By the name of Allah

Sudan University of Science and Technology

College of Science

Scientific Laboratories Department-Chemistry

A complementary research for BA (HONS):

Development and Validation of Spectrophotometric Method for Determination of Penicillamine (PA) in Pharmaceutical Formulation Using 4-Chloro-7-Nitrobenzo-2-Oxa-1, 3-Diazol (NBD-Cl)

By:

- Maaza Alnazeer Babikir.
- Mariam Abd-almonem Abd-allah.
- Mohammed Hamid Ahmed.

Supervisor:

Amira Anwar Babikir

2015-1437

الآيـة

﴿وقُلْ رَبِي زِدْنِي عِلْماً﴾

سورة طه

(الآية 114)

Dedication

To my parents.

Who inspired me with faith in Allah, love and respect towards all people

and sincerity in work.

To my Friends

for their sacrifice and encouragement.

Acknowledgment

Thanks to Allah

First I would like to express my thanks to my supervisor Amira Anwar Babikir, for

her interest in the research project and guidance and help during the research.

My thanks extend to the technicians of University of Khartoum laboratories (shambat).

مستخلص البحث:

تم إقتراح طريقة طيفية بسيطة وحساسة لتحديد (D-Penicillamine) كمركب دوائي. تقوم هذه الطريقة على أساس ناتج الإضافة البني-الأرجواني لتفاعل (PA) مع 4-كلورو-7-نيتروبنزو-2-اوكسا-3,1-ديازول (NBD-Cl)(4-chloro-7-nitrobenzo-2-oxa-1, 3-diazole) في وسط قاعدي (pH=10.5), في مدى إمتصاصية قصوى (λ_{max}) 465nm. تحت ظروف التفاعل المثلى, كانت الطريقة في علاقة خطية مع التركيز في مدى تركيز 1-15µg. تم تطبيق الطريقة بنجاح لإيجاد تركيز PA في شكل جرعات الدوائية. وكانت النتائج متقاربة مع نتائج الطريقة الرسمية لدستور الولايات المتحدة الأمريكية للأدوية. هذه الطريقة مفيدة للتحليل الروتيني للبنيسلامين (PA) في مختبرات ضبط الجودة.

Abstract:

A sensitive and simple spectrophotometric method has been proposed for the determination of D-Penicillamine (PA) in pharmaceutical formulation. The proposed method is based on the reaction between the PA and 4-chloro-7-nitrobenzo-2-oxa-1, 3-diazole (NBD-Cl) at alkaline medium (pH=10.5) to form deep brown-purple adduct, exhibiting maximum absorption (λ_{max}) at 465nm. Under optimized reaction condition, the method was linear in the concentration rage 1-15µgmL^{-1}. The method was applied successfully to the determination if PA in pharmaceutical dosage form. The results were in a good agreement with the official USP method. The method is useful for routine analysis of PA in quality control laboratories.

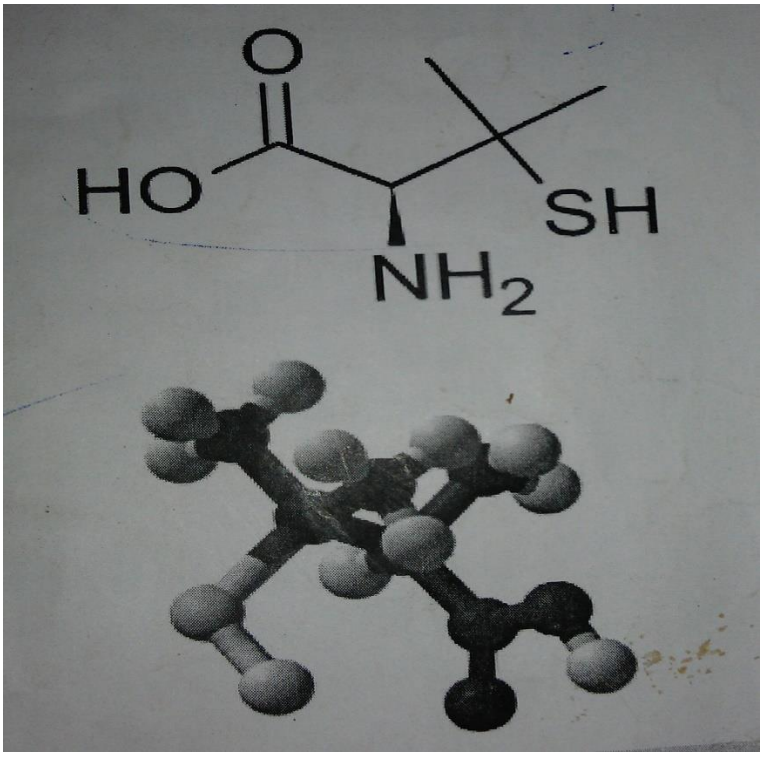
Contents:

