International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 1861-1876www.ijprajournal.com ISSN: 2249-7781

Analytical method development of methotrexate by UV visible spectroscopy

KhanSudip[1], S.Tejashwini[2], Chawla Ayushi[3]
[1,2] *Research Scholar, Nargund collage of pharmacy,Bangalore, Karnataka, India*
[3].*Asst professor, Nargund collage of pharmacy,Bangalore, Karnataka, India*

Submitted: 05-08-2023 Accepted: 15-08-2023

ABSTRACT:
A simple,first accurate and precise UV spectroscopic method were developed and validated for the estimation of Methotrexate as per ICH Guidelines Phosphate Buffer (6.4, 7.4), NAOH (0.1N) were used as the solvent. The λmax of Methotrexate was found to be 300 nm approximately in all solvent used (NaOH, Phosphate buffer) and it was proved linear in concentration range of 10, 20, 30, 40, 50 µg/ml in 0.1 NaOH and for phosphate buffer in the range of 50-100 µg/ml with a co-relation coefficient value of 0.999. Accuracy and Precise studies of UV spectroscopies method was performed at 3 differentlevels that is 50%, 100%, 150% And recovery was found to be in the range of 90-100% for methotrexate. The optimum conditions of methotrexate were developed. Beer's law was obeyed in the concentration range of 10-100 µg/ml. Calibration curve shows linear relationship between the absorbance and concentration with r^2 value of 0.999 was obtained. Validation was performed according to the ICH guidelines for Linearity, accuracy, precision, LOD and LOQ.The sample solution was stable in 0.1 N NaOH but was not stable in phosphate buffer 7.4 after 24 hours at controlled temperature conditions. The proposed method may be suitable for the Qualitative and Quantitative analysis including stability studies and method development of methotrexate for the quality control purposes.

I. INTRODUCTION:

Cancer is a group of Disease characterized by uncontrolled and unregulated growth of cells. Although cancer is often considered a disease of gaining with the majority of cases diagnosed in those over age 55years, it occurs in people of all ages.According to the Indian council of Medical research estimates, by 2020 there would be over 17.3 lakh new cases of cancers with cancer of breast, lung and cervix topping the list.Cancer may be regarded as a group of disease characterized by 1) Abnormal growth of cells 2) ability to invade adjacent tissues and even distant organ, and 3) the eventual death of the affected patient if the tumour has progressive beyond that stage when it can be successfully removed. [1]

1.1 Anticancer:

Anticancer drug also called anti-Neoplastic drug, any drug that is effect in the malignant disease.A type of anticancer drug that blocks cell growth by interfering with DNA the genetic material in cell. [1,2]

1.2 Anticancer drugs have been found till now:

150 anticancer drugs approved by the US Food and Drug Administration (FDA). Based on drug mechanism of action, these agents are divided into two groups: 61 cytotoxic-based drugs and 89 target-based drugs.From above 150 drugs, we built a drug-cancer network, which contained 183 nodes (150 drugs and 33 cancer types) and 248 drug-cancer associations. The network indicated that the cytotoxic drugs tended to be used to treat more cancer types than targeted drugs. [3,4]

1.3

Methodvalidation: Methodvalidationistheprocessof establishingtheperformance characteristics and limitations of a method andthe identification of the influences, which may change these characteristics,andtowhatextent.
Itisalsotheprocessofverifyingthatamethodisfitfor purposeincludes**.** Formulationdevelopmentmethods, Analyticalmethods**,** Cleaningmethods. [13]

1.4 Cleaningvalidation

Cleaning validation (CLV) is written evidence that determines
aspecifiedcleaningprocedurewillleadtoreliableandre peatableresults in the cleaning of surfaces with and without contact with theproduct.Itisshownthatthefollowingcriteriaarefulf illed:Theconcentrationofactivesubstancesonproduct contactsurfaceswillnotexceedspecifiedlimits.

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 1861-1876www.ijprajournal.com ISSN: 2249-7781

Theconcentrationofhighlyactivesubstances(e.g.,hormonesorcytostatic)onsurfaceswithoutcontactwiththeproductwillnotexceedspecifiedlimits. Theconcentrationofotherpharmacologicallyactivesubstances(e.g.,processandcleaningmaterialsordisinfectants) in the product to follow will not exceed specifiedlimits. Thenumberofgermson productcontact surfaceswillnot exceed specified limit. [13,14]

1.5 CALIBRATION

To maintain the accuracy and precision of test equipment atalltimes, ensure highest level of confidence in all measurementthat affect materials disposition decision, with unbrokenchainoftraceabilitytonationalstandard., determine whether the equipment is still fit for itsintendedpurpose. It is based on the comparison of a primary standard orinstrument of known accuracy with another equipment (tobecalibrated) and used to detect correlate, report or eliminate byadjustment of any variation in the accuracy of theequipmentbeingcalibrated. [14,15]

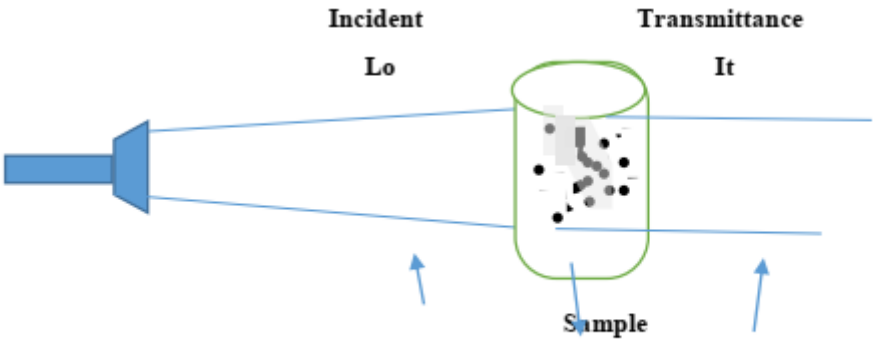
1.6 SPECTROSCOPY

Spectroscopy is the study of interaction of electromagnetic radiation with matter. These interactions involve absorption and emission of radiation (energy) by the matter. Spectroscopy are of two types, absorption spectroscopy and emission spectroscopy. The study of electromagnetic radiation absorbed by the sample, in the form of spectra is called absorption spectroscopy (UV-visible, IR, NMR, microwave and Radio wave spectroscopy). The study of electromagnetic radiation emitted by the sample, in the form of spectra is called emission spectroscopy (flame photometry and fluorimetry). Spectroscopy is useful for the study of atomic and molecular structure and used in the analysis of a wide range of samples. Atomic spectroscopy is the study of interaction of electromagnetic radiation with atoms, changes in energy takes place at atomic level. [17]

1.7 UV-VIS spectroscopy

It is also known as electronic spectroscopy; it is a type of absorption spectroscopy in which the amount of light absorbed at each wavelength of UV region [200-400nm] of electromagnetic spectrum is absorbed by the molecule is measure

PRINCIPLE-The principle of UV-spectroscopy is based on the absorption spectrum and 0it is based on the principle of Beer`s Lamberts law. [16, 17]



Absorption = Io - It

II. MATERIALS AND METHODS

2.1 METHOD -1

Use of 0.1N NaOH (Standard)

Solvent used 0.1N NaoH

PART-A:

Preparation of 0.1N NaOH

Weigh 4gm of sodium hydroxide pellets and transfer it into 1000ml of volumetric flask. Dissolve sodium hydroxide using distilled water and make the volume up to the mark using distilled water

PART-B

Stock solution -1

Weigh 10mg of pure drug and dissolve in 100ml volumetric flask using 0.1N NaOH and make up the volume up to the mark with 0.1N NaOH **(1000µg/ml)**

PART –C

Stock solution -2

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 1861-1876www.ijprajournal.com ISSN: 2249-7781

Take 10ml of stock solution -1 in 100ml of volumetric flask and make up the volume up to the mark using 0.1N NaOH(**100µg/ml)**

PART –D
DILUTIONS
Take 5ml ,6ml,7ml ,8ml and 9ml of stock solution -2 in 10ml of volumetric flask separately. make up the volume up to the mark(10ml) using 0.1N NaOH respectively.
50µg/ml,60µg/ml, 70µg/ml,80µg/ml,90µg/ml of series dilutions have been prepared.

