National Pharmaceutical Control Bureau
MINISTRY OF HEALTH MALAYSIA

World Health Organization
WHO Collaborating Centre for Regulatory Control of Pharmaceuticals

PIC/S
Pharmaceutical Inspection Convention and Pharmaceutical Inspection Co-operation Scheme

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[illegible]

Analytical Method Validation & Common Problem 1

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OUTLINE

- Introduction
- Common Problems

INTERNATIONAL GUIDELINE

- Validation of Analytical Procedures:

  Text and Methodology Q2(R1)

  (ICH 2006)

VALIDATION OF ANALYTICAL PROCEDURE

- The process by which it is established by the laboratory studies, demonstrate that the method is suitable for its intended purpose

Creates confidence in results and quality of products'

What are the types of analytical procedures to be validated?

1. Identification

2. Assay (content & dissolution measurement only)

3. Impurities (quantitative & limit test)

What is Identification?

- Identification tests are intended to ensure the identity of an analyte in a sample

- This is normally achieved by comparison of a property of the sample (spectrum, chromatogram, chemical reactivity etc) to that of a reference standard

NPCB
MOH

Example of Identification

2. Identification (Ref.: B.P)	**(a) *By IR*** *The infrared spectrum should be identical to that of Atenolol CRS.* **Apparatus: IR: Shimadzu FTIR-8400 QC-003(A)** **Weighing Balance** **Carver Laboratory Press-Model C QC-003(D)** **Reagents:** **HCl Lot.:... Art.:...** **NaOH Lot.:... Art.:...** **MeOH Lot.:... Art.:...** **Chloroform Lot.:... Art.:...** **Anhydrous sodium sulphate Lot.:... Art.:...** **Potassium Bromide Lot.: ... Art.:...** **0.1 M HCl Ref.:SOL-001Ma** **1M NaOH Ref.:...SOL-011Ma** Test preparation: Accurately weigh 557mg Atenolol. Place it into a 50ml conical flask. Add 15ml Methanol. Heat at 50°C, shake for 5 minutes and filter. Evaporate the filtrate to dryness. Add to the residue 10ml 0.1M HCl, warm it, shake and filter. Add to the filtrate sufficient 1M NaOH to make it alkaline. Extract with 10ml chloroform, dry it by shaking with anhydrous sodium sulphate, filter and evaporate the filtrate to dryness. Dry the residue at 105°C for 1 hour. Weigh ~1.5mg and ~350mg of potassium bromide. Mix and prepare a KBr disc. Run the IR Spectrum. **w=...mg** **KBr = ... mg** First prepare a Blank KBr disc and run the background Compare the spectra WITH **Atenolol** CRS. **Result: Complies: Yes / No** ***(b) By UV*** By UV, *the light Absorbsion in the range 230 to 350nm of the test solution in the Assay exhibits maxima at 275nm and 282nm.*

2A. Identification (by HPLC)	: The retention time of the major peak in the chromatogram of Assay preparation corresponds to the chromatogram of the Standard preparation as obtained in the Assay.
2B. Identification by UV	: The absorption maxima of the Test preparation should fall within ±2 nm of the absorption maxima of the standard preparation.

What is Assay?

- A quantitative analysis to determine the purity or the amount of a drug substance in a drug sample.
- Method of assay test :

a. Ultraviolet and Visible Absorption (UV)
b. High Pressure Liquid Chromatography (HPLC)
c. Gas Chromatography (GC)
d. Titration

Example of Assay by HPLC

5) Assay of active ingredient

Requirement: 90.0-110.0%L.A. Of Roxithromycin

Chromatographic system

Column	:	Ultracarb 7 ODS (20) size 250x4.6 nm Brandname: Phenomenex
Mobile phase	:	Mix 307 ml of acetonitrile and 693 ml of 49.1 g/l solution of ammonium dihydrogen phophate, adjusted to pH 5.3 with dilute sodium hydroxide solution.
Flow rate	:	1.50 ml/min
Detector	:	205 nm.
Injection volume:		20 microlitre

System suitability

Requirement .:

1. Relative standard deviation for replicate injections is not more than 2.0%
2. The theorectical plates is not less than 2000.
3. The tailing factor is not more than 1.8.

Standard preparation

Accurately weigh standard Roxithromycin 50 mg to a 25 ml volumetric flask, dissolve with mobile phase and sonicate for 15 minutes. Dilute to volume with mobile phase.

Sample preparation

Accurately weigh sample about 74 mg (equivalent to 50 mg of Roxithromycin) to a to a 25 ml volumetric flask, dissolve with mobile phase and sonicate for 15 minutes. Dilute to volume wit h mobile phase, mix and filter through filter papaer whatman No. 4.

Calculation

$$\%L.A. = \frac{Au \times wt\ std \times \%std \times 25 \times wt/tab \times 100}{As \quad 25 \quad wt\ sam \quad 300}$$

whereas;	Au,As	= Peak area of sample and standard, respectively
	wt std	= Weight of standard (mg)
	wt/tab	= Average weight per tablet (mg)
	wt sam	= Weight of sample (mg)

Reference : Manufacturer's

Example of Assay by Ultraviolet and Visible Absorption Spectrophotometry (UV)

Quantitation of Hypermellose (0.7%w/v):

Test method:

- Test preparation: dilute 2ml of sample to 25ml with water. Further dilute 10ml of this solution to 50ml with water (0.1mg/ml)

- Standard preparation: weight 20mg of hypromellose WS and dilute to 100ml with water. Dilute 25ml of this solution to 50ml with water (0.1 mg/ml)

- Blank solution: water
- Wavelength: 635nm
- What to look for in protocol of analysis: UV spectrum (if company able to provide)

Example of Assay by Titration

Quantitation of Total Chloride (0.45%w/v):

Test method:

- Test preparation: Pipette out 10ml of sample into 250ml conical flask, add about 50ml water. Neutralize the solution with dilute nitric acid using red litmus paper and titrate with 0.1M silver nitrate using potassium chromate solution as indicator

- Each ml of 0.1M silver nitrate is equivalent to 0.003545g of chloride

Calculation:

$$\text{Content of Total chloride (\%w/v)} = \frac{V \times 0.003545 \times MF \times 100}{10}$$

Where,

V = Titre value (ml)

MF= Molarity factor of 0.1M silver nitrate solution

Dissolution Test

WHY DO WE PERFORM DISSOLUTION TEST?

- To determine the compliance with dissolution requirement where stated in the individual monograph or protocol of analysis for tablet or capsule dosage form
- Optimisation of therapeutic effectiveness during product development and stability assessment
- Routine assessment of production quality to ensure uniformity between production lots

•WHAT ARE WE MEASURING?

- Dissolution rate: The amount of active ingredient in a solid dosage form dissolved in unit time under experimental conditions of temperature, media composition and rotation of basket/paddle
- Dissolution profile: The charting of the release of the drug during dissolution over time

Example of Dissolution (1)

Specification:

The requirements for dosage uniformity are if the acceptance value of the first 10 dosage units is less than or equal to L1%. If the acceptance value is >L1%, test the next 20 units, and calculate the acceptance value. The requirements are met if the final acceptance value of the 30 dosage units is ≤ L1% and no individual content of any dosage unit is less than [1-(0.01) (L2)] M not more [1 + (0.01) (L2)] M as specified in the *Calculation of Acceptance Value* under *Content Uniformity* or *Under Weight* variation. Unless otherwise specified, L1 is 15.0 and is 25.0.

8. DISSOLUTION

8.1 Method: HPLC

Medium	:	*Water*; 900ml
Apparatus	:	2 (Paddle)
Speed	:	75 rpm
Time	:	30 minutes

8.2 Chromatographic conditions
Prepare as directed in the Assay.

8.3 Standard Preparation (Use within 1 hour of preparation)
Weigh accurately 0.032g of [redacted] and 0.033g of [redacted] and transfer to 100mL volumetric flask. Dissolve in and dilute to volume with *water*.

8.4 Sample Preparation
Transfer 6 tablets individually to six dissolution vessels having 900ml of *water* equilibrated to 37.0 ± 0.5°C and start the apparatus. At 30 minutes, filter about 25mL of the solution with 0.45µm nylon membrane filter and use the filtrate as **test solution a (for** [redacted]**)**. Dilute 5 ml of the **test solution a** to 10ml with *water* and mix well and use as **test solution b (for** [redacted]**)**.Filter both test solution through 0.45µm nylon membrane filter.

Example of Dissolution (2)

8.5 Procedure

Inject separately equal volumes (20µL) of the Standard Solution and Test Solutions into the HPLC. Record the chromatograms and measure the responses for the major peak.

i) One Standard solution: 6 injections
ii) Six Samples solution : 1 injection of each

8.5.1 Acceptance Criteria

i. The RSD area of six replicate injections is not more than 2.0%.
ii. The RSD retention time of six replicate injections is not more than 1.0%.

8.6 System Suitability

Follow as same under Assay

8.7 Calculation

a) % content of [redacted]; ***(Test solution b)***

$$\frac{\text{Spl. Area}}{\text{Std. Area}} \times \frac{\text{Std. Weight}/100}{500/900 \times 5/10} \times \text{Potency of working standard} \times 100$$

b) % content of [redacted] ***(Test solution a)***

$$\frac{\text{Spl. Area}}{\text{Std. Area}} \times \frac{\text{Std. Weight}/100}{125/900} \times \text{Potency of working standard} \times 100$$

Specification:

i) [redacted]: Not less than 85% (Q) of the labeled amount of [redacted] is dissolved in 30 minutes.

ii) [redacted]: Not less than 80% (Q) of the labeled amount of [redacted] is dissolved in 30 minutes.

What is Related Substances?

"……. molecular variants of the desired product formed during manufacture and/or storage, which are active and have no deleterious effect on the safety and efficacy of the drug substance/drug product."

"……..properties comparable to the desired product and are not considered impurities."