Chapter one

Theoretical section

By the name of Allah

1 -1Penicillamine:



1-1-1 Systematic name:

(2S)-2-amino-3-methyl-3-sulfanyl-butanoic acid.

1-1-2 Penicillamine definition:

It is a pharmaceutical of the chelator class[1]. It has been sold under the trade names of **Cuprimine** and **Depen**. The pharmaceutical form is *D-Penicillamine, as L-Penicillamine* is toxic (it inhibits the action of

pyridoxine)[2]. It is an α-amino acid metabolite of penicillin, although it has no antibiotic properties[3].

1-1-3 Clinical data:

Trade names	Cuprimine
AHFS/Drug.com	monograph
Pregnancy category	- AU: D. - US: D (Evidence of risk)
Routes of administration	Oral

1-1-4 Chemical data:

Formula	$C_5H_{11}NO_2S$
Molecular mass	149.212 g/mol

1-1-5 prescription drug:

- Treats Wilson's disease (too much copper in your body) and rheumatoid arthritis. Also helps to prevent kidney stones in people who have cystinuria (too much of the amino acid cysteine in your urine).

  Side effects –Warnings –How to use

National Library of medicine.

- Brand names: Cuprimine, Depen.
- Molar mass: 149.212 g/mol.
- Pregnancy risk: Category D (Positive evidence of risk).
- May treat: Rheumatoid arthritis, Cystinuria, Lead poisoning, Mercury poisoning, copper toxicity, Biliary cirrhosis.
- Drug class: Antirheumatic Agent.
- Other drugs in same class: Hydroxychloroquine, Leflunomide, Hydroxychloroquine sulfate.

It is on the World Health Organization's List of Essential Medicines, a list of the most important medication needed a basic health system.

1-1-6 Uses:

This medication is used to treat rheumatoid arthritis, Wilson's disease (a condition in which high levels of copper in the body cause damage to the liver, brain and other organs), and a certain disorder which cause kidney stones (cystinuria). For the treatment of rheumatoid arthritis, Penicillamine is known as a disease-modifying Antirheumatic drug (DMARD). It helps to decrease pain/tenderness/swelling in the joints. For the treatment of Wilson's disease, Penicillamine binds to copper and helps it to be removed from the body.

Decreasing copper levels helps to improve liver function and the mental/mood/nerve problems (such as confusion, difficulty speaking/walking) caused by the disease. For the treatment of cystinuria,

Penicillamine helps to decrease the amount of certain substance (cysteine) in the urine which can cause kidney stones.

1-1-7 Medical Uses:

Penicillamine is used as a form of immunosuppression to treat rheumatoid arthritis. It works by reducing numbers of T-lymphocytes, inhibiting macrophage function, decreasing IL-1, decreasing rheumatoid factor, and preventing collagen from cross-linking[5].

It is used as chelating agent:

- In Wilson's disease a rare genetic disorder of copper metabolism, Penicillamine treatment relies on its binding to accumulated copper and elimination through urine[1].
- In cystinuria, a hereditary disorder featuring formation of cysteine stones, Penicillamine binds with cysteine to yield a mixed disulfide which is more soluble than cystine[6].
- Penicillamine has been used to treat scleroderma[7].
- Penicillamine was the second line treatment for arsenic poisoning, after dimercaprol (BAL)[8]. It is no longer recommended.