PART –E
CALIBRATION CURVE
Take 50,60,70,80 and 90µg/ml individually for analysis and the readings have been record and calculated r2 value and plot the graph between concentration and absorbance. (fig:1)

PART-F
ACCURACY
For the accuracy determination we selected two dilutions one is highest and another one is lowest i.e. 50 µg/ml and 90 µg/ml.After that we calculated its absorbance and observed the spectrum between absorbance and wavelength for three times and determined its reproducibility. (fig:3)

PART –G
PRECISION
For the precision value we took two dilution series i.e. 50,60 .70 ,80,90 µg/ml and observed its spectrum by using UV probe and this procedure we repeated for two times in same day (intra- day precision) (fig:5) and next day (inter-day precision) (fig: 6). [12,13]

2.2 METHOD -2
Use of phosphate buffer 6.4 (Standard)
Solvents used is phosphate buffer 6.4

PART-A
Preparation of phosphate buffer solution pH 6.4
Dissolve 2.5gm of disodium hydrogen phosphate ,2.5 gm of sodium dihydrogen phosphate and 8.2gm of sodium chloride in 950ml of water adjust the Ph. of the solution to 6.4 with 1M sodium hydroxide or1M hydrochloric acid if necessary. Dilute to 100ml with water.

PART-B
STOCK SOLUTION -1
Weigh 10mg of pure drug and dissolve in 100ml of volumetric flask and make up the volume up to the mark using phosphate buffer 6.4**(1000µg/ml)**

PART-C
STOCK SOLUTION -2
Take 10ml of stock solution-1, and dissolve in 100ml of volumetric flask and make up the volume up to the mark using phosphate buffer 6.4**(100µg/ml)**

PART –D
DILUTIONS
Take 1ml ,2ml,3ml ,4ml and 5ml of stock solution -2 in 10ml of volumetric flask separately. make up the volume up to the mark(10ml) using 0.1N NaOH respectively.
10µg/ml,20µg/ml, 30µg/ml,40µg/ml,50µg/ml of series dilutions have been prepared.

PART –E
CALIBRATION CURVE
Take 10,20,30,40and 50µg/ml individually for analysis and the readings have been recorded and calculate r2 value and plot the graph between concentration and absorbance. (fig:9)

PART-F
ACCURACY
For the accuracy determination we selected two dilutions one is highest and another one is lowest i.e. 10 µg/ml and 50 µg/ml. After that we calculated its absorbance and observed the spectrum between absorbance and wavelength for three times and determined its reproducibility. (fig:11)

PART –G
PRECISION
For the precision value we took two dilution series i.e. 10,20 .30 ,40,50 µg/ml and observed its spectrum by using UV probe and this procedure we repeated for two times in same day (intra- day precision) (fig:13) and next day (inter-day precision) (fig:14). [10,11]

2.3 METHOD -3
Use of phosphate buffer 7.4 (Standard)
Solvents used is phosphate buffer 7.4

PART-A
Preparation of phosphate buffer solution pH 7.4

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 1861-1876www.ijprajournal.com ISSN: 2249-7781

Dissolve 2.5gm of disodium hydrogen phosphate,2.5 gm of sodium dihydrogen phosphate and 8.2gm of sodium chloride in 950ml of water adjust the pH of the solution to 6.4 with 1M sodium hydroxide or1M hydrochloric acid if necessary. Dilute to 100ml with water.

PART-B
STOCK SOLUTION -1

Weigh 10mg of pure drug and dissolve in 100ml of volumetric flask and make up the volume up to the mark using phosphate buffer 7.4**(1000µg/ml)**

PART-C
STOCK SOLUTION -2

Take 10ml of stock solution-1, and dissolve in 100ml of volumetric flask and make up the volume up to the mark using phosphate buffer 7.4**(100µg/ml)**

PART –D
DILUTIONS

Take 1ml ,2ml,3ml ,4ml and 5ml of stock solution -2 in 10ml of volumetric flask separately. make up the volume up to the mark(10ml) using phosphate buffer 7.4respectively.
10µg/ml,20µg/ml, 30µg/ml,40µg/ml,50µg/ml of series dilutions have been prepared.

PART –E
CALIBRATION CURVE

Take 10,20,30,40and 50µg/ml individually for analysis and the readings have been recorded and calculate r2 value and plot the graph between concentration and absorbance. (fig:17).

PART-F
ACCURACY

For the accuracy determination we selected two dilutions one is highest and another one is lowest i.e. 10 µg/ml and 50 µg/mlAfter that we calculated its absorbance and observed the spectrum between absorbance and wavelength for three times and determined its reproducibility(fig:19).

PART –G
PRECISION

For the precision value we took two dilution series i.e. 10,20 .30 ,40,50 µg/mland observed its spectrum by using UV probe and this procedure we repeated for two times in same day (intra- day precision) (fig:21) and next day (inter-day precision). [14,15]

2.4 METHOD-4
Use of 0.1N NaOH (Standard)
Solvent used 0.1N NaOH

PART-A
PREPARATION OF 0.1N NaOH
Weigh 4gm of sodium hydroxide pellets and transfer it into 1000ml of volumetric flask. Dissolve sodium hydroxide using distilled water and make the volume up to the mark using distilled water

PART-B
STOCK SOLUTION -1

Weigh 50mg of tablet powder of Methotrexate(10mg) and dissolve in 50ml volumetric flask using 0.1N NaOH and make up the volume up to the mark with 0.1N NaOH (1000µg/ml)

PART-C
STOCK SOLUTION- 2

Take 10ml of stock solution-1 in 100ml of volumetric flask and make up the volume up to the mark using 0.1N NaOH (100µg/ml)

PART-D
DILUTIONS

Take 5ml, 6ml, 7ml, 8ml and 9ml of stock solution-2 in 10ml of volumetric flask separately make up the volume up to the mark (10ml) using 0.1N NaOH respectively.
50µg/ml, 60µg/ml, 70µg/ml, 80µg/ml and 90µg/ml of series dilutions have been prepared

PART-E
CALIBRATION CURVE

Take 50,60,70,80 and 90µg/ml individually for analysis and the readings have been record and calculate R^2 value and plot the graph between Concentration and Absorbance. (fig:2)

PART-F
ACCURACY

For the accuracy determination we selected two dilutions one is lowest and another one is lowest i.e. 50µg/ml and 90µg/ml.

After that we calculate its absorbance and observed the spectrum between absorbance and

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 1861-1876www.ijprajournal.com ISSN: 2249-7781

wavelength for three times and determined its reproducibility(fig:4)

PART-G
PRECISION

For the precision value we took two dilution series i.e. 50,60,70,80 and 90µg/ml and observed its spectrum by using UV probe and this procedure we repeated for two times in same day (intra-day precision) (fig:7) and next day (inter-day precision) (fig:8). [5;6]

2.5 METHOD – 5
Use of Phosphate buffer 6.4 (Standard)
Solvents used is phosphate buffer 6.4

PART-A
Preparation of Phosphate buffer solution pH 6.4

Dissolve 2.5gm of disodium hydrogen phosphate, 2.5gm of sodium dihydrogen phosphate and 8.2gm of sodium chloride in 950ml of water adjust the pH of solution to 6.4 with 1M sodium hydroxide or 1M hydrochloric acid if necessary dilute to 100ml with water

PART-B
STOCK SOLUTION – 1

Weigh 0.05gm of market formulation of Methotrexate tablet(10mg) and dissolve in 50ml of volumetric flask and make up the volume up to the mark using phosphate buffer 6.4 (1000µg/ml)

PART-C
STOCK SOLUTION – 2

Take 10ml of stock solution-1, and dissolve in 100ml of volumetric flask and make up the volume up to the mark using phosphate buffer 6.4 (100µg/ml)

PART-D
DILUTIONS

Take 5ml, 6ml, 7ml, 8ml and 9ml of stock solution-2 in 10ml of volumetric flask separately make up the volume up to the mark using phosphate buffer 6.4 respectively.
50µg/ml, 60µg/ml, 70µg/ml, 80µg/ml, 90µg/ml of series dilutions have been prepared

PART-E
CALIBRATION CURVE

Take 50,60,70,80 and 90µg/ml individually for analysis and the readings have been recorded and calculate R^2 value and plot the graph between Concentration and Absorbance (fig: 10)

PART-F
ACCURACY

For the accuracy determination we selected two dilutions one is lowest and another one is highest i.e. 50µg/ml and 90µg/ml

After that we calculate its absorbance and observed the spectrum between absorbance and wavelength for three times and determined its reproducibility(fig:12).

