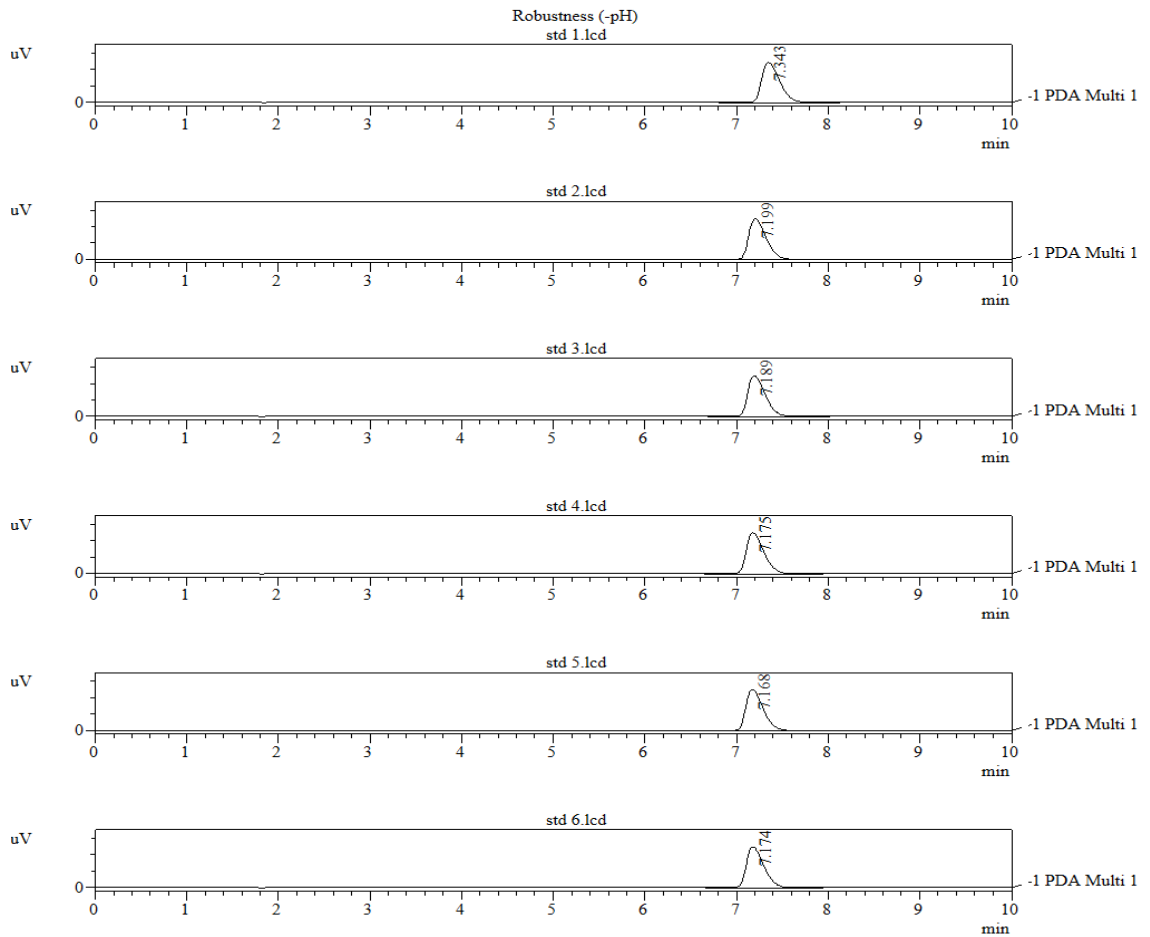
Title	Sample Name	Sample ID	Retention Time	Area	Theoretical Plate	Tailing Factor	Resolution
smpl 1.lcd	Imipress Tablet	Method Validation	8.46	6869456.6	6363.0	1.61	0.00
smpl 2.lcd	Imipress Tablet	Method Validation	8.47	6863053.0	6395.5	1.60	0.00
smpl 3.lcd	Imipress Tablet	Method Validation	8.44	6869658.8	6415.5	1.61	0.00
smpl 4.lcd	Imipress Tablet	Method Validation	8.40	6890296.6	6452.6	1.61	0.00
smpl 5.lcd	Imipress Tablet	Method Validation	8.34	6854493.3	6469.0	1.63	0.00
smpl 6.lcd	Imipress Tablet	Method Validation	8.33	6890279.3	6479.9	1.61	0.00
Average			8.41	6872872.9	6429.3	1.61	0.00
%RSD			0.720	0.212	0.710	0.610	0.000
Maximum			8.47	6890296.6	6479.9	1.63	0.00
Minimum			8.33	6854493.3	6363.0	1.60	0.00
Standard Deviation			0.06	14579.5	45.6	0.01	0.00