USP Guideline for Submitting Requests for Revision to *USP-NF V4 July 2009*
http://www.usp.org/pdf/EN/USPNF/glossary.pdf

What is Related Substances?

Related substances are structurally related to a drug substance. These substances may be

a) **Identified or unidentified impurity arising from synthesis manufacturing process such as intermediates or by-products and DO NOT increase on storage**

b) **Identified or unidentified degradation products that result from drug substance or drug product manufacturing processes or arise during storage of a material.**

Example of Related Substances (1)

Preparation of buffer (pH 8.0)

Dissolve about 5.88 g of sodium citrate dihydrate and 2.84 g of anhydrous dibasic sodium phosphate to a 2 L volumetric flask, dilute with water to volume and mix. Adjust the pH of this solution to 8.0 ± 0.05 with *ortho*phosphoric acid. Filter through 0.45 µ or finer porosity membrane filter.

Preparation of Eluant A

Prepare a degassed mixture containing 1700 mL of buffer (pH 8.0) and 300 mL of acetonitrile.

Preparation of Eluant B

Prepare a degassed mixture containing 300 mL of buffer (pH 8.0) and 700 mL of acetonitrile.

Preparation of 0.4 % 9-Fluorenylmethyl chloroformate solution

Accurately weigh and transfer about 200 mg of 9-Fluorenylmethyl chloroformate to a 50 mL volumetric flask. Dilute with acetonitrile to volume and mix. Prepare this solution fresh prior to use.

Preparation of diluent

Transfer 29.4 g of sodium citrate dihydrate to a 1000 mL volumetric flask, dissolve in and dilute with water to volume and mix.

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Preparation of 0.05 M borate solution

Transfer about 19.1 g of sodium borate to a 1000 mL volumetric flask, dissolve in and dilute with water to volume and mix.

Preparation of standard stock solution

Accurately weigh and transfer 60 mg of [redacted] working standard equivalent to about 55 mg of [redacted] to a 100 mL volumetric flask. Dissolve and dilute to volume with diluent.

Preparation of standard solution

Transfer 5 mL of standard stock solution to a 50 mL glass stoppered tube containing 5 mL of 0.05 M borate solution and 5 mL of acetonitrile. Stopper the tube and shake the solution for 3 min. Add 5 mL of 0.4 % 9-Fluorenylmethyl chloroformate solution. Stopper the tube and shake the solution for 45 sec. Allow the solution to stand at room temperature for 30 min. Add 20 mL of methylene chloride. Stopper the tube tightly and shake for 1 min. Allow the solution to stand for 10 min. Filter the upper aqueous layer through 0.45 µ nylon filter.

Example of Related Substances (2)

Preparation of dilute standard solution

Dilute 5 mL of standard stock solution to 50 mL with diluent. Dilute 5 mL of this solution to 50 mL with diluent. Finally dilute 5 mL of this solution to 50 mL with diluent. Transfer 5 mL of this solution to a 50 mL glass stoppered tube containing 5 mL of 0.05 M borate solution and 5 mL of acetonitrile. Stopper the tube and shake the solution for 3 min. Add 5 mL of 0.4 % 9-Fluorenylmethyl chloroformate solution. Stopper the tube and shake the solution for 45 sec. Allow the solution to stand at room temperature for 30 min. Add 20 mL of methylene chloride. Stopper the tube tightly and shake for 1 min. Allow the solution to stand for 10 min. Filter the upper aqueous layer through 0.45 µ nylon filter.

Preparation of sample stock solution

Determine average weight of 20 tablets and crush the tablets to fine powder. Accurately weigh and transfer tablet powder equivalent to about 55 mg of alendronic acid to a 100 mL volumetric flask. Add 50 mL of diluent, sonicate for about 30 min (with intermittent shaking) and make up the volume with diluent and mix.

Example of Related Substances (3)

Preparation of sample solution

Transfer about 5 mL of sample stock solution to a 50 mL glass stoppered tube containing 5 mL of 0.05 M borate solution and 5 mL of acetonitrile. Stopper the tube and shake the solution for 3 min. Add 5 mL of 0.4% 9-Fluorenylmethyl chloroformate solution. Stopper the tube and shake the solution for 45 sec. Allow the solution to stand at room temperature for 30 min. Add 20 mL of methylene chloride. Stopper the tube tightly and shake for 1 min. Allow the solution to stand for 10 min. Filter the upper aqueous layer through 0.45 µ nylon filter.

Preparation of placebo solution

Accurately weigh and transfer placebo powder equivalent to about 55 mg of alendronic acid to a 100 mL dry volumetric flask. Add 50 mL of diluent, sonicate for about 30 min. (with intermittent shaking) and make up the volume with diluent and mix.

Transfer about 5 mL of placebo solution to a 50 mL glass stoppered tube containing 5 mL of 0.05 M borate solution and 5 mL of acetonitrile. Stopper the tube and shake the solution for 3 min. Add 5 mL of 0.4 % 9-Fluorenylmethyl chloroformate solution. Stopper the tube and shake the solution for 45 sec. Allow the solution to stand at room temperature for 30 min. Add 20 mL of methylene chloride. Stopper the tube tightly and shake for 1 min. Allow the solution to stand for 10 min. Filter the upper aqueous layer through 0.45 µ nylon filter.

Example of Related Substances (4)

Chromatographic parameters

Column	:	Hamilton PRP-1, 10 µ (250 mm x 4.1 mm)
Column oven temperature	:	45 °C
Mobile phase	:	

Time (min.)	Eluant A	Eluant B
0	100	0
15	50	50
25	0	100
27	100	0
32	100	0

Flow rate	:	1.8 mL/min.
Detector	:	UV at 266 nm
Injection volume	:	20 µL
Run time	:	32 min.

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Example of Related Substances (5)

Evaluation of system suitability

a. Inject the standard solution into the chromatograph and record the chromatogram. The tailing factor for [redacted] peak should not be more than 2.5.

b. Inject the diluted standard solution into the chromatograph and record the chromatogram. The signal to noise ratio should not be less than 3.

Make adjustments, if necessary to meet system suitability parameters.

Procedure

Inject the placebo and sample solution into the chromatograph and record the chromatograms. Examine the placebo chromatogram for any extraneous peaks and disregard any corresponding peaks observed in the chromatogram of the sample solution. The retention time of [redacted] peak is between 4.0 min.-5.5 min. (with delay volume of the instrument about 1 mL).

Calculations

i) Any individual impurity (% w/w) = $\frac{AT}{AS} \times \frac{DS}{DT} \times \frac{P}{100} \times \frac{A}{C} \times \frac{249.10}{271.08}$

ii) Total impurities (% w/w) = Sum of all individual impurities

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FOR ACCURACY

Protocol of analysis

What is protocol of analysis?

- The way of performing the analysis
- Describe in detail the steps necessary to perform each test

Common Problems – Protocol of analysis

Common problem 1: Protocol of analysis is copied **directly** from pharmacopeia

- Please take note that NPCB does NOT accept any documents that are copied directly from any pharmacopeia (B.P, U.S.P, E.P etc)

Example of Direct Copied of POA from U.S Pharmacopeia

Bromocriptine Mesylate Tablets

» Bromocriptine Mesylate Tablets contain bromocriptine mesylate ($C_{32}H_{40}BrN_5O_5 \cdot CH_4SO_3$) equivalent to not less than 90.0 percent and not more than 110.0 percent of the labeled amount of bromocriptine ($C_{32}H_{40}BrN_5O_5$).

Packaging and storage— Preserve in tight, light-resistant containers.

Labeling— The labeling indicates the *Dissolution Test* with which the product complies.

USP REFERENCE STANDARDS ⟨11⟩ —
USP Bromocriptine Mesylate RS

Identification— Examine the chromatograms obtained in the test for *Related compounds*: the principal spot obtained from the test solution is similar in R_F value, and color to that obtained from the Standard solution.

DISSOLUTION ⟨711⟩ —

Test 1: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 1*.

Medium: 0.1 N hydrochloric acid; 500 mL.

Apparatus 1: 120 rpm.

Time: 60 minutes.

Procedure— Determine the amount in solution in portions of the solution under test previously have been passed through a glass-fiber filter from fluorometric measurements at an excitation wavelength of [illegible] nm and an emission wavelength of 445 nm, using *Dissolution Medium* as the blank, in comparison with a Standard solution having a known concentration of USP Bromocriptine Mesylate RS in the same *Medium*. A volume of alcohol not to exceed 5% of the total volume of the Standard solution may be used to bring the standard into solution prior to dilution with 0.1 N hydrochloric acid.

Tolerances— Not less than 80% *(Q)* of the labeled amount of bromocriptine ($C_{32}H_{40}BrN_5O_5$) is dissolved in 60 minutes.

Test 2: If the product complies with this test, the labeling indicates that it meets USP *Dissolution Test 2*.

Medium: 0.1 N hydrochloric acid; 500 mL.

Apparatus 2: 50 rpm.

Standard preparation— Dissolve an accurately weighed quantity of USP Bromocriptine Mesylate RS in methanol, and quantitatively dilute with *Dissolution Medium* to obtain a solution having a known concentration similar to the expected concentration of the test solution.

Mobile phase— Prepare a filtered and degassed mixture of acetonitrile and 0.01 M ammonium carbonate (65:35). Make adjustments if necessary (see *System Suitability* under *Chromatography* ⟨621⟩).

Chromatography system (see *Chromatography* ⟨621⟩)*—*The liquid chromatograph is equipped with a 300-nm detector and a 3.9-mm × 30 cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph replicate injections of the *Standard preparation*, and record the peak responses as directed for *Procedure:* the relative standard deviation is not more than 2.0%.

Procedure— Separately inject equal volumes (about 100 µL) of the *Standard preparation* and the solution under test into the chromatograph, record the chromatograms, and measure the responses for the major peaks. Calculate the quantity of $C_{32}H_{40}BrN_5O_5$ dissolved by comparison of the peak responses obtained from the *Standard preparation* and the solution under test.