PART-G
PRECISION

For the precision value we took two dilution series i.e. 50,60,70,80 and 90µg/ml and observed its spectrum by using UV probe and this procedure we repeated for two times in same day (intra-day precision) (fig:15) and next day (inter-day precision) (fig:16). [8,9]

2.6 METHOD – 6
Use of Phosphate buffer 7.4 (Standard)
Solvents used is phosphate buffer 7.4

PART-A
Preparation of Phosphate buffer solution pH 7.4

Dissolve 2.5gm of disodium hydrogen phosphate, 2.5gm of sodium dihydrogen phosphate and 8.2gm of sodium chloride in 950ml of water adjust the pH of solution to 6.4 with 1M sodium hydroxide or 1M hydrochloric acid if necessary dilute to 100ml with water

PART-B
STOCK SOLUTION – 1

Weigh 0.05gm of market formulation of Methotrexate tablet(7.5mg) and dissolve in 50ml of volumetric flask and make up the volume up to the mark using phosphate buffer 7.4(1000µg/ml)

PART C
STOCK SOLUTION – 2

Take 10ml of stock solution-1, and dissolve in 100ml of volumetric flask and make up the volume up to the mark using phosphate buffer 7.4 (100µg/ml)

PART D
DILUTIONS

Take 5ml, 6ml, 7ml, 8ml and 9ml of stock solution-2 in 10ml of volumetric flask separately

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 1861-1876www.ijprajournal.com ISSN: 2249-7781

make up the volume up to the mark using phosphate buffer 7.4 respectively.
50µg/ml, 60µg/ml, 70µg/ml, 80µg/ml, 90µg/ml of series dilutions have been prepared

PART E
CALIBRATION CURVE

Take 50,60,70,80 and 90µg/ml individually for analysis and the readings have been recorded and calculate R^2 value and plot the graph between Concentration and Absorbance (fig:18).

PART-F
ACCURACY

For the accuracy determination we selected two dilutions one is lowest and another one is highest i.e. 50µg/ml and 90µg/ml

After that we calculate its absorbance, observed the spectrum between absorbance and wavelength for three times, and determined its reproducibility(fig:20).

PART-G
PRECISION

For the precision value we took two dilution series i.e. 50,60,70,80 and 90µg/ml and observed its spectrum by using UV probe and this procedure we repeated for two times in same day (intra-day precision)(fig:22) and next day (inter-day precision)(fig:23). [6,7]

III. RESULT AND DISCUSSION

3.1 CALIBRATION CURVE OF STANDARD (0.1 N NaoH)

Calibration curve was plotted between concentration an absorbance by using the dilution series of 50, 60,70, 80, 90 µg/ ml and observe its r^2 value and slope value. [

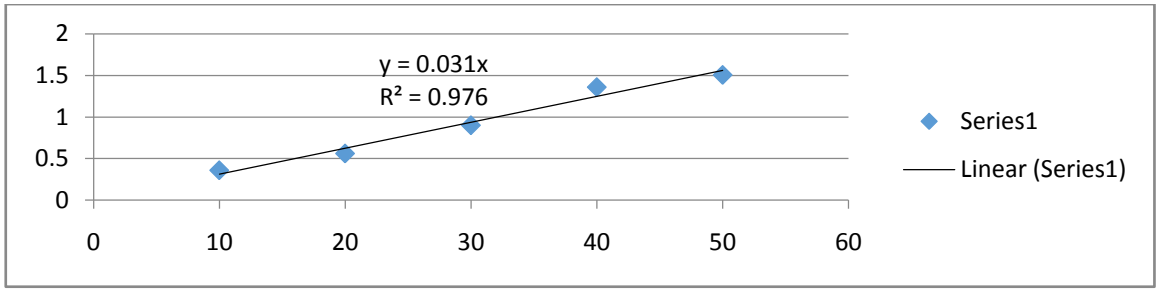


Figure 1 Calibration curve of Std drug by using solvent 0.1N NaOH

3.2 CALIBRATION CURVE OF MARKET FORMULATION (0.1N NaOH)

Calibration curve was plotted between concentration an absorbance by using the dilution series of 50, 60,70, 80, 90 µg/ ml and observed its r^2 value and slope value.

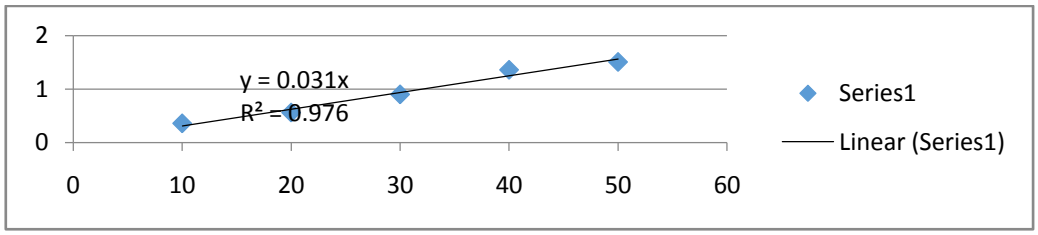


Figure 2 Calibration curve of market drug by using solvent 0.1N NaOH

3.3 ACCURACY OF STANDARD DRUG ((0.1N NaOH)

The spectra were observed between absorbance and wavelength. The highest 90µg/ml and the lowest 50µg/ml was chosen to check the accuracy.

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 1861-1876www.ijprajournal.com ISSN: 2249-7781

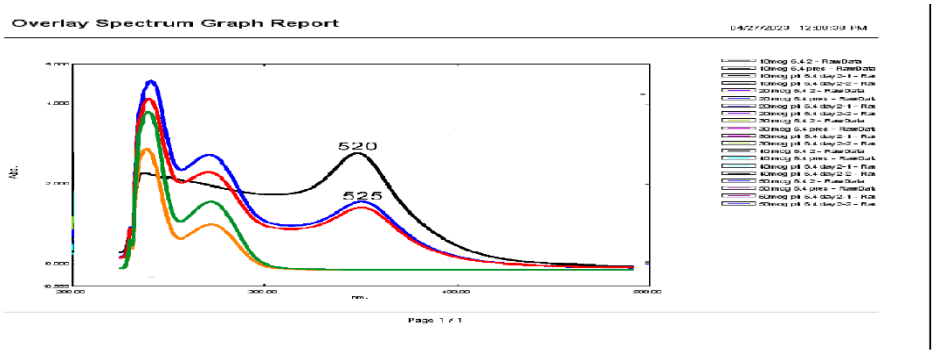


Figure 3 ACCURACY OF STANDARD DRUG ((0.1N NaOH)

3.4ACCURACY OF MARKET FORMULATION ((0.1N NaOH)

The spectra were observed between absorbance and wavelength. The highest 90µg/ml and the lowest 50µg/ml was chosen to check the accuracy.

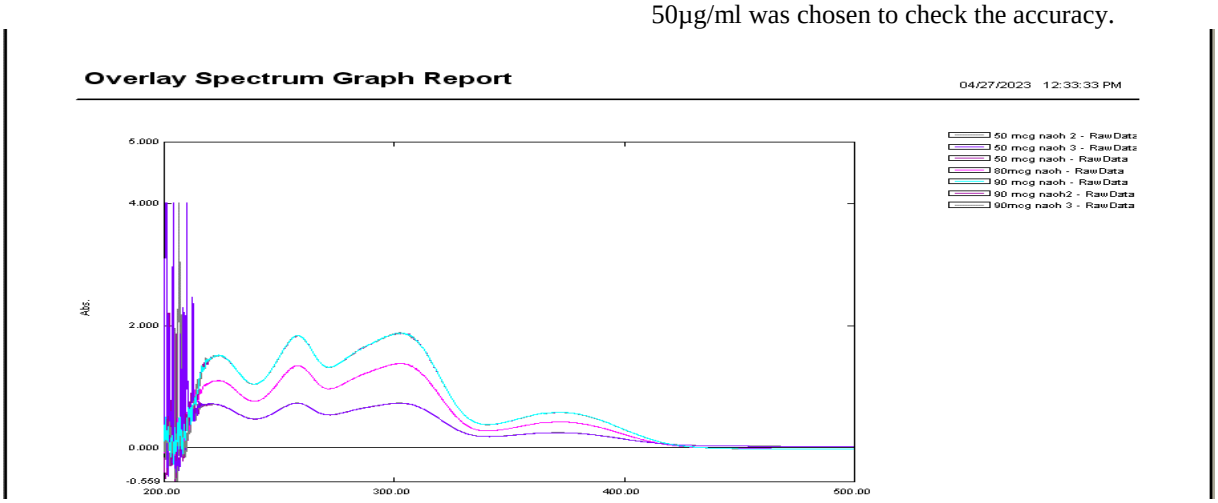


Figure 4 ACCURACY OF MARKET FORMULATION ((0.1N NaOH)