Chromatogram 35: Chromatogram of 6 Injections of Standard at Condition G

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

Acquired by : Radiatul Nadiah Razali
 Sample Name : Imipress Tablet (Assay)
 Sample ID : Method Validation
 Mobile phase : 0.06M Sodium Perchlorate: Acetonitrile: Triethylamine (625 : 375: 1)
 Column : Phenomenex C18, Gemini, 5 um, 150 mm, 4.6 mm
 Flow rate : 1.0 min/ mL

<Chromatogram>



<< PDA >>

ID#1 Compound Name: Imipramine Hydrochloride

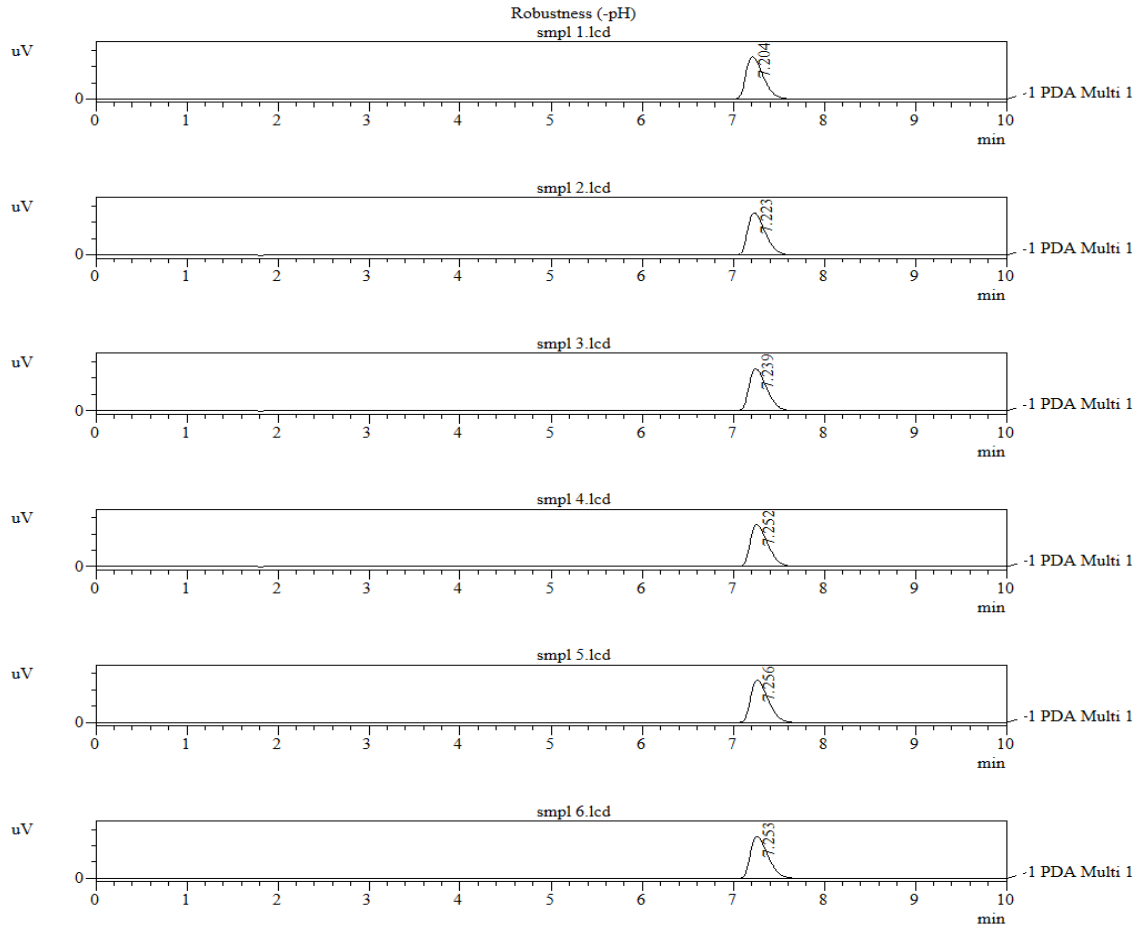
Title	Sample Name	Sample ID	Retention Time	Area	Theoretical Plate	Tailing Factor	Resolution
std 1.lcd	Imipress Tablet	Method Validation	7.34	6899972.7	6323.6	1.55	0.00
std 2.lcd	Imipress Tablet	Method Validation	7.20	6846506.3	6303.0	1.56	0.00
std 3.lcd	Imipress Tablet	Method Validation	7.19	6892477.0	6308.9	1.55	0.00
std 4.lcd	Imipress Tablet	Method Validation	7.17	6884739.6	6301.7	1.54	0.00
std 5.lcd	Imipress Tablet	Method Validation	7.17	6821804.9	6335.5	1.55	0.00
std 6.lcd	Imipress Tablet	Method Validation	7.17	6890622.2	6299.7	1.54	0.00
Average			7.21	6872687.1	6312.1	1.55	0.00
%RSD			0.923	0.454	0.228	0.486	0.000
Maximum			7.34	6899972.7	6335.5	1.56	0.00
Minimum			7.17	6821804.9	6299.7	1.54	0.00
Standard Deviation			0.07	31233.0	14.4	0.01	0.00