Tolerances— Not less than 80% (Q) of the labeled amount of bromocriptine ($C_{32}H_{40}BrN_5O_5$) is dissolved in 30 minutes.

UNIFORMITY OF DOSAGE UNITS ⟨905⟩ : meet the requirements.

Procedure for content uniformity— [Caution — Protect all solutions from light.]

Solvent solution— Dissolve 1.0 g of tartaric acid in 500 mL of water, add 500 mL of methanol, and mix.

Standard solution— Using an accurately weighed quantity of USP Bromocriptine Mesylate RS, prepare a solution in the *Solvent solution* having a known concentration of about 0.04 mg per mL.

Test solution— Transfer 1 Tablet into a 25-mL volumetric flask. Add about 15 mL of *Solvent solution*, and shake by mechanical means for 30 minutes. Dilute with *Solvent solution* to volume, and mix. Filter and dilute 10.0 mL of the clear filtrate with *Solvent solution* to 50.0 mL.

Procedure— Concomitantly determine the absorbances of the *Test solution* and the *Standard solution* in 1-cm cells at the wavelength of maximum absorbance at about 306 nm, with a suitable spectrophotometer, using *Solvent solution* as the blank. Calculate the quantity, in mg, of bromocriptine ($C_{32}H_{40}BrN_5O_5$) in the Tablet taken by the formula:

$$(654.59 / 750.70)(TC / D)(A_U / A_S)$$

in which 654.59 and 750.70 are the molecular weights of bromocriptine and bromocriptine mesylate, respectively; *T* is the labeled quantity, in mg, of bromocriptine in the Tablet; *C* is the concentration, in µg per mL, of USP Bromocriptine Mesylate RS in the *Standard solution; D* is the

Not Acceptable

Common Problems – Protocol of analysis

Common problem 2: Do not provide sufficient detail on the preparation of solutions (e.g. Standard, sample, placebo, mobile phase, buffer solution etc)

- It is important that the company should provide the details of preparation of solutions in the POA.

Example of Preparation of Mobile Phase (1)

PREPARATION OF MOBILE PHASE

MOBILE PHASE:

Mix the buffer and acetonitrile in the ratio of 67:33 and mix well. Sonicate for 2 minutes.

0.1 M SODIUM HYDROXIDE:

Weigh accurately 4.0 g of sodium hydroxide and transfer to 1000 mL volumetric flask, add 500 mL water, dissolve and make up to volume with water.

DILUENT:

Use the mobil phase.

What is wrong with the preparation of mobile phase above?

Ans: Preparation of BUFFER solution in the mobile phase is not provided

Example of Preparation of Mobile Phase (2)

PREPARATION OF MOBILE PHASE

BUFFER:

Weigh accurately 3.12 g of sodium dihydrogen orthophosphate dihydrate ($NaH_2PO_4.2H_2O$) and 0.52 g of disodium hydrogen orthophosphate dihydrate into a beaker containing 1000 mL of Milli-Q- grade water. Adjust the pH to 6.0 ± 0.05 with orthophosphoric acid. Filter through 0.45 µm or finer porosity membrane filter and degas.

MOBILE PHASE:

Mix the buffer and acetonitrile in the ratio of 67:33 and mix well. Sonicate for 2 minutes.

0.1 M SODIUM HYDROXIDE:

Weigh accurately 4.0 g of sodium hydroxide and transfer to 1000 mL volumetric flask, add 500 mL water, dissolve and make up to volume with water.

DILUENT:

Use the mobil phase.

Common Problems – Protocol of analysis

Common problem 3: Do not provide chromatogram of solutions such as standard, sample, blank/placebo (if any), system suitability solution (if any).

- It is important that the company should provide the chromatograms of respective standard, sample, blank, system suitability solution and etc in the POA as well as in the method validation.

Common Problems – Protocol of analysis

Common problem 4: Method provided is not specific (especially for titration technique)

- Please refer to the example on the next slide.

Example of the testing procedure that is not detail enough

iii) 0.1M Disodium Edetate VS Solution

Dissolve 37.5g of disodium edetate in sufficient water to produce 500ml, add 100ml of 1M sodium hydroxide and dilute to 1000ml with water.

Standardization:

Dissolve 0.120g of [redacted] in granulas, in 4ml of 7M hydrochloric acid (721g of HCl in 1000ml of water) and add 0.1ml of bromine water (A saturated solution obtained by shaking occasionally for 24 hours 3ml of bromine with 100ml of water and allowing to separate. Store solution over and excess of bromine, protected from light). Boil to remove excess bromine, cool, add 2M sodium hydroxide until the solution is weakly acidic or neutral. Dilute the above to 200ml with water and add about 50mg of xylenol orange triturate and sufficient hexamine to produce a violet-pink colour. Add a further 2g of hexamine and titrate with 0.1M disodium edetate VS until the colour changes to yellow. Each ml of 0.1M disodium edeteate VS is equivalent to 6.54mg of [redacted]

Procedure:

Heat 0.5g of the cream gently is porcelain dish over a small flame until the basis is completely volatilised or charred. Increase the heat until all the carbon is removed. Dissolve the residue in 10ml of 2M acetic acid and add sufficient water to produce 50ml. To the resulting solution add 50mg of xylenol orange triturate and sufficient hexamine to produce a violet-pink colour. Add a further 2g of hexamine and titrate with 0.1M disodium edetate VS until the solution becomes yellow. Each ml of 0.1M disodium edetate VS is equivalent to 8.138mg of [redacted].

The procedure states that "Heat 0.5g of the cream gently…"

i) How long does it need to heat up?
ii) What apparatus is used to heat up?
iii) Any specific temperature?

Calculation:

$$\%w/w \text{ of } [\text{redacted}] = Vs \times F \times \frac{0.008138}{Sw} \times 100$$

Where:

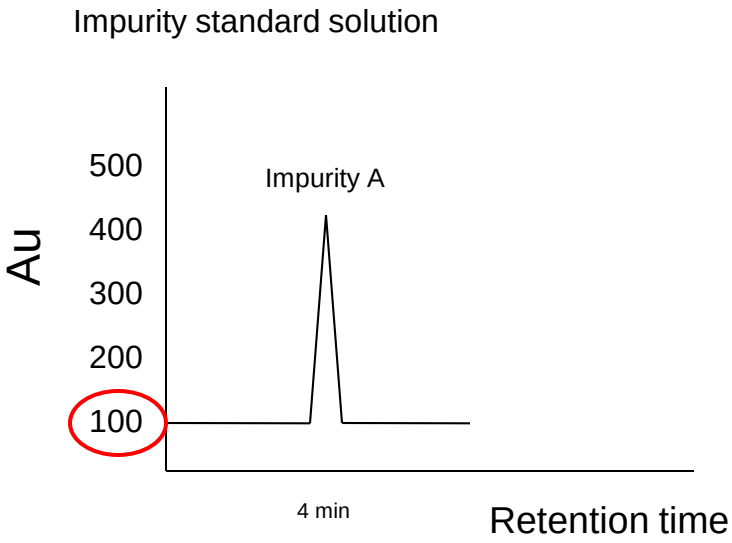
Vs = Volume of titrant consumed by sample
F = Standardization factor for 0.1M disodium edetate VS
Sw = Weight of sample used

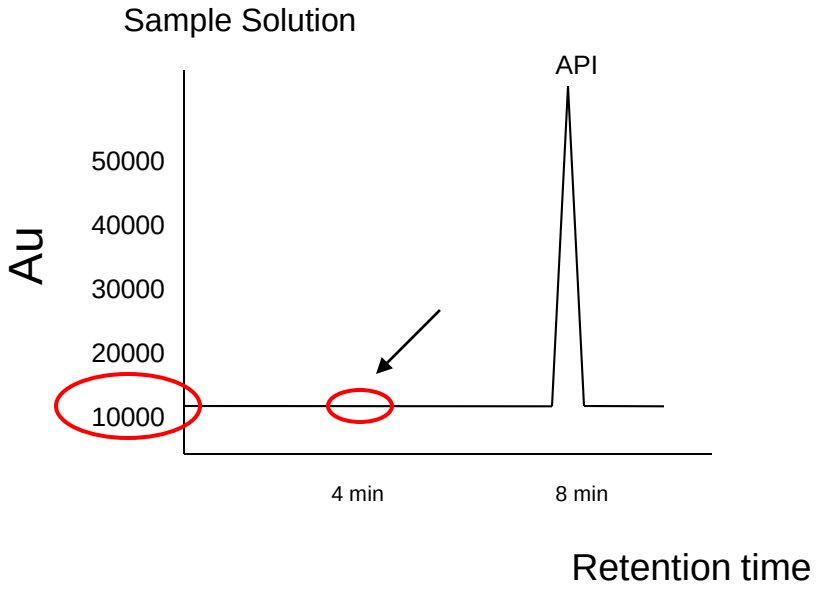
Common Problems – Protocol of analysis

Common problem 5: Chromatograms are not in the same scales particularly for related substances test

- In protocol of analysis, some chromatograms provided are not in the same scales (particularly for related substances test).
- Please refer next slide for example.

Example of the chromatogram that is not in the same scale





In the chromatogram of sample solution, the scale provided is 10000Au which is 100 times larger than the chromatogram of impurity standard solution. This may cause some of the impurity peaks undetected.

METHOD VALIDATION

- Process of demonstrating that analytical procedures are suitable for their intended use.

- It begins with the planned & systematic collection of the validation data to support the analytical procedures.

Common Problems – Method Validation

Common problem: Do not provide on how to prepare test solution for each validation parameter

- Company only provides validation report and raw data but description on how to prepare the test solution for each validation parameter is not provided

Example of Method Validation (1)

6.0 SYSTEM PRECISION:

6.1 Weigh and transfer accurately about 67mg of [redacted] working standard into 200ml volumetric flask, add 25ml of methanol and sonicate to dissolve, cool it, and dilute to volume with dissolution media. Pipette out 5ml of this solution into 50ml volumetric flask and dilute to volume with dissolution media and mix.