1-1-8 Adverse effects:

Bone marrow suppression, dysgeusia, anorexia, vomiting and diarrhea are the most common side effect, occurring in ~20-30% of the patients treated with Penicillamine[5][10].

Other possible adverse effects include:

- Nephropathy[5][6].
- Hepatotoxicity[11].
- Membranous glomerulonephritis[12].
- A plastic anemia (idiosyncratic)[11].
- Antibody-mediated myasthenia gravis[5] and Lambert-Eaton myasthenic syndrome, which may persist even after is withdrawal.
- Drug-induced systemic lupus erythematosus[13].
- Elastosis perforans serpiginosa[14].
- Toxic myopathies[15].
- Unwanted breast growth[16].

Besides, people allergic to penicillin may have hypersensitivity to Penicillamine.

1-1-9 Penicillamine side effects:

If you experience any of the following serious side effects seek emergency medical attention or contact your doctor immediately:

- An allergic reaction (shortness of breath; closing of your throat; difficulty breathing; swelling of your lips, face, or tongue; or hives).
- Fever or chills.
- A sore throat.
- Unusual bleeding or bruising.
- Blood in the urine.
- Unexplained shortness of breath, coughing, or wheezing.
- Abdominal pain.
- Yellow skin or eyes.

- Muscle weakness.
- Double vision.

Otherwise, less serious side effects may be more likely to occur. Continue to take Penicillamine and notify your doctor if you experience

- Itching or a rash.
- Nausea, vomiting, diarrhea, or decreased appetite.
- Ringing in ears.
- Decreased taste.
- Sores in the mouth.
- Poor wound healing.
- Increased wrinkling of the skin.

Side effects other than those listed here may also occur. Talk to your doctor about any side effect that seems unusual or that is especially bothersome. You may report side effects to **FDA** at **1-800-FDA-1088**.

1-1-10 Penicillamine Pregnancy Warnings:

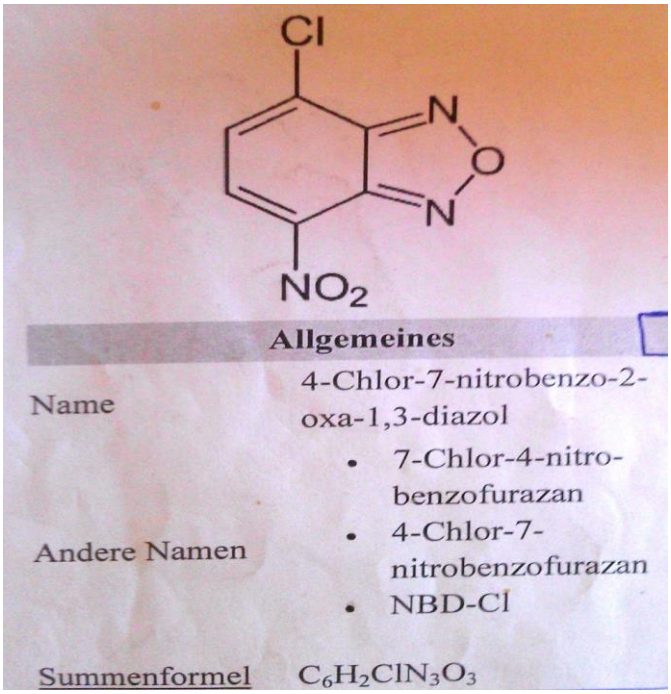
Penicillamine has been assigned to pregnancy category D by the **FDA**.

Penicillamine can cause fetal harm when administered to a pregnant woman.