3.5 PRECISION OF STANDARD DRUG (0.1 N NaOH)

The spectra were observed between wavelength and absorbance for both inter and intraday precision. [12,13]

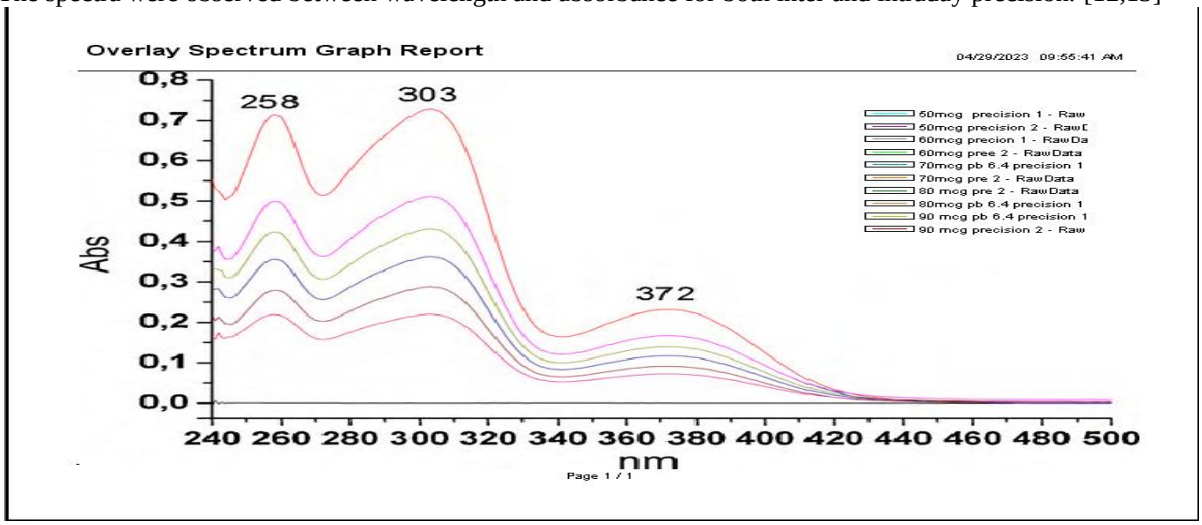


Figure 5 PRECISION OF STANDARD DRUG (0.1 N NaOH) day 1

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 1861-1876www.ijprajournal.com ISSN: 2249-7781

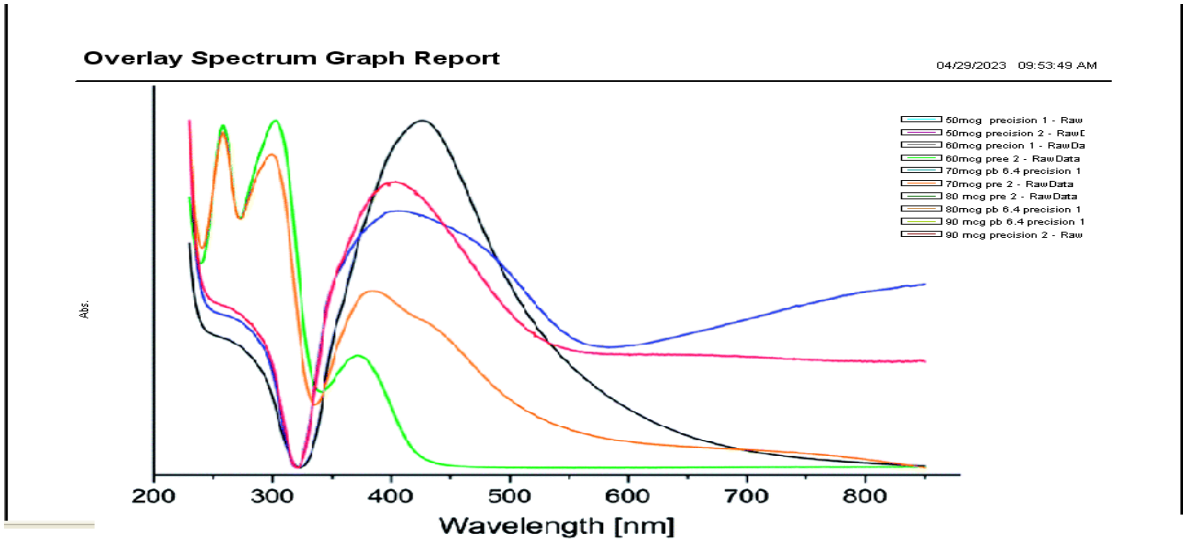


Figure 6Day 2 PRECISION OF STANDARD DRUG (0.1 N NaOH)

3.6PRECISION OF MARKET FORMULATION (0.1 N NaOH)

The spectra were observed between wavelength and absorbance for both inter and intraday precision

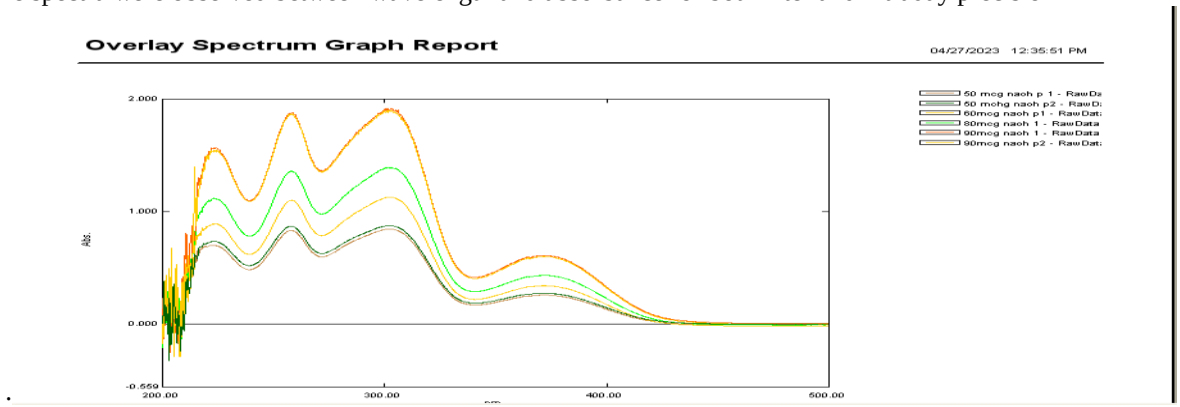


Figure 7 Day 1 Precision of NaOH

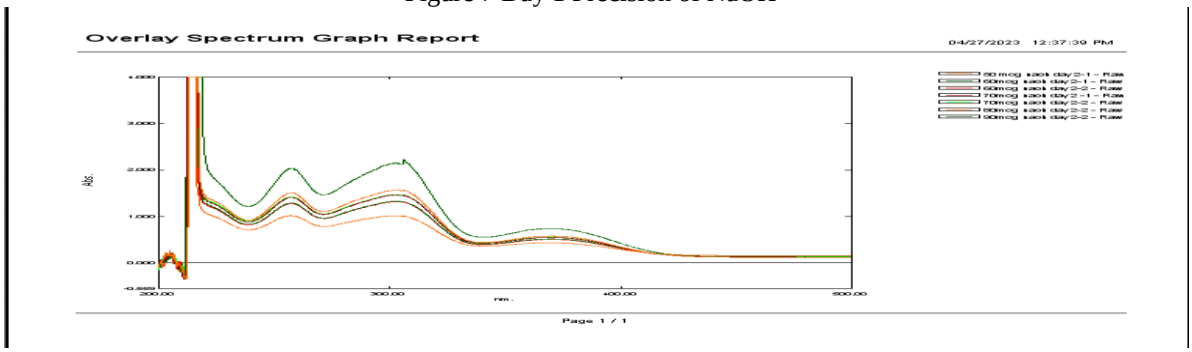


Figure 8 Day 2 Precision of NaOH

3.7 CALIBRATION CURVE OF STANDARD (PHOSPHATE BUFFER 6.4)

Calibration curve was plotted between concentration an absorbance by using the dilution series of 10, 20,30, 40, 50 µg/ ml and observe its r^2 value and slope value.