6.2 Inject 20µl of dissolution media as blank and above solution 6 times, calculate the Relative Standard deviation for the area of [redacted].

6.3 Acceptance Criteria:

The relative standard deviation for the Six replicate injections of standard preparation should not be more than 2%.

7.0 SYSTEM SUITABILITY:

7.1 Weigh and transfer accurately about 67mg of [redacted] working standard into 200ml volumetric flask, add 25ml of methanol and sonicate to dissolve, cool it, and dilute to volume with dissolution media. Pipette out 5ml of this solution into 50ml volumetric flask and dilute to volume with dissolution media and mix.

7.2 Inject 20µl of dissolution media as blank and above solution 5 times, calculate the System suitability parameters.

7.3 Acceptance Criteria:

The RSD for the five replicate injections of standard preparation should not be more than 2%.

The tailing factor for [redacted] should be in between 0.8 and 2.0.

Theoretical plates of [redacted] peak should be not less than 3000.

Approximate retention time of [redacted] is about 4.5 minutes.

Example of description on how to prepare the test solution

Example of Method Validation (2)

10.0 SPECIFICITY:

10.1 Sample preparation:

Perform the test on six tablets. Place one tablet in each dissolution vessel containing 900ml of medium maintained at 37±0.5°C. After each interval, filter the solution through 0.45µm nylon filter and collect the sample after discarding 2-3ml of filtrate.

10.2 Placebo preparation: Transfer placebo powder equivalent to one tablet into 500 ml volumetric flask. Add to it about 350 ml of dissolution media and sonicate for 10-15 minutes. Filter the solution through 0.45µm nylon filter, collect the sample after discarding first 2-3 ml of the filtrate. Separately prepare the placebo solutions.

10.3 Preparation of [redacted] Related compound F: Prepare the [redacted] related compound F solutions at the specification level in dissolution media.

10.4 Procedure: Separately inject in single 20 µl of blank as dissolution media, placebo preparation, sample preparation and individual impurity F preparations. Inject all solutions in single. Calculate the purity angle and Purity threshold for Analyte peak.

10.5 Acceptance Criteria: The purity angle should less than that of purity threshold for standard & sample preparations.

Placebo interference should not more than 2.0 % placebo & [redacted] related compound F preparations when compared with standard area.

12.0 DEMONSTRATION OF LINEARITY AND RANGE :

12.1 Linearity is expressed in terms of variance around the slope of the regression line calculated in accordance to established mathematical relationship between the test results obtained by the analysis varying concentrations of [redacted].

12.2 Linearity solutions preparation:

Weigh and transfer accurately about 67mg of [redacted] working standard into 200ml volumetric flask, add 25ml of methanol and sonicate to dissolve, cool it, and dilute to volume with dissolution media. Further dilute this solution successively as per the table to achieve the target concentrations.

Validation Characteristics- Specificity

What is Specificity?

ICH 2005

"...the ability to assess unequivocally the analyte in the presence of components which may be expected to be present"

Eg: impurities, degradants, matrix

- solvent blank or diluents do not have the same retention time as the analyte.

- The analyte should have **no interference** from other extraneous components and should be well resolved from them.

Specificity – by High Pressure Liquid Chromatography

Method:

- Inject blank/ diluent, standard, sample and placebo solution

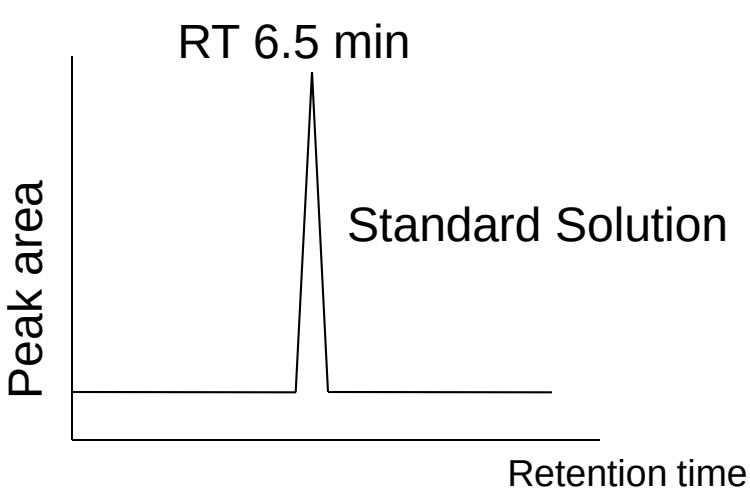
Acceptance criteria:

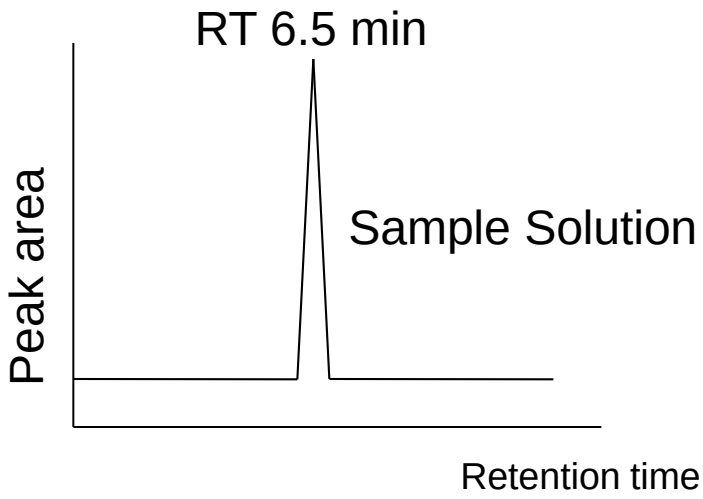
- No interference of blank/ diluent and placebo solution at the retention time of active peak

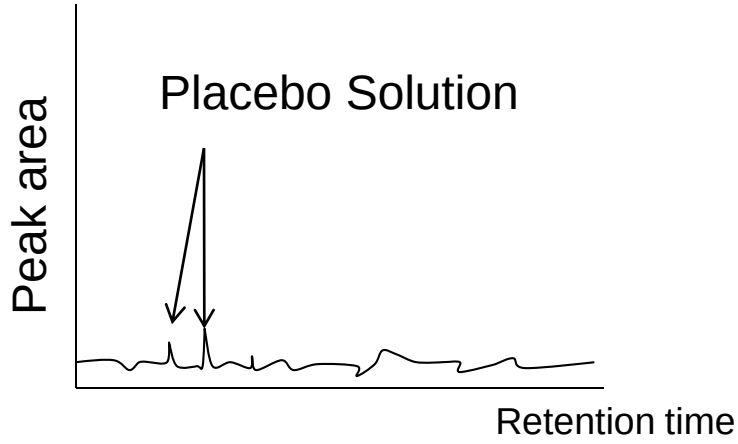
What to look for:

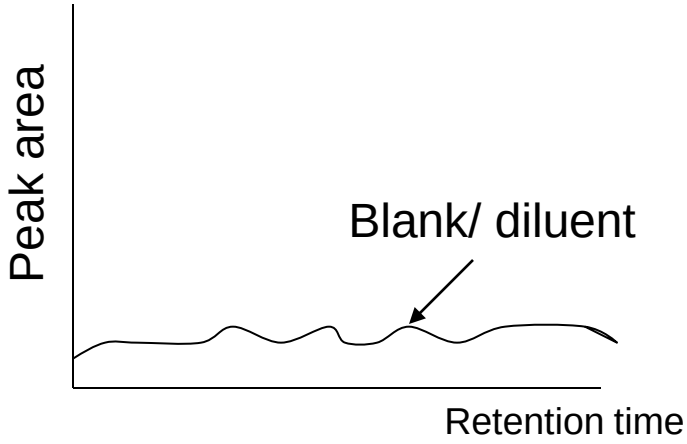
- HPLC chromatogram
- Same retention time for the active peak in sample and standard solution
- Blank/ diluent and placebo solution → peak was not observed at the retention time of active peak

Specificity – by High Pressure Liquid Chromatography









Type of analytical procedure characteristics	Identification	Testing For Impurities Quantitation Limit	Assay - dissolution (measurement only) - content/ potency
Accuracy	-	+ -	+
Precision			
Repeatability	-	+ -	+
Interm. Precision	-	+ (1) -	+ (1)
Specificity (2)	+	+ +	+
Detection Limit	-	- (3) +	-
Quantitation Limit	-	+ -	-
Linearity	-	+ -	+
Range	-	+ -	+

- signifies that this characteristic is not normally evaluated

+ signifies that this characteristic 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 procedure(s)

(3) may be needed in some cases

Common Problems - Specificity

Common problem 1: Chromatograms NOT provided for HPLC/GC method

- Company only provide details like method of carrying out the Specificity test and result in tabulated form
- Chromatograms are MANDATORY for specificity

Example of Chromatograms for Specificity (1)

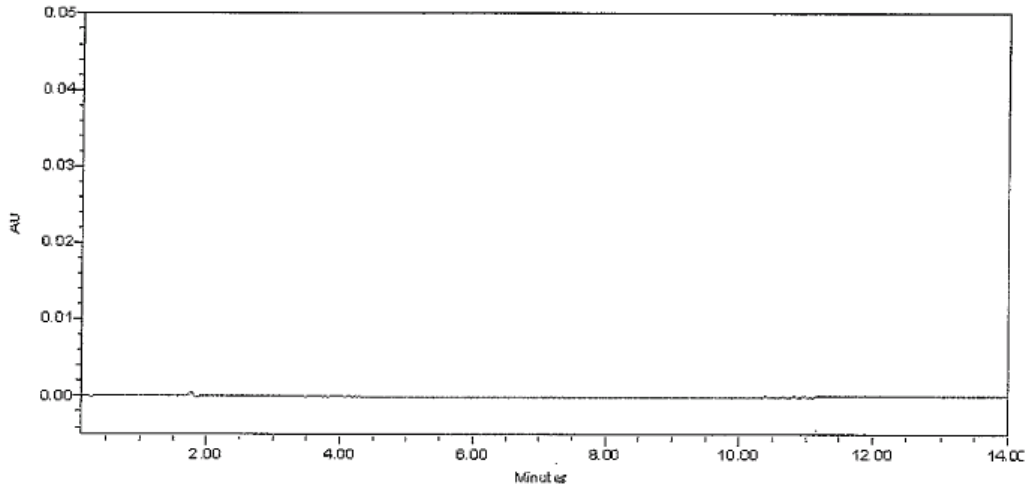


Figure 11 Chromatogram of a solution of excipient mixture (placebo)