Chromatogram 36: Chromatogram of 6 Samples Injection at Condition G

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

Acquired by : Radiatul Nadiah Razali
 Sample Name : Imipress Tablet (Assay)
 Sample ID : Method Validation
 Mobile phase : 0.06M Sodium Perchlorate: Acetonitrile: Triethylamine (625 : 375: 1)
 Column : Phenomenex C18, Gemini, 5 um, 150 mm, 4.6 mm
 Flow rate : 1.0 min/ mL

<Chromatogram>



<< PDA >>

ID#1 Compound Name: Imipramine Hydrochloride

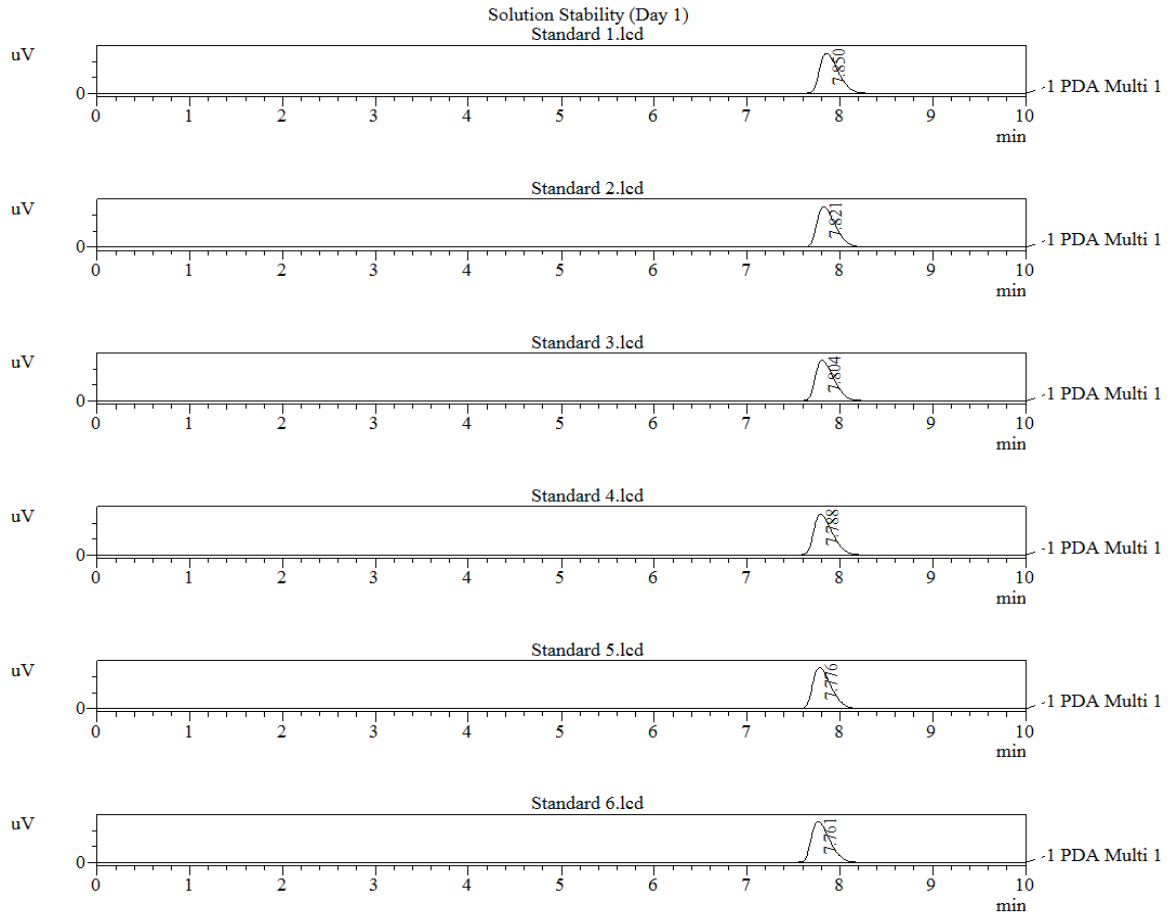
Title	Sample Name	Sample ID	Retention Time	Area	Theoretical Plate	Tailing Factor	Resolution
smpl 1.lcd	Imipress Tablet	Method Validation	7.20	6899655.2	6301.8	1.55	0.00
smpl 2.lcd	Imipress Tablet	Method Validation	7.22	6864122.3	6298.7	1.54	0.00
smpl 3.lcd	Imipress Tablet	Method Validation	7.24	6854939.3	6318.1	1.53	0.00
smpl 4.lcd	Imipress Tablet	Method Validation	7.25	6858980.6	6310.1	1.56	0.00
smpl 5.lcd	Imipress Tablet	Method Validation	7.26	6870210.0	6283.4	1.54	0.00
smpl 6.lcd	Imipress Tablet	Method Validation	7.25	6866849.3	6276.4	1.55	0.00
Average			7.24	6869126.1	6298.1	1.54	0.00
%RSD			0.311	0.232	0.251	0.679	0.000
Maximum			7.26	6899655.2	6318.1	1.56	0.00
Minimum			7.20	6854939.3	6276.4	1.53	0.00
Standard Deviation			0.02	15922.4	15.8	0.01	0.00

Chromatogram 37: Chromatogram of 6 injections of standard at initial

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

Acquired by : Radiatul Nadiah Razali
 Sample Name : Imipress Tablet (Assay)
 Sample ID : Method Validation
 Mobile phase : 0.06M Sodium Perchlorate: Acetonitrile: Triethylamine (625 : 375: 1)
 Column : Phenomenex C18, Gemini, 5 um, 150 mm, 4.6 mm
 Flow rate : 1.0 min/ mL