Penicillamine has been shown to be empryotoxic and teratogenic in rats. There are no controlled data in human pregnancy. Although normal outcomes have been reported, characteristics congenital cutis laxa and associated birth defects have been reported, in infants born of mothers who received therapy with Penicillamine during pregnancy. Several conflicting

recommendations have appeared in the literature concerning the use of Penicillamine during pregnancy. Although evidence is incomplete, maintaining the daily dose at 500mg or less may reduce the incidence of Penicillamine-induced toxicity in the newborn. The manufacturer recommends, however, that the daily dose be limited to 750 mg and, if cesarean section is planned, the dose should be limited to 250 mg/day for 6 weeks before delivery and postoperatively until wound healing is complete.

1-2 NBD-Cl Indicator:



1-2-1 Properties:

Property	
Related	Biochemicals and Reagents, Building Blocks, Chemical Synthesis.
Categories	Derivatization agents, Fluorescent Probes, Labels, Particles and Stains.
Assay	98%.
M.P	97-99◦C (lit.).
Solubility	Chloroform: soluble 50mg/mL, clear to slightly hazy, faintly yellow to yellow.

1-2-2 Applications:

4-chloro-7-nitrobenzofurazan (NBD-chloride) was used in the following studies:

- Synthesis of fluorescent phospholipid-derivative, NBD-didecanoylphosphoatidylethanolamine.
- Synthesis of functionalized hydroxynaphtofurazan.
- Spectrophotometric and spectroflourometric determination of clementine hydrogen fumarate, loratadine, losartan potassium and ramipril in pharmaceutical formulations.
- Synthesis of 7-nitrobenzofurazan (NBD)-labeled maleimide, via Diels-Alder reaction.

1-2-3 General description:

4-chloro-7-nitrobenzofurazan is a highly sensitive chromogenic and fluorogenic reagent. It is reported to react spontaneously with *Trans*-1-methoxy-3-(trimethylsilyloxy)-1, 3-butadiene (Danishefsky's diene), to afford regioselectively the silyl enol ether, via normal electron-demand Diels-Alder (NEDDA) reaction.

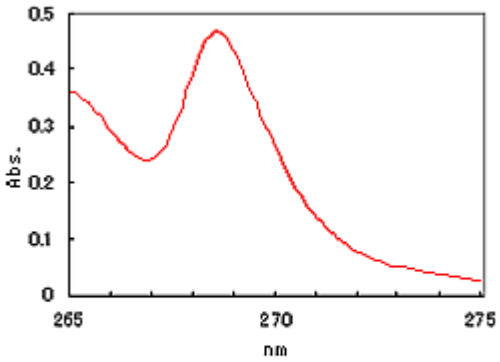
1-3 UV-1800

Successor of UV-1650, 1700: the new UV-1800 UV-VIS spectrophotometer achieves a resolution of 1 nm, the highest in its class, in a compact design.

Designed in accordance with the governing Japanese And European Pharmacopoeia, the new UV-1800 UV-VIS Spectrophotometer achieves a resolution of 1 nm, the highest in its class, in a compact design.

Offering an array of user-friendly features, the UV-1800 can be Used either as a stand-alone instrument or as a PC-controlled Instrument.

High resolution – 1 nm

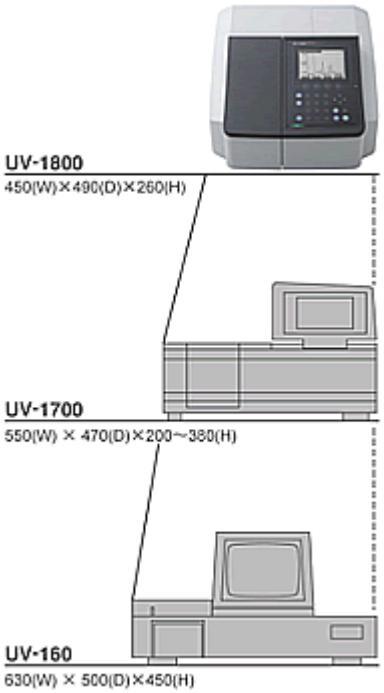


Toluene