Placebo

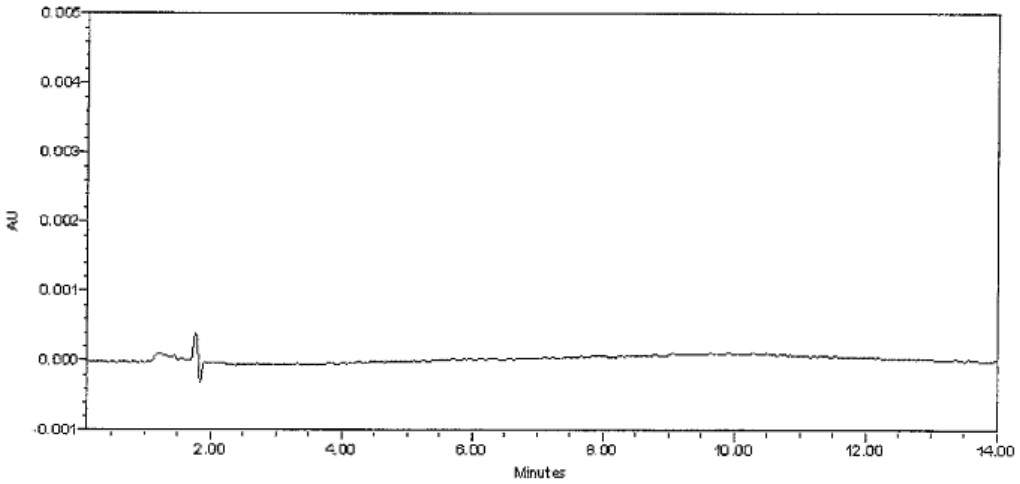


Figure 12 Chromatogram of a solution of excipient mixture (placebo), magnified

Example of Chromatograms for Specificity (2)

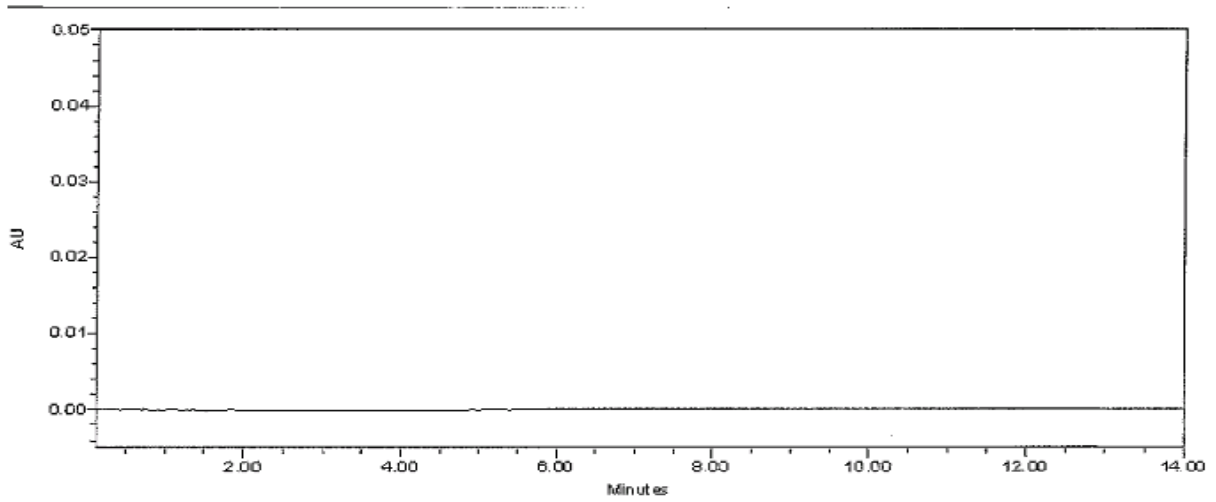


Figure 9 Chromatogram of a blank solution (5% diluent in mobile phase, used for the dilution of samples)

Blank Solution

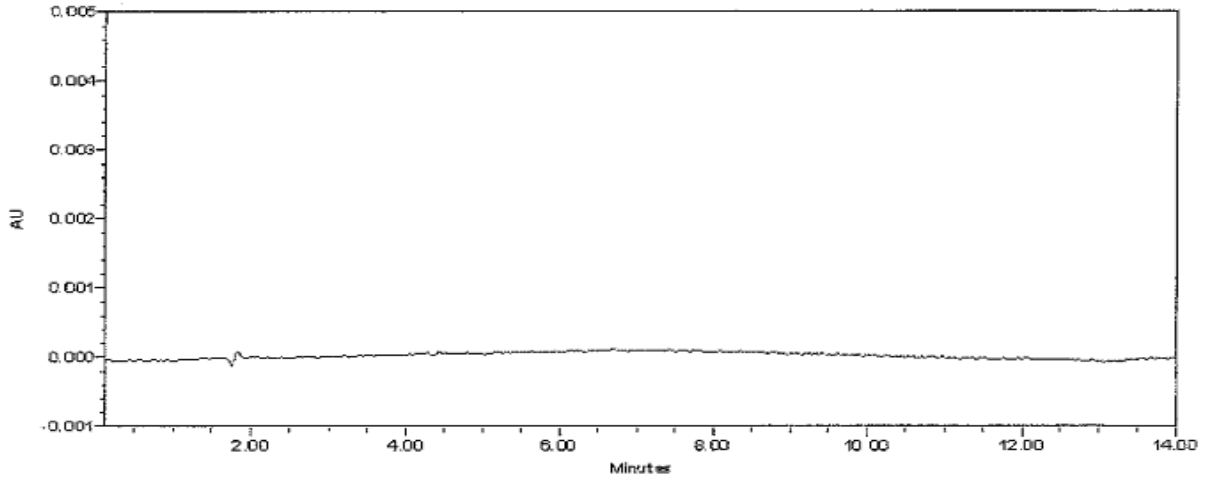


Figure 10 Chromatogram of a blank solution (5% diluent in mobile phase, used for the dilution of samples), magnified

Example of Chromatograms for Specificity (3)

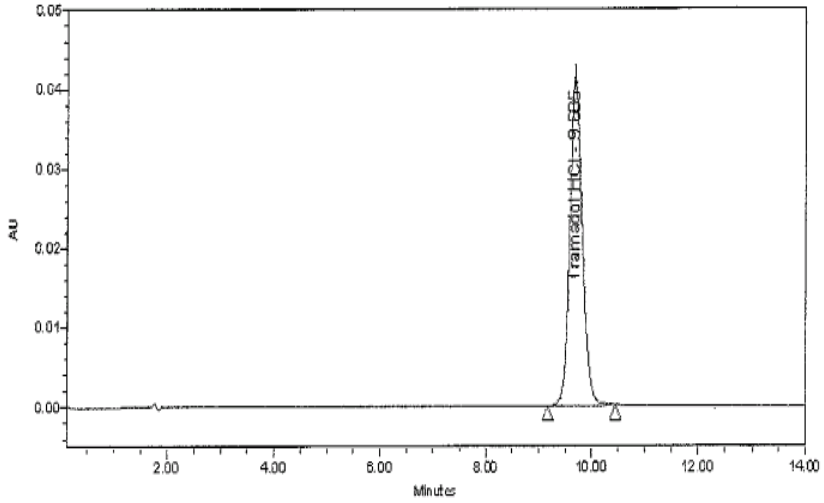


Figure 2. Chromatogram of a solution consisting of ~100 µg/mL Tramadol HCl RS and excipient mixture (placebo)

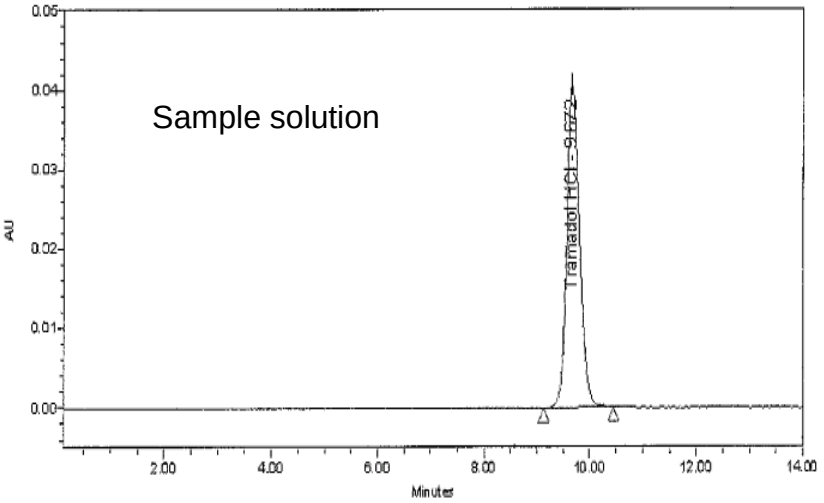


Figure 3 Chromatogram of a solution of sample (100 mg tablets)