<Chromatogram>



<< PDA >>

ID#1 Compound Name: Imipramine Hydrochloride

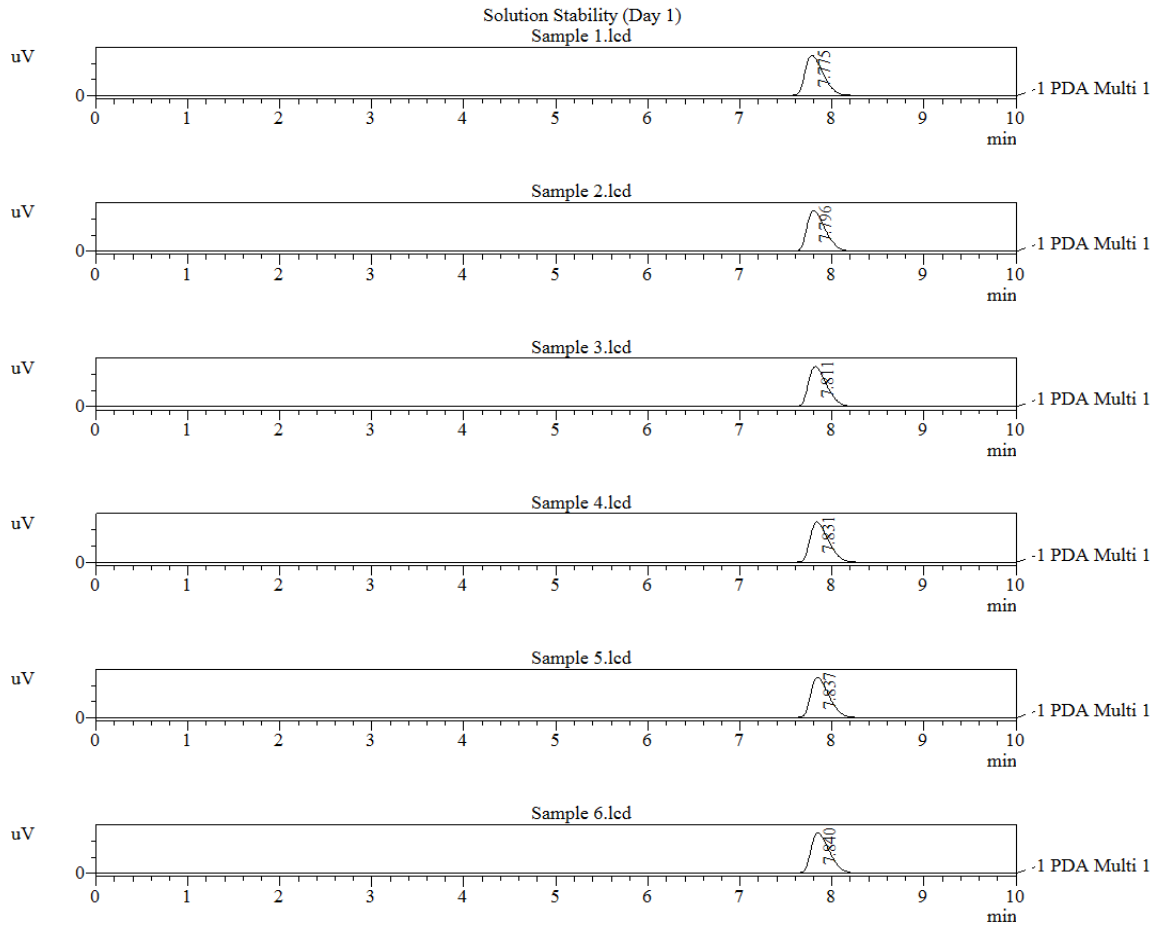
Title	Sample Name	Sample ID	Retention Time	Area	Theoretical Plate	Tailing Factor	Resolution
Standard 1.lcd	Imipress Tablet	Method Validation	7.85	7144443.2	6638.5	1.57	0.00
Standard 2.lcd	Imipress Tablet	Method Validation	7.82	7147948.4	6655.5	1.56	0.00
Standard 3.lcd	Imipress Tablet	Method Validation	7.80	7148073.9	6683.0	1.57	0.00
Standard 4.lcd	Imipress Tablet	Method Validation	7.79	7148737.2	6688.4	1.56	0.00
Standard 5.lcd	Imipress Tablet	Method Validation	7.78	7148939.2	6686.1	1.56	0.00
Standard 6.lcd	Imipress Tablet	Method Validation	7.76	7146315.3	6709.5	1.56	0.00
Average			7.80	7147409.5	6676.8	1.56	0.00
%RSD			0.405	0.024	0.382	0.330	0.000
Maximum			7.85	7148939.2	6709.5	1.57	0.00
Minimum			7.76	7144443.2	6638.5	1.56	0.00
Standard Deviation			0.03	1722.3	25.5	0.01	0.00