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 1861-1876www.ijprajournal.com ISSN: 2249-7781

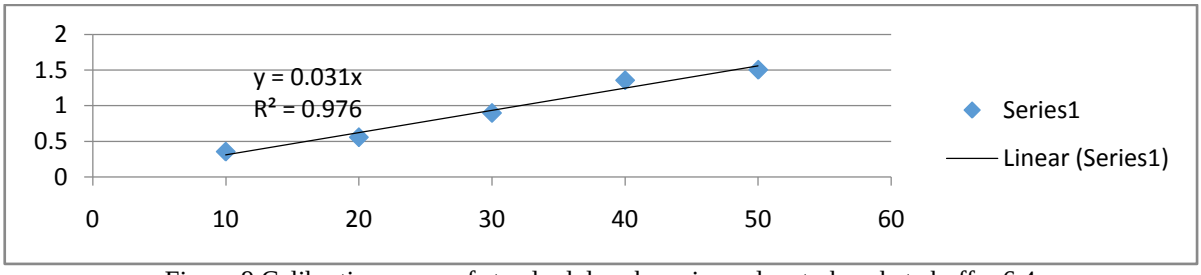


Figure 9 Calibration curve of standard drug by using solvent phosphate buffer 6.4

3.8CALIBRATION CURVE OF MARKET FORMULATION (PHOSPHATE BUFFER 6.4)

Calibration curve was plotted between concentration an absorbance by using the dilution series of 50, 60, 70, 80, 90 µg/ ml and observe its r^2 value and slope value.

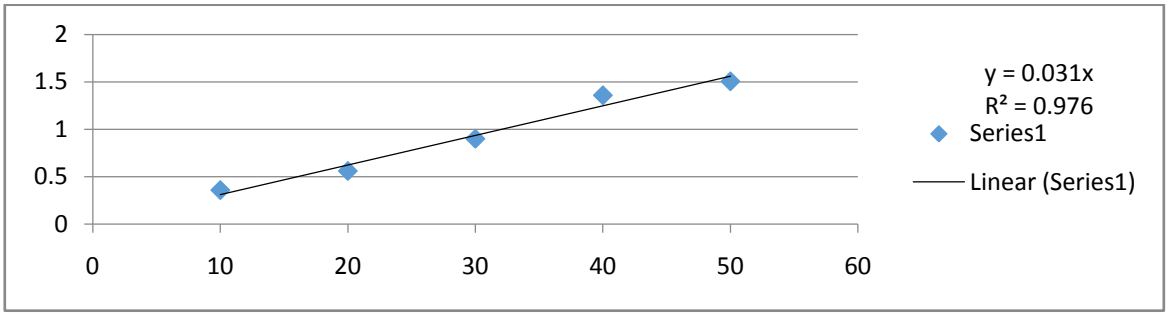


Figure 10 Calibration curve of market drug using solvent phosphate buffer 6.4

3.9 ACCURACY OF STANDARD DRUG (PHOSPHATE BUFFER 6.4)

The spectra were observed between absorbance and wavelength. The highest 50µg/ml and the lowest 10µg/ml was chosen to check the accuracy.

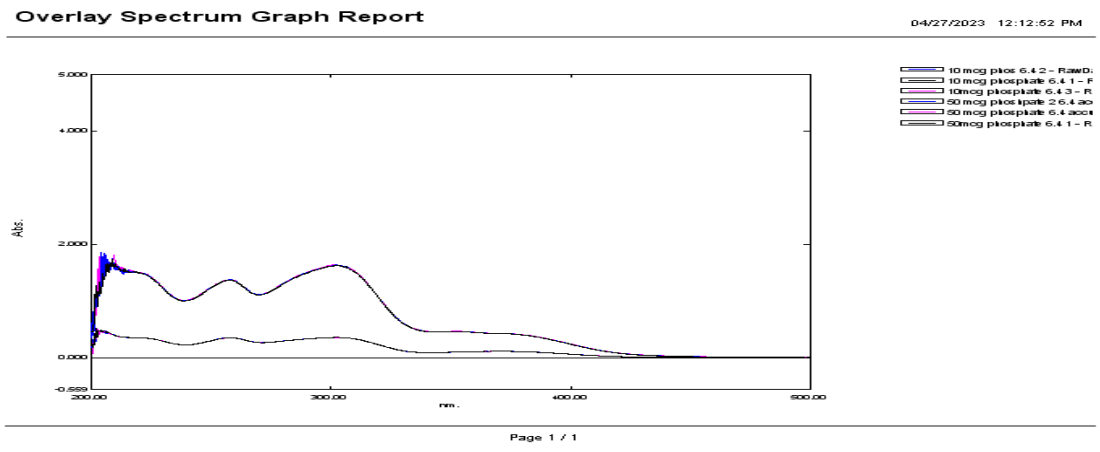


Figure 11 ACCURACY OF STANDARD DRUG (PHOSPHATE BUFFER 6.4)

3.10 ACCURACY OF MARKET FORMULATION (PHOSPHATE BUFFER 6.4)

The spectra were observed between absorbance and wavelength. The highest 90µg/ml and the lowest 50µg/ml was chosen to check the accuracy.

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 1861-1876www.ijprajournal.com ISSN: 2249-7781

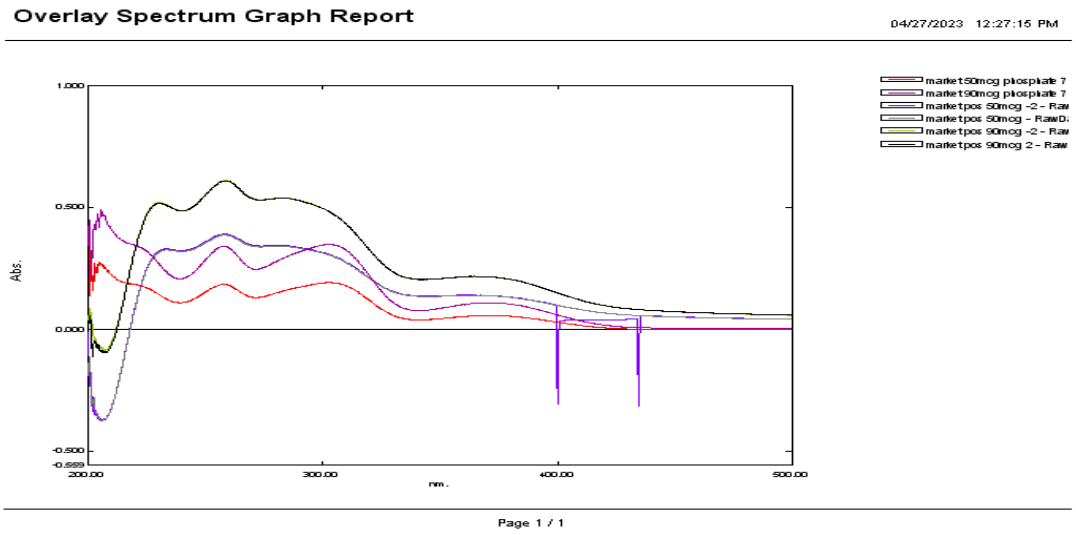


Figure 12 ACCURACY OF MARKET FORMULATION (PHOSPHATE BUFFER 6.4)

3.11 PRECISION OF STANDARD DRUG (PHOSPHATE BUFFER 6.4)

The spectra were observed between wavelength and absorbance for both inter and intraday precision. [5,6]