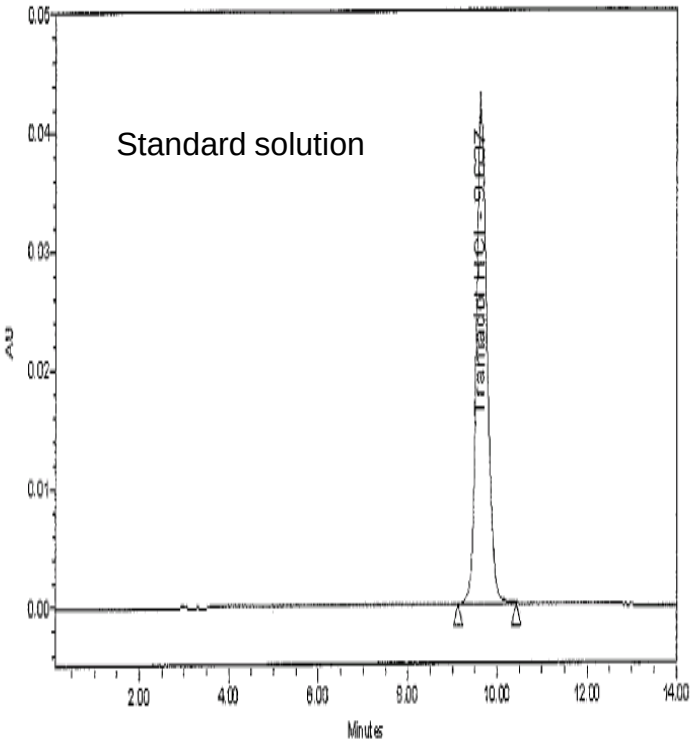
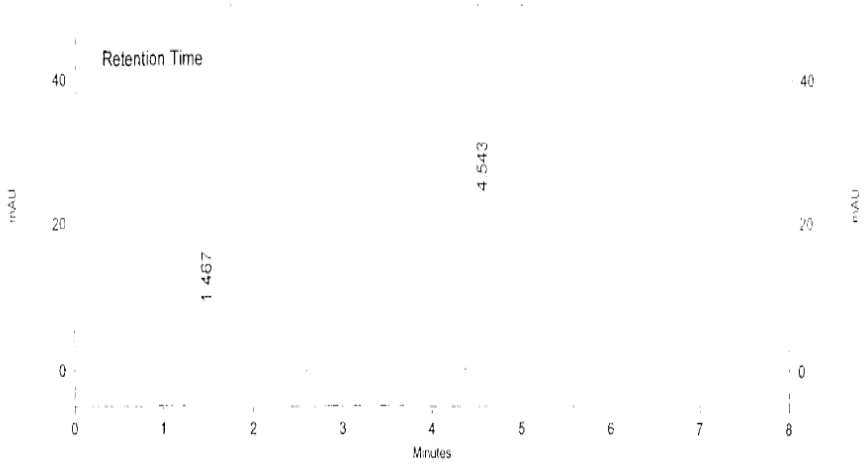


Figure 1. Chromatogram of ~100 µg/mL Tramadol HCl RS solution

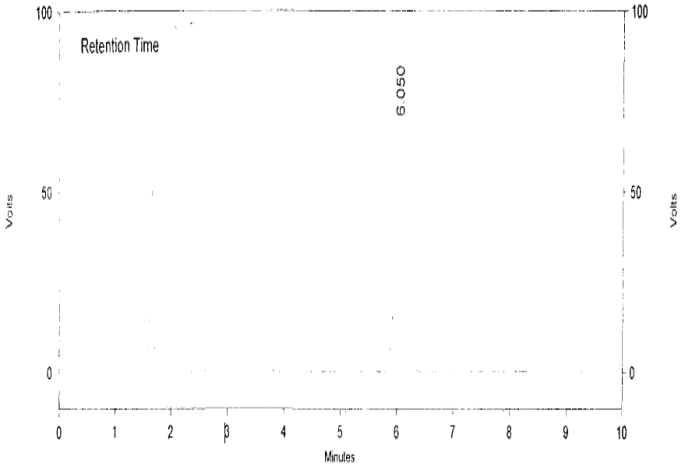
•Note:
Please include the identity of each chromatograms!

Any problem with these chromatograms?



VWD: Signal A, 273 nm Results

Name	Retention Time	Area	Area %
Fumerate Peak	1.467	411853	12.56
Bisoprolol	4.543	2868117	87.44
Totals		3279970	100.00



VWD: Signal A, 227 nm Results

Name	Retention Time	Area	Area %
Bisoprolol	6.050	13530803	100.00
Totals		13530803	100.00

Ans: YES, the peak of interest CANNOT be seen.
Soln: Please provide clear chromatogram for evaluation

Common Problems - Specificity

Common problem 2: No Stress test done for Unknown Impurities (specifically for Related Substances Test)

- If impurity or degradation products standards are unavailable, specificity may be demonstrated by comparing the test results of samples containing impurities or degradation products to a second well-characterized procedure e.g: Pharmacopeia or other validated analytical procedure. As appropriate, this should include samples stored under relevant stress conditions: **Light, heat, humidity, acid/base hydrolysis and oxidation.**
- (**Forced Degradation Study**)

Forced Degradation Study

- Why forced degradation studies are carried out?
  - ✓ To develop and validate a stability indicating method
  - ✓ To identify impurities related to drug substances or excipients

- Typically, a stressed sample of about 10-20% of degradation is used to demonstrate the resolution among degradation products.
- A 10-20% degraded sample is used because it has a sufficiently high concentration level of critical related substances ⟶ rel. substances can be detected easily.

Example of Stress Test (1)

9.0 FORCED DEGRADATION: (Before starting every experiment ensure that system suitability should pass)

9.1 **Preparation for Impurity stock solution:** Weigh and transfer about 3.2 mg of [redacted] related compound F into 20 ml volumetric flask. Add about 7 ml of Acetonitrile and sonicate to dissolve and dilute to volume with water and mix.

9.2 **Preparation of Resolution Solution:** Weigh and transfer about 50 mg of [redacted] working standard in 250 ml volumetric flask, add 90 ml of acetonitrile and dilute to volume with water and mix. Dilute 5 ml of this solution to 100 ml with diluent and mix. Transfer 5 ml of this solution to 10 ml volumetric flask, add 1 ml of impurity stock solution and dilute to volume with diluent and mix.

9.3 **Standard preparation:** Weigh and transfer about 40mg of [redacted] working standard into a 100ml volumetric flask, add about 35ml of Acetonitrile and sonicate to dissolve, dilute to volume with water and mix. Dilute 2ml of this solution to 50ml with diluent and further dilute 5ml of this solution to 50ml with diluent and mix.

9.4 **Unstressed Sample preparation:** Weigh and finely crush not less than 20 tablets. Weigh and transfer equivalent to 80mg of [redacted] into 100ml volumetric flask, add about 35ml of Acetonitrile and sonicate for 15 minutes. Cool and dilute to volume with water and mix. Filter the supernatant solution through 0.45µm nylon filter; collect the sample after discarding 2-3ml of the filtrate (Inject freshly prepared solution).

9.5 **Procedure :** Inject blank & resolution solution in single. Inject standard preparation in six replicates and unstressed sample in single.

Example of Stress Test (2)

10.0 Acid Hydrolysis:

10.1 **Sample preparation:** Weigh and finely crush not less than 20 tablets. Weigh and transfer equivalent to 80 mg of [redacted] into 100ml volumetric flask, add 5 ml of 0.1N HCl & 35 ml of Acetonitrile and sonicate for 10-15minutes in cool water. Keep aside for 30 minutes. Add 5 ml of 0.1N NaOH solution and dilute up to the mark with water and mix. Filter the supernatant solution through 0.45µm nylon filter; collect the sample after discarding 2-3ml of the filtrate. (Inject freshly prepared solution). (Note: Stop the experiment when 10 to 30 % of degradation is achieved when compared to area of unstressed sample or repeat the experiment with mild or strong conditions)

10.2 **Procedure:** Inject sample in single and calculate the purity angle and Purity threshold. Report % degradation achieved after each experiments by comparing with unstressed area. Also report % [redacted] related compound F single maximum impurity & % total impurities after each experiment.

Table -24 (Results summary) Acid Hydrolysis

Impurities	Purity Angle	Purity threshold	Acceptance criteria
Unknown	59.245	90.000	Purity Angle < Purity threshold
Impurity F	80.899	90.000	
[redacted]	2.470	3.311	

Example of Stress Test (3)

12.0 Oxidation:

12.1 **Sample preparation:** Weigh and finely crush not less than 20 tablets. Weigh and transfer equivalent to 80mg of [redacted] into 100ml volumetric flask, add 5 ml of 5 % H_2O_2 solution & 35 ml of Acetonitrile and sonicate for 10-15minutes in cool water. Keep aside for 30 minutes and dilute up to the mark with water and mix. Filter the supernatant solution through 0.45µm nylon filter; collect the sample after discarding 2-3ml of the filtrate. (Inject freshly prepared solution). (Note: Stop the experiment when 10 to 30 % of degradation is achieved when compared to area of unstressed sample or repeat the experiment with mild or strong conditions)