Chromatogram 38: Chromatogram of 6 samples injection at initial

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

Acquired by : Radiatul Nadiyah Razali
 Sample Name : Imipress Tablet (Assay)
 Sample ID : Method Validation
 Mobile phase : 0.06M Sodium Perchlorate: Acetonitrile: Triethylamine (625 : 375: 1)
 Column : Phenomenex C18, Gemini, 5 um, 150 mm, 4.6 mm
 Flow rate : 1.0 min/ mL

<Chromatogram>



<< PDA >>

ID#1 Compound Name: Imipramine Hydrochloride

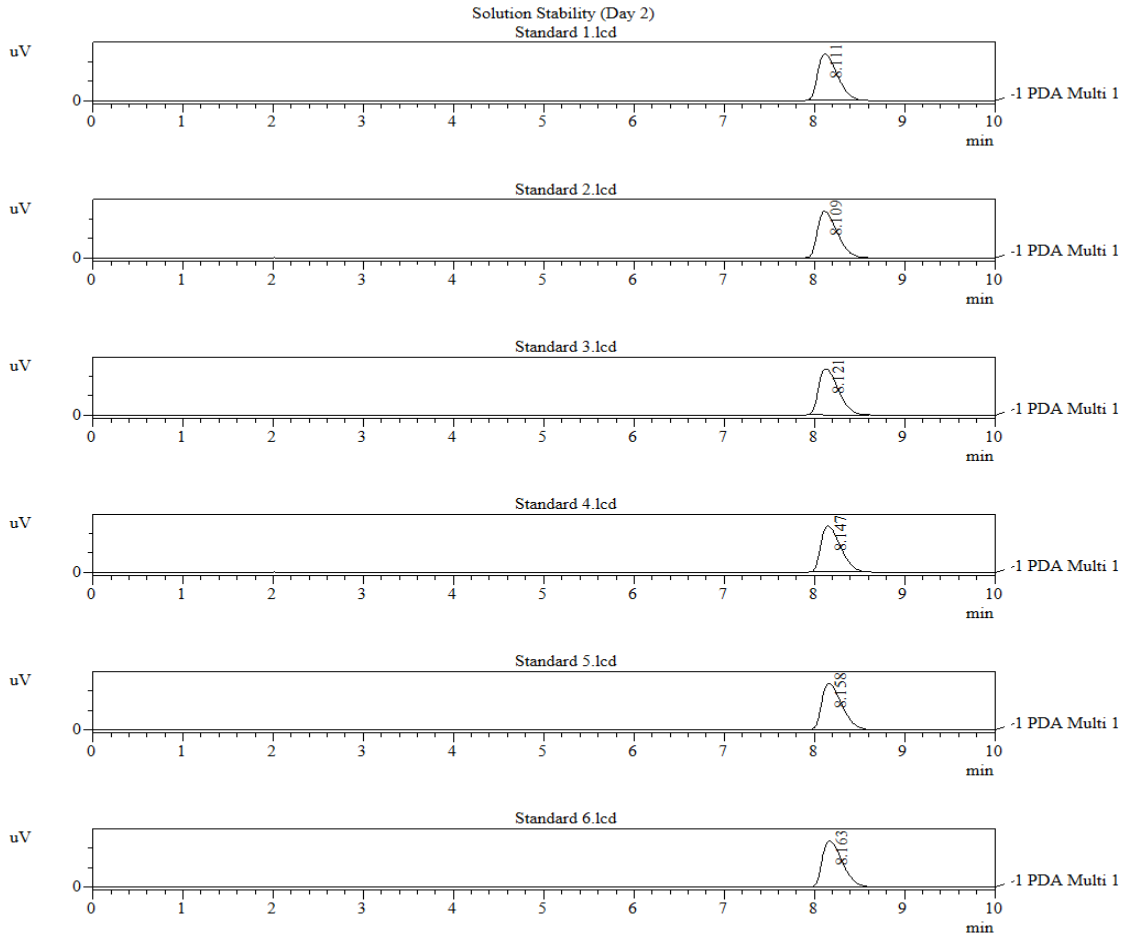
Title	Sample Name	Sample ID	Retention Time	Area	Theoretical Plate	Tailing Factor	Resolution
Sample 1.lcd	Imipress Tablet	Method Validation	7.78	6949239.2	6802.7	1.54	0.00
Sample 2.lcd	Imipress Tablet	Method Validation	7.80	6990946.0	6784.6	1.54	0.00
Sample 3.lcd	Imipress Tablet	Method Validation	7.81	6958568.6	6763.9	1.54	0.00
Sample 4.lcd	Imipress Tablet	Method Validation	7.83	6992220.7	6751.0	1.54	0.00
Sample 5.lcd	Imipress Tablet	Method Validation	7.84	7000723.6	6779.9	1.54	0.00
Sample 6.lcd	Imipress Tablet	Method Validation	7.84	6997234.1	6760.9	1.54	0.00
Average			7.82	6981488.7	6773.8	1.54	0.00
%RSD			0.310	0.313	0.278	0.000	0.000
Maximum			7.84	7000723.6	6802.7	1.54	0.00
Minimum			7.78	6949239.2	6751.0	1.54	0.00
Standard Deviation			0.02	21853.5	18.8	0.00	0.00