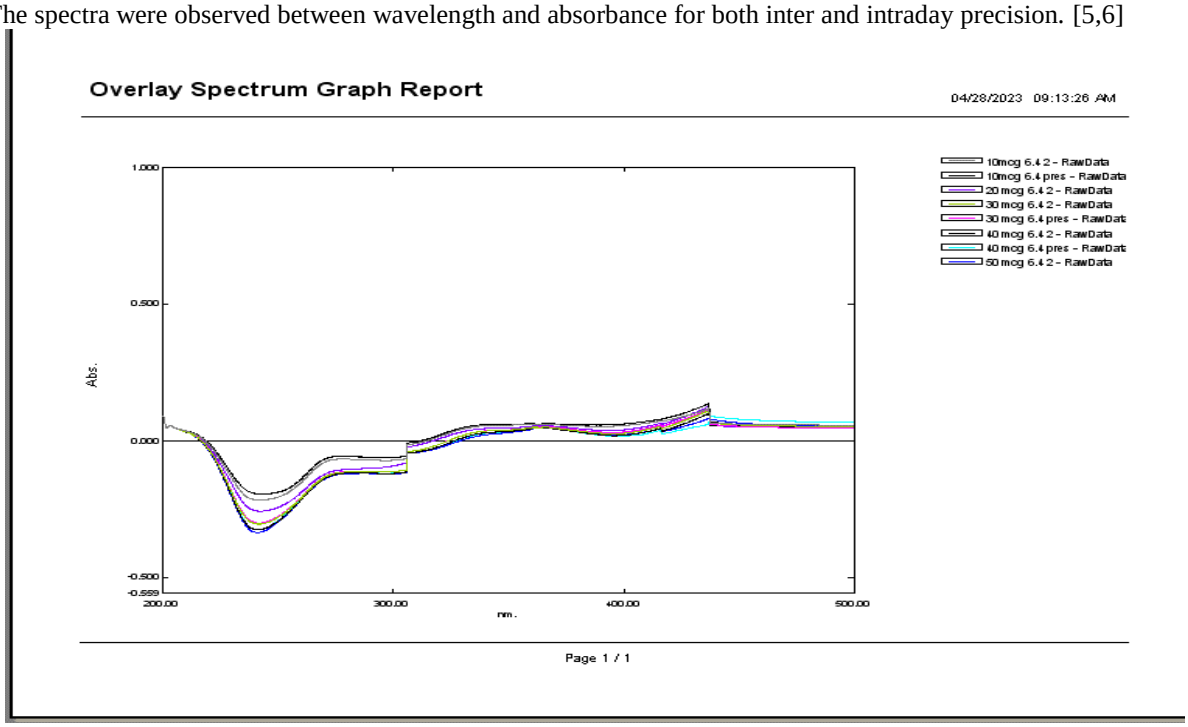


Figure 13 PRECISION OF STANDARD DRUG (PHOSPHATE BUFFER 6.4) day-1

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 1861-1876www.ijprajournal.com ISSN: 2249-7781

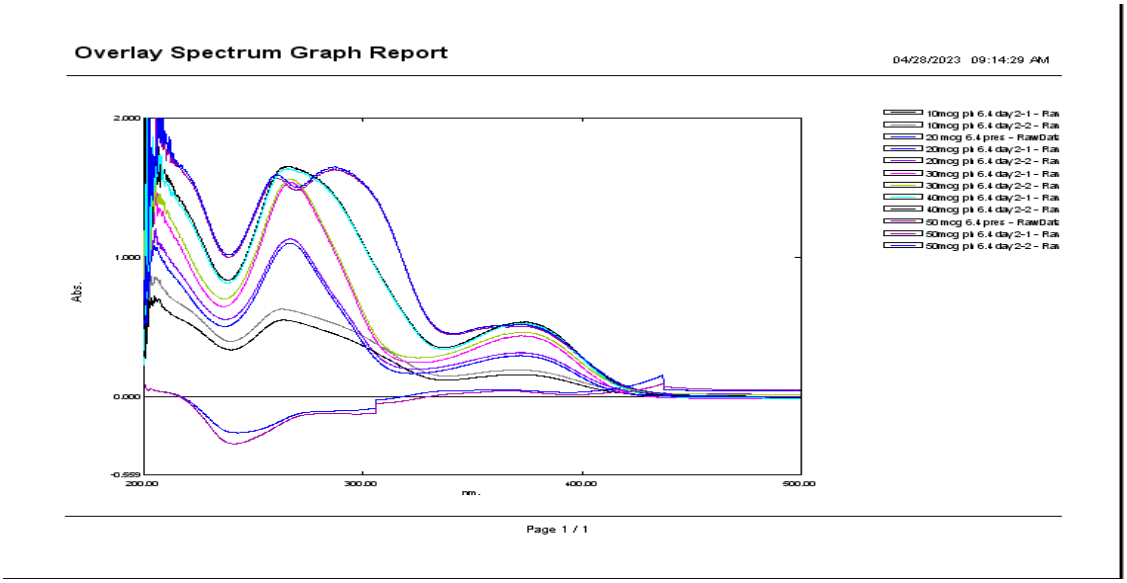


Figure 14 PRECISION OF STANDARD DRUG (PHOSPHATE BUFFER 6.4) day-2

3.12 PRECISION OF MARKET FORMULATION (PHOSPHATE BUFFER 6.4)

The spectra were observed between wavelength and absorbance for both inter and intraday precision. [8,9]

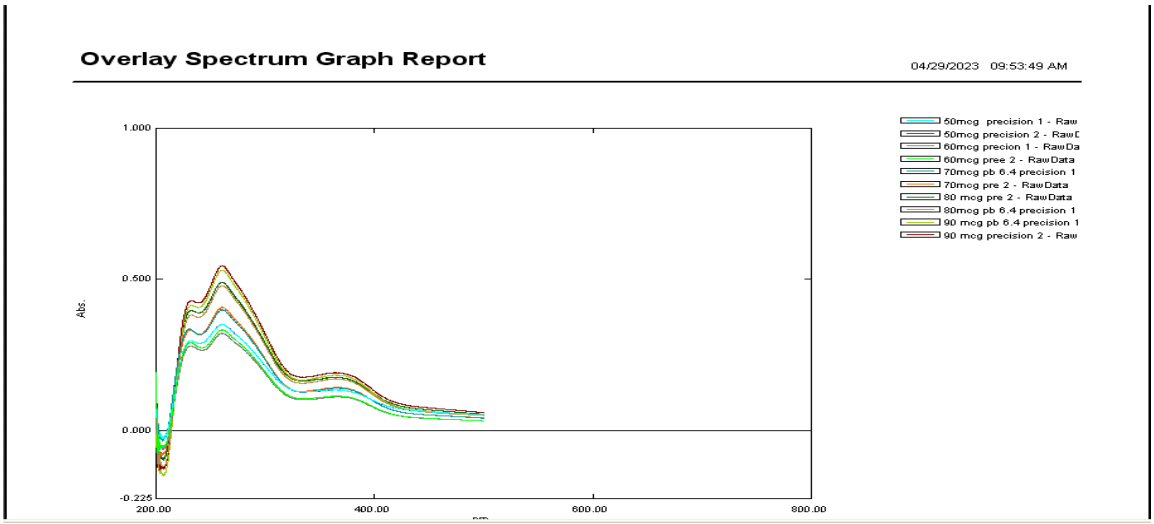


Figure 15 PRECISION OF MARKET FORMULATION (PHOSPHATE BUFFER 6.4) Day 1

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 1861-1876www.ijprajournal.com ISSN: 2249-7781

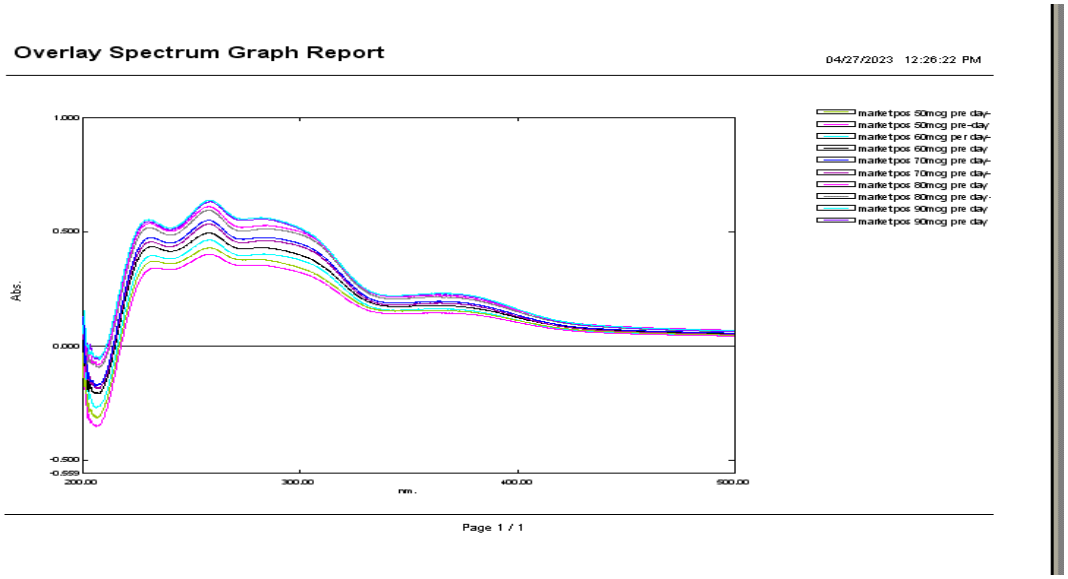


Figure 16 PRECISION OF MARKET FORMULATION (PHOSPHATE BUFFER 6.4) Day 2

3.13 CALIBRATION CURVE OF STANDARD (PHOSPHATE BUFFER 7.4)

Calibration curve was plotted between concentration an absorbance by using the dilution series of 10, 20,30 , 40, 50 µg/ ml and observe its r^2 value and slope value.

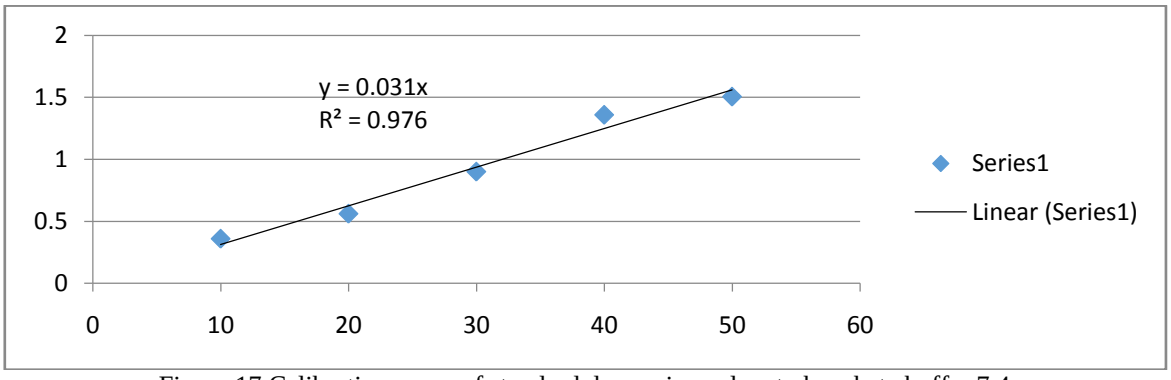


Figure 17 Calibration curve of standard drug using solvent phosphate buffer 7.4

3.14 CALIBRATION CURVE OF MARKET FORMULATION (PHOSPHATE BUFFER 7.4)

Calibration curve was plotted between concentration an absorbance by using the dilution series of 50, 60, 70, 80, 90 µg/ml and observe its r^2 value and slope value.

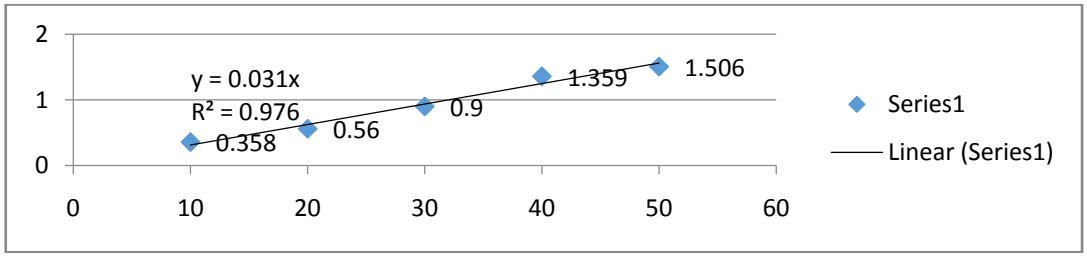


Figure 18 Calibration curve of market formulation using solvent phosphate buffer 7.4

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 1861-1876www.ijprajournal.com ISSN: 2249-7781

3.15 ACCURACY OF STANDARD DRUG (PHOSPHATE BUFFER 7.4)

The spectra were observed between absorbance and wavelength. The highest 50µg/ml and the lowest 10µg/ml was chosen to check the accuracy.

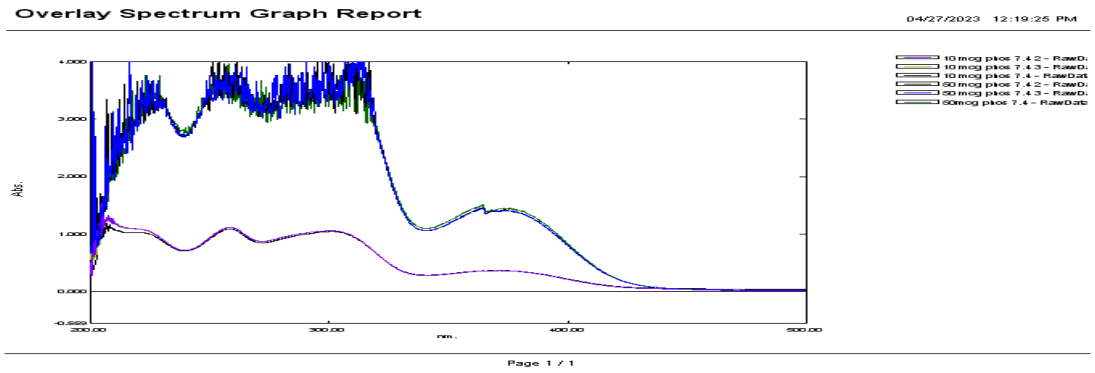


Figure 19 ACCURACY OF STANDARD DRUG (PHOSPHATE BUFFER 7.4)

3.16 ACCURACY OF MARKET FORMULATION (PHOSPHATE BUFFER 7.4)

The spectra were observed between absorbance and wavelength. The highest 90µg/ml and the lowest 50µg/ml was chosen to check the accuracy.

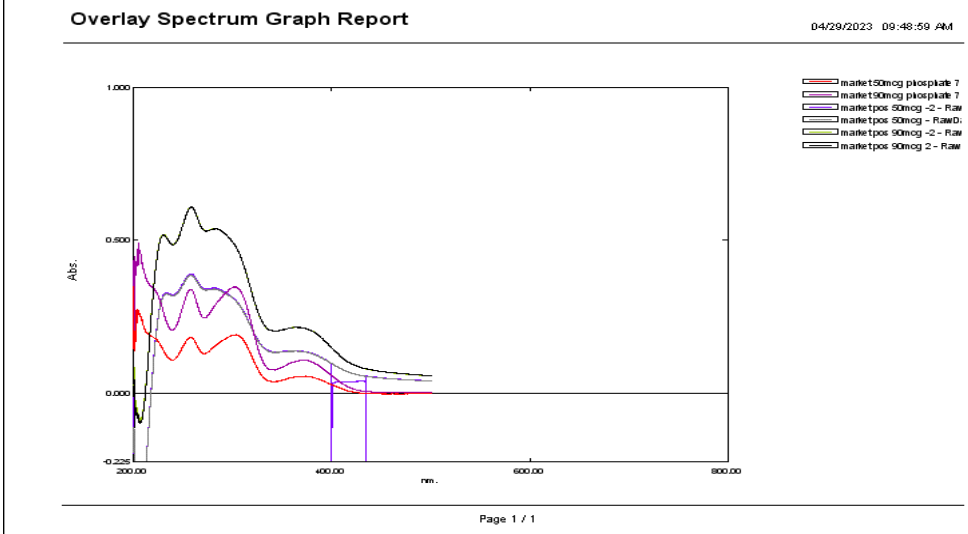


Figure 20 ACCURACY OF MARKET FORMULATION (PHOSPHATE BUFFER 7.4)

3.17 PRECISION OF STANDARD DRUG (PHOSPHATE BUFFER 7.4)

The spectra were observed between wavelength and absorbance for both inter and intraday precision. [14,15]

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 1861-1876www.ijprajournal.com ISSN: 2249-7781

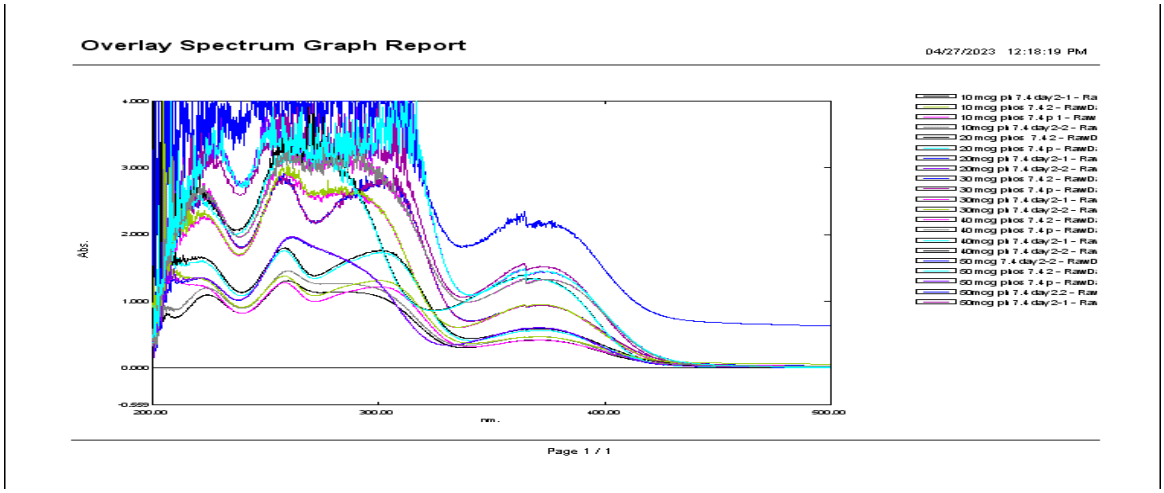


Figure 21 PRECISION OF STANDARD DRUG (PHOSPHATE BUFFER 7.4)

3.17 PRECISION OF MARKET FORMULATION (PHOSPHATE BUFFER 7.4)

The spectra were observed between wavelength and absorbance for both inter and intraday precision. [14,15]

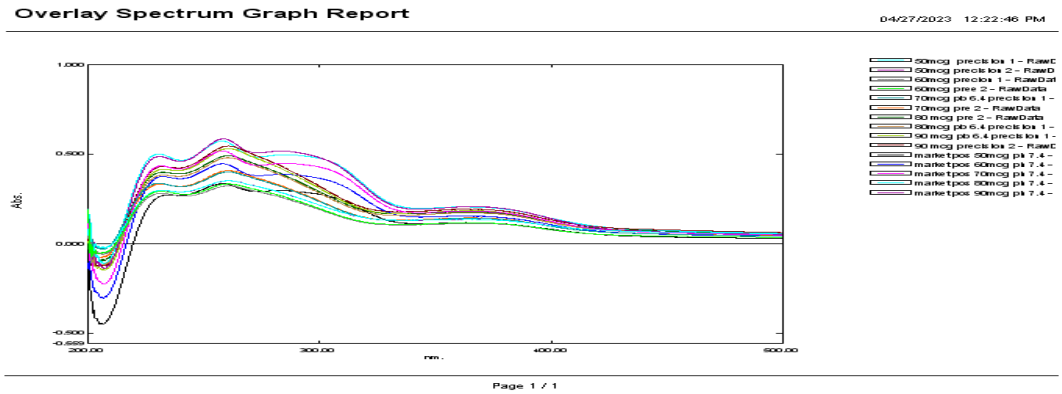


Figure 22 PRECISION OF MARKET FORMULATION (PHOSPHATE BUFFER 7.4) Day 1

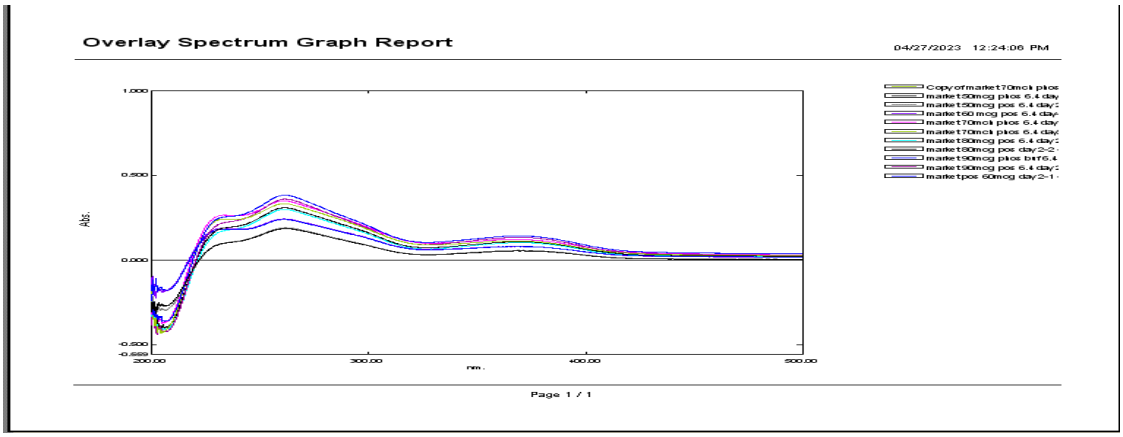


Figure 23 PRECISION OF MARKET FORMULATION (PHOSPHATE BUFFER 7.4) Day 2

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 4 July-Aug 2023, pp: 1861-1876www.ijprajournal.com ISSN: 2249-7781

IV. CONCLUSION:

Simple, specific, precise, accurate and economic method was developed by using UV – Visible spectrophotometer. Several analytical methods were developed by using UV –Visible spectrophotometer of different models. The method was developed by studying various research and review paper of last five years and following ICH guidelines. In the process of method development, we observed the various parameters like regression coefficient, precision, accuracy etc. by using solvents like sodium hydroxide, phosphate buffer of different paper. Till now what paper we studied that the methotrexate is sensitive to basic medium but while developing the method we concluded it is stable in acidic medium also but for shorter duration means it is not sensitive to inter-day precision i.e. observing negative absorbance value. This method took about 20 minutes for each dilution series and observed various parameters like precision, accuracy, standard deviation. In future, this method will be helpful in the qualitative, quantitative analysis and stability studies of methotrexate by using solvents in different p H medium.

V. ACKNOWLEDGEMENT

A heartfelt THANKS...

The work presented in this thesis would not have been possible without my close association with many people. I take this opportunity to extend my sincere gratitude and appreciation to all those who made this B-pharmacy project possible.

First and foremost, I would like to express my sincere and hearty gratitude, to my respected guide,Nargundcollege of Pharmacy, DattatreyaNagar, Bangalore, Karnataka, Dept. of Pharmaceutical Analysis, for introducing me to this exciting field of science and for the continuous support of my project related research, for her dedicated help, advice, patience, motivation, and immense knowledge. Her guidance helped me in all the time of research and conducting of this project. I could not have imagined having a better advisor and mentor for my B-pharmacy study, and thanks to lab assistant. Special thanks to the drug control department for providing us the pure form of the drug for conducting the research.we would heartfully thank our beloved m pharm students and our fellow mates for their continuous support we could have not imagined the success of this project without them.

REFERENCES:

[1]. Lewis medical surgical, nursing
[2]. Sharma A, Saini S; Process Validation of Solid Dosage Form: A Review. International Journal of Research in Pharmacy and Science, 2013; 3 (2): 12-30.
[3]. . Kaur H, Singh G, Seth N; Pharmaceutical Process Validation: A Review. 2013; 3 (4): 189-194.
[4]. Thaduvai R, Rao BS, Jeybaskaran M; Process Validation of Pantoprazole 40 mg Tablets: The Pharma Innovation. 2012; 1 (5): 50-52.
[5]. Kavita, Khurana G, Chaudhary S; Process Validation of Solid Dosage Form: A Review. Pharma Science Monitor, An International Journal of Pharmaceutical Sciences, September 2013; 4 (4): 390-391.
[6]. Chapman K. G: A History of Validation in the United States, Part I, Pharma Technology. November 1991; 39-98
[7]. Nash R. A., Wachter A. H: In Pharmaceutical Process Validation. Third Edition; Revised and Expanded, Marcel Dekker Inc., New York, 1993.
[8]. WHO Expert Committee on Specifications for Pharmaceutical Preparations. WHO technical report, series no. 863 – 34th report, Annex 6 –GMP: Guidelines on the validation of manufacturing processes: 4-7.
[9]. ICH Q7A Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients: 33.
[10]. Potdar MA; Current Good Manufacturing Practices for Pharmaceuticals. 2nd Edition, PharmaMed Press Publication, Delhi, December 2007: 8.2-8.3.
[11]. Validation of Solid Dosage Forms the FDA view, 1989; 15(6-7): 1119-1133.
[12]. Folsom AR,DemissieZ,Harnack L:Guidelines for Process Validation of Pharmaceutical Dosage Forms. Saudi Food and Drug Authority, Kingdom of Saudi Arabia, 2010; 9-15.
[13]. Validation Guidelines for Pharmaceutical Dosage Form; Health products and Food branch Inspectorate, Canada, December 2009; 8-13.
[14]. www.processvalidation.com.
[15]. Von Doehren PJ, St. John; An Approach to the Characterization and Technology

Transfer of Solid Dosage Form Processes: Pharma Technology, 1982; 6 (9): 139–156.

[16]. Patel RC, Bhuva CK; Pharmaceutical Process Validation: Pharmatutor, ART - 105

[17]. DIPLI MATOLE,HRISHIKESH H RAJPUT "ULTRAVIOLET SPECTROSCOPY