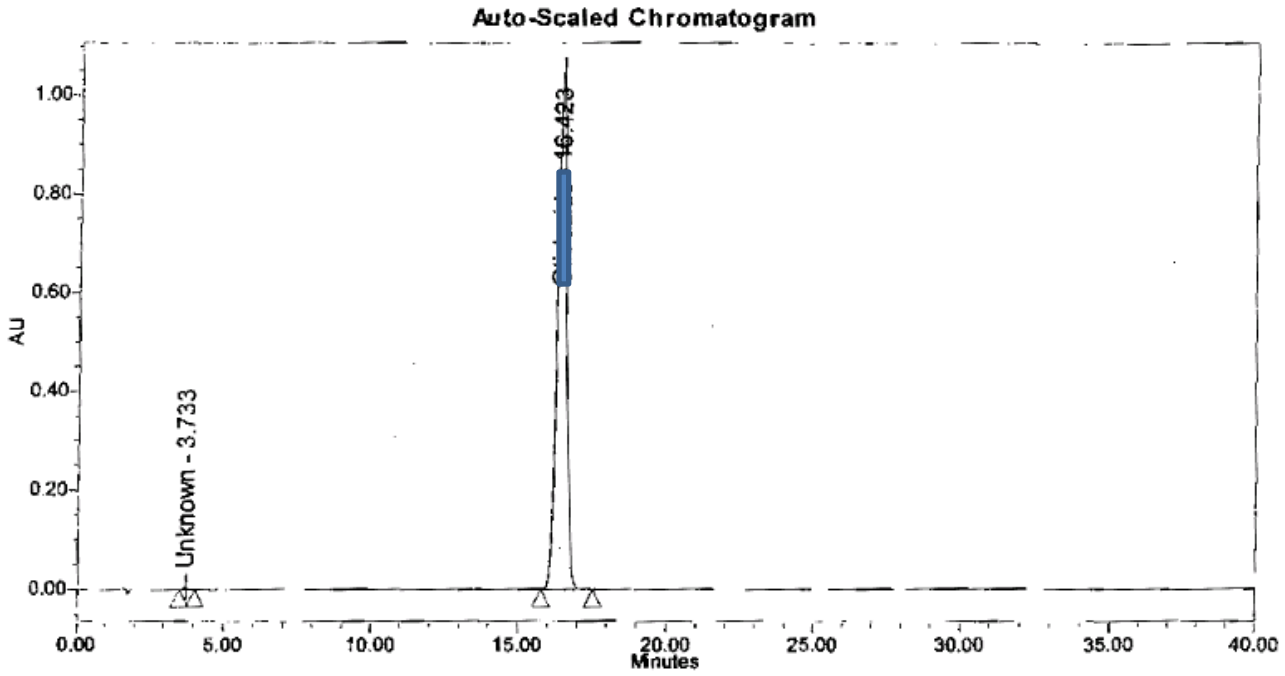
12.2 **Procedure:** Inject sample in single and calculate the purity angle and Purity threshold. Report % degradation achieved after each experiments by comparing with unstressed area. Also report % Gliclazide related compound F single maximum impurity & % total impurities after each experiment.

12.3 **Acceptance Criteria:** Purity angle should less than that of purity threshold for [redacted] & [redacted] related compound F from sample preparation.

Table -30 (Results summary) Oxidation

Impurities	Purity Angle	Purity threshold	Acceptance criteria
Unknown	59.996	90.00	Purity Angle < Purity threshold
Impurity F	70.237	90.00	
[redacted]	2.477	3.284	

Example of Stress Test (4) : With Chromatograms

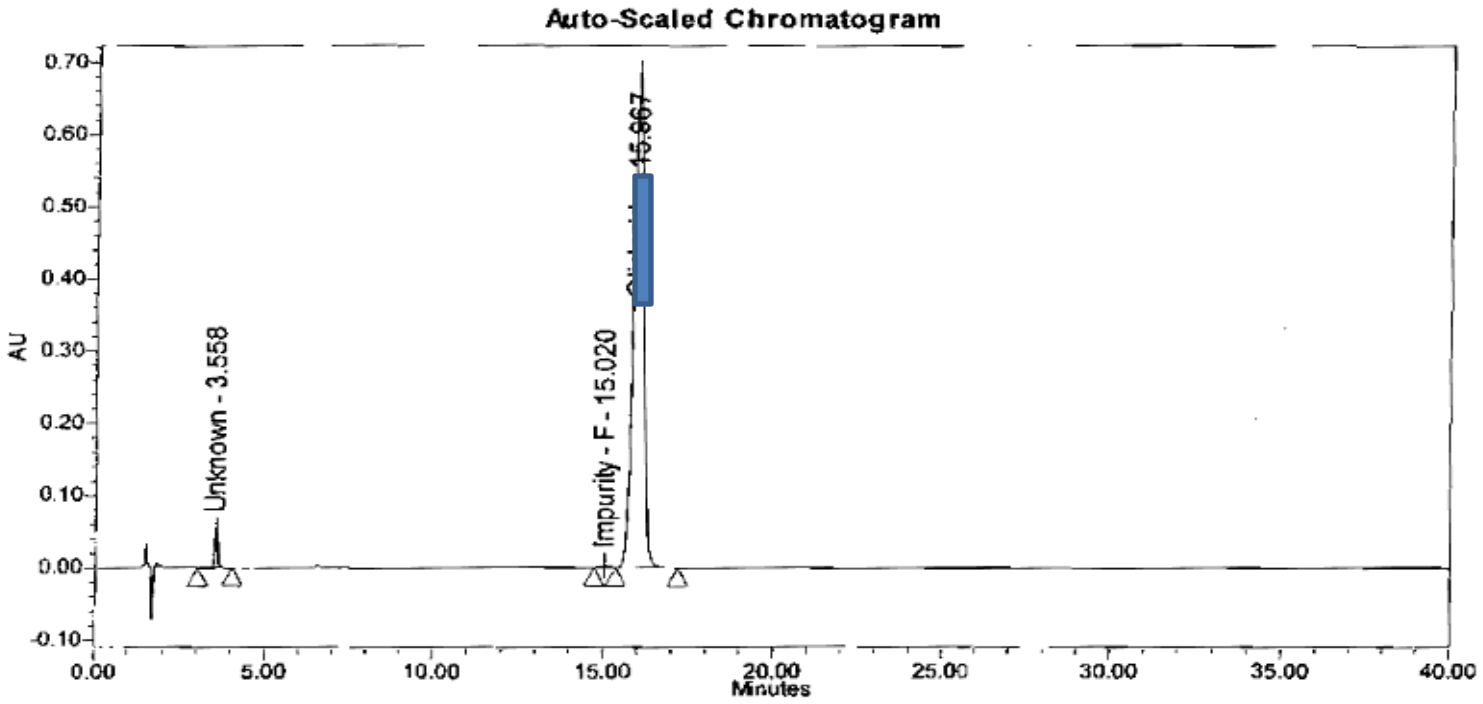


Peak Results

	Name	RT	Area	% Area	Purity1 Angle	Purity1 Threshold	USP Plate Count	USP Tailing	USP Resolution	RT Ratio
1	Unknown	3.733	35489	0.16	80.025	90.000	12603	1.18		0.23
2	[redacted]	16.423	21754174	99.84	1.529	1.972	14730	1.02	36.79	

Unstressed

Example of Stress Test (5) : With Chromatograms

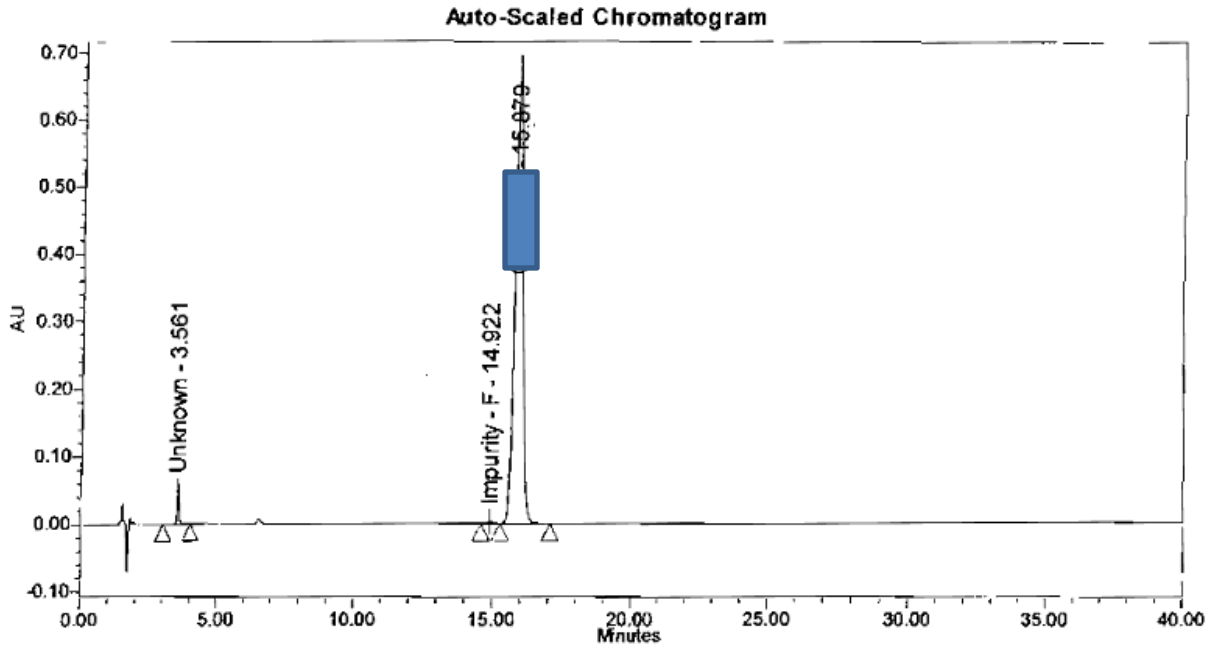


Peak Results

	Name	RT	Area	% Area	Purity1 Angle	Purity1 Threshold	USP Plate Count	USP Tailing	USP Resolution	RT Ratio
1	Unknown	3.558	266921	1.93	59.245	90.000	13380	1.35		0.22
2	Impurity - F	15.020	49256	0.36	80.899	90.000	15069	1.02	38.07	0.94
3		15.967	13489039	97.71	2.470	3.311	15425	1.03	1.88	

Acid hydrolysis

Example of Stress Test (6) : With Chromatograms



Peak Results

	Name	RT	Area	% Area	Purity1 Angle	Purity1 Threshold	USP Plate Count	USP Tailing	USP Resolution	RT Ratio
1	Unknown	3.561	265195	1.97	59.996	90.000	13311	1.32		0.22
2	Impurity - F	14.922	48886	0.36	70.237	90.000	18110	1.03	38.27	0.94
3	[redacted]	15.879	13121469	97.66	2.477	3.284	15727	1.02	1.94	

Oxidation

Common Problems - Specificity

Common problem 3: Does not provide peak purity test for impurities (specifically for related substance test)

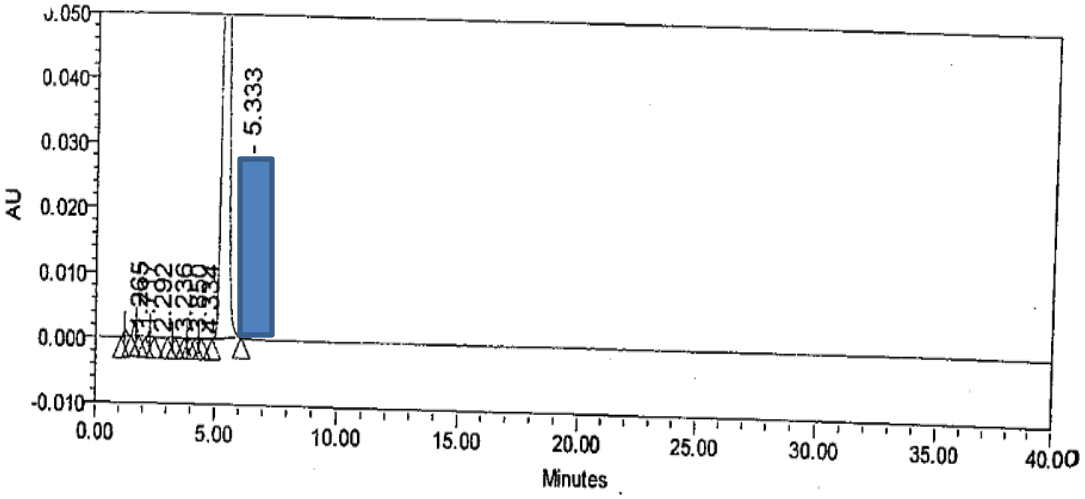
- For impurity tests, the impurity profiles should be compared
- Peak purity tests are useful to show that the analyte chromatographic peak is not attributable to more than one component (e.g. photodiode array, mass spectrometry)

Peak Purity Test

- The purity angle is a measure of the spectral heterogeneity of a peak based on the comparison of spectra over all the peak, using the spectral contrast angle.
- The non-ideal effects are quantified and provided as a value of the Threshold angle.
- When the **peak is pure,** the Purity angle is **lower** than the Threshold angle.

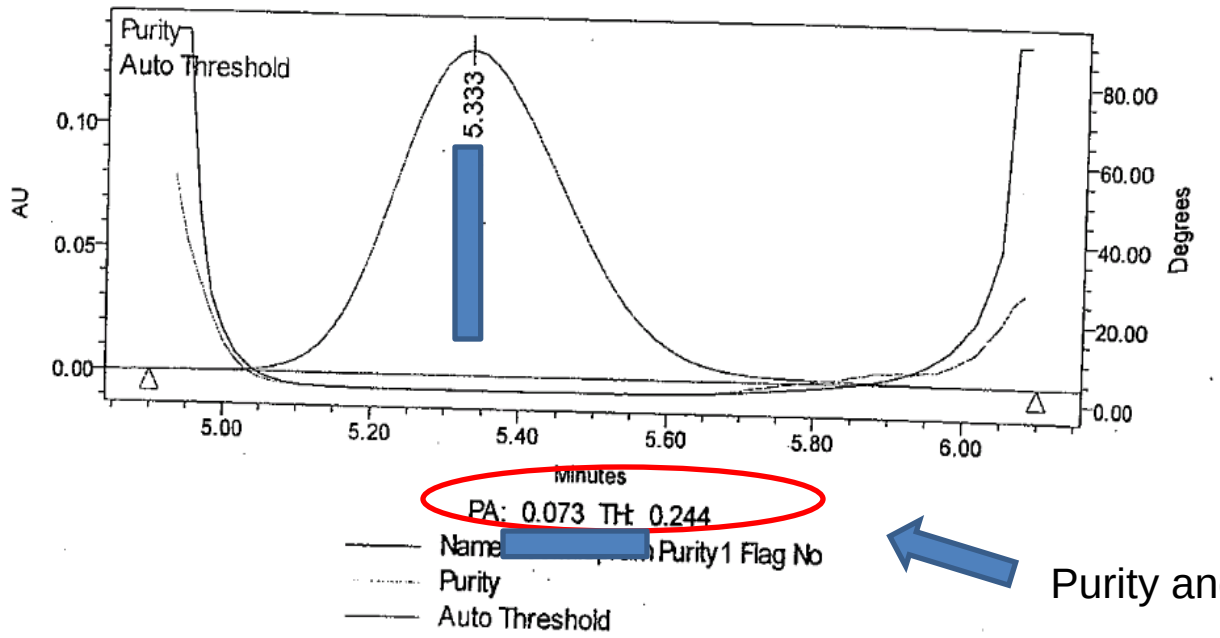
Example of peak purity test (1)

Figure 3A: Chromatogram of Acid stressed [redacted] Tablets Test



Example of peak purity test (2)

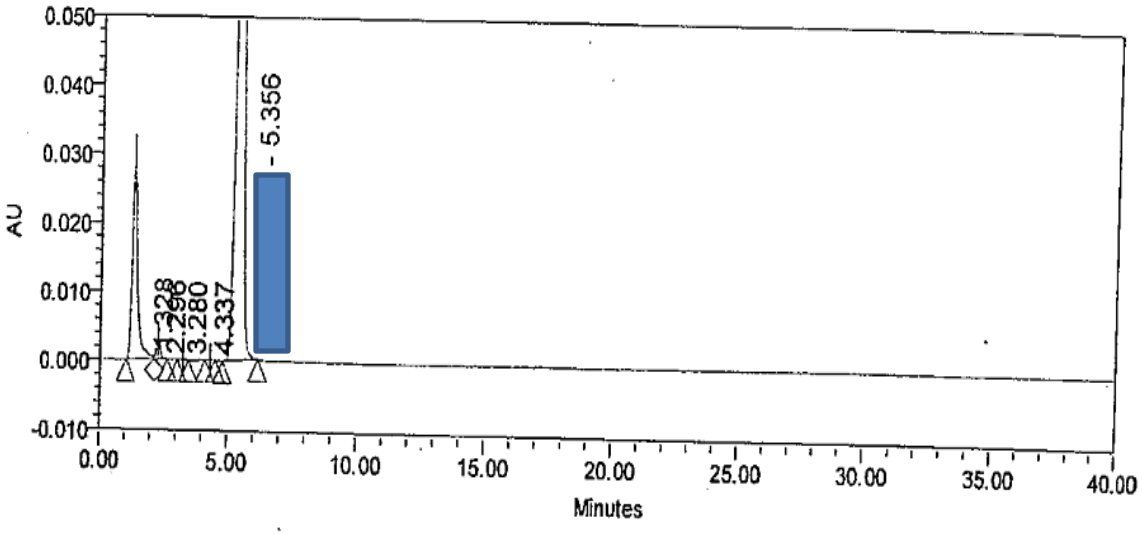
Figure 3B: Purity plot of Acid stressed [redacted] Tablets Test



Purity angle < Purity Threshold

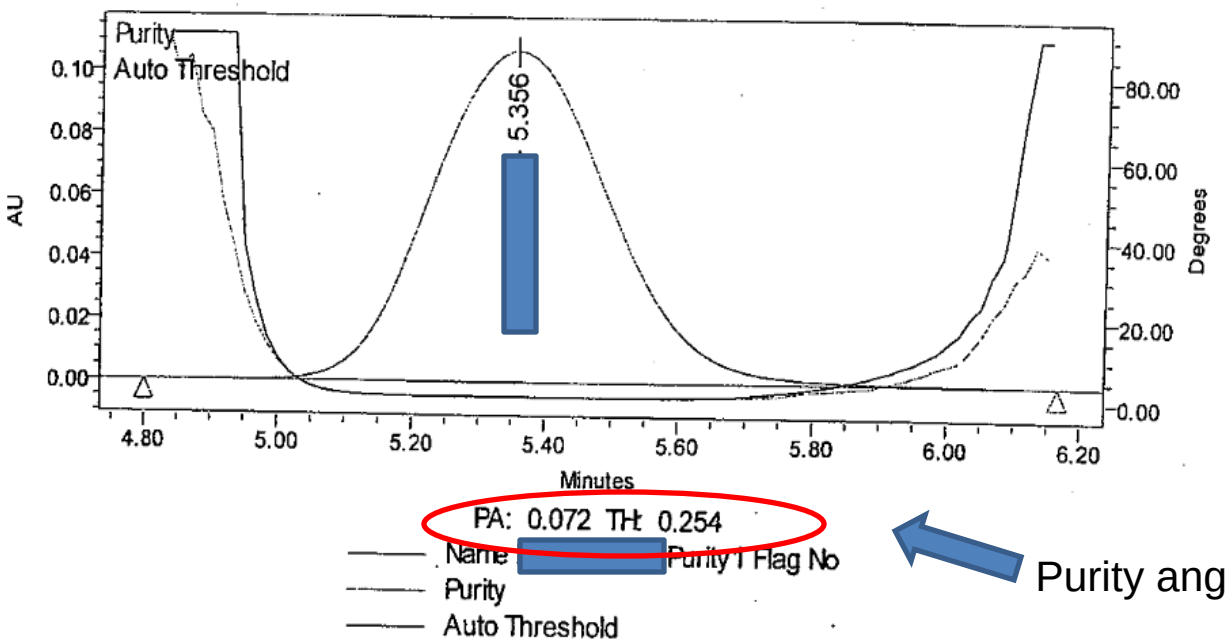
Example of peak purity test (3)

Figure 4A: Chromatogram of Base stressed [redacted] Tablets Test



Example of peak purity test (4)

Figure 4B: Purity plot of Base stressed [redacted] Tablets Test



Purity angle < Purity Threshold

Thank you