Chromatogram 39: Chromatogram of 6 injections of fresh standard

==== Shimadzu LcSolution Analysis Report ====

Acquired by : Radiatul Nadiah Razali
 Sample Name : Imipress Tablet (Assay)
 Sample ID : Method Validation
 Mobile phase : 0.06M Sodium Perchlorate: Acetonitrile: Triethylamine (625 : 375: 1)
 Column : Phenomenex C18, Gemini, 5 um, 150 mm, 4.6 mm
 Flow rate : 1.0 min/ mL

<Chromatogram>



<< PDA >>

ID#1 Compound Name: Imipramine Hydrochloride

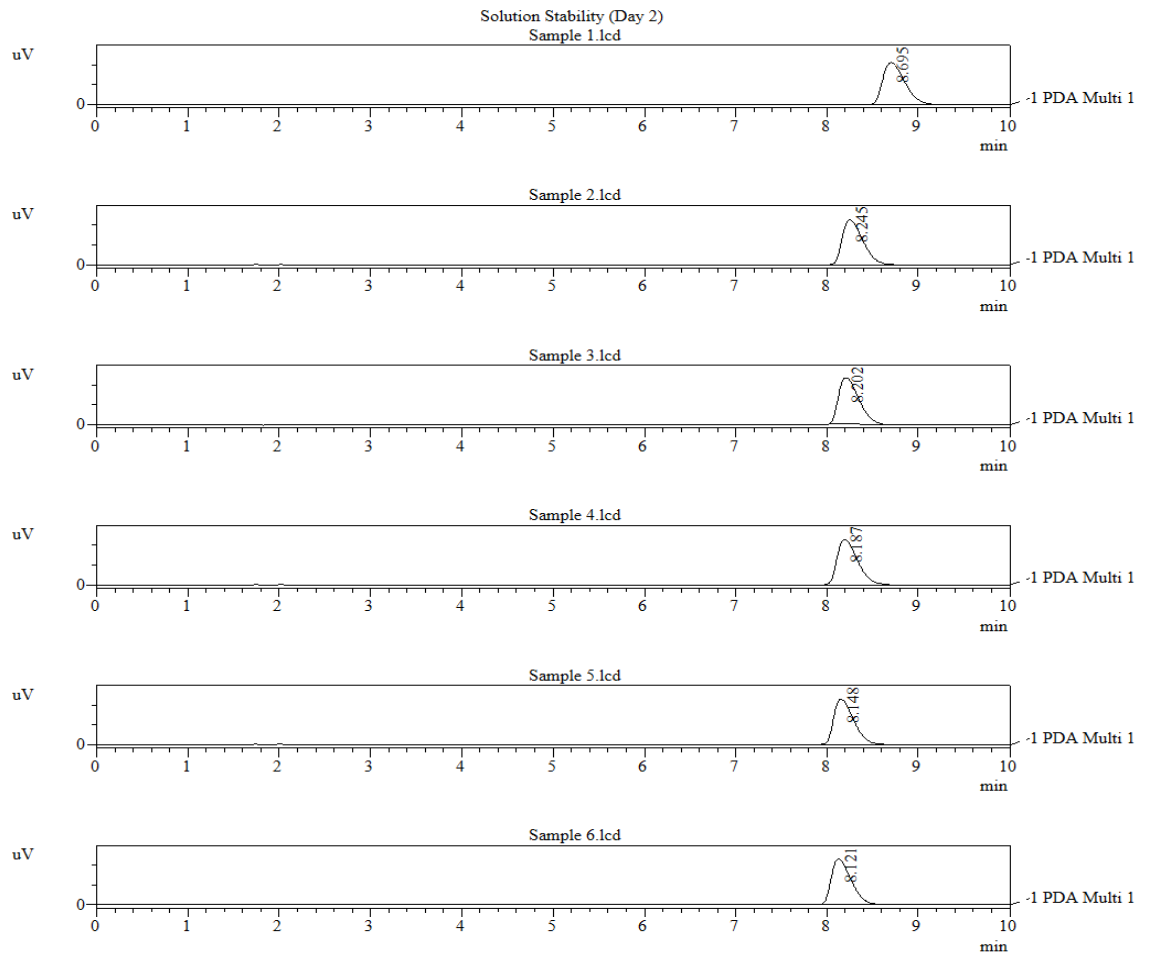
Title	Sample Name	Sample ID	Retention Time	Area	Theoretical Plate	Tailing Factor	Resolution
Standard 1.lcd	Imipress Tablet	Method Validation	8.11	7134729.5	6310.0	1.60	0.00
Standard 2.lcd	Imipress Tablet	Method Validation	8.11	7282552.2	6208.4	1.61	0.00
Standard 3.lcd	Imipress Tablet	Method Validation	8.12	7227620.4	6212.1	1.61	0.00
Standard 4.lcd	Imipress Tablet	Method Validation	8.15	7230680.7	6200.9	1.59	0.00
Standard 5.lcd	Imipress Tablet	Method Validation	8.16	7220042.4	6205.3	1.60	0.00
Standard 6.lcd	Imipress Tablet	Method Validation	8.16	7220295.0	6217.1	1.61	0.00
Average			8.13	7219320.0	6225.6	1.60	0.00
%RSD			0.299	0.660	0.670	0.509	0.000
Maximum			8.16	7282552.2	6310.0	1.61	0.00
Minimum			8.11	7134729.5	6200.9	1.59	0.00
Standard Deviation			0.02	47651.4	41.7	0.01	0.00

Chromatogram 40: Chromatogram of 6 samples injection at 24 hours

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

Acquired by : Radiatul Nadiah Razali
 Sample Name : Imipress Tablet (Assay)
 Sample ID : Method Validation
 Mobile phase : 0.06M Sodium Perchlorate: Acetonitrile: Triethylamine (625 : 375: 1)
 Column : Phenomenex C18, Gemini, 5 um, 150 mm, 4.6 mm
 Flow rate : 1.0 min/ mL

<Chromatogram>



<< PDA >>

ID#1 Compound Name: Imipramine Hydrochloride

Title	Sample Name	Sample ID	Retention Time	Area	Theoretical Plate	Tailing Factor	Resolution
Sample 1.lcd	Imipress Tablet	Method Validation	8.70	7106053.5	6023.4	1.55	0.00
Sample 2.lcd	Imipress Tablet	Method Validation	8.24	7087123.4	6176.5	1.58	0.00
Sample 3.lcd	Imipress Tablet	Method Validation	8.20	7219952.4	6245.0	1.62	0.00
Sample 4.lcd	Imipress Tablet	Method Validation	8.19	7069441.1	6230.1	1.59	0.00
Sample 5.lcd	Imipress Tablet	Method Validation	8.15	7074559.1	6230.7	1.59	0.00
Sample 6.lcd	Imipress Tablet	Method Validation	8.12	7075949.4	6263.2	1.59	0.00
Average			8.27	7105513.1	6194.8	1.59	0.00
%RSD			2.617	0.810	1.434	1.419	0.000
Maximum			8.70	7219952.4	6263.2	1.62	0.00
Minimum			8.12	7069441.1	6023.4	1.55	0.00
Standard Deviation			0.22	57564.2	88.8	0.02	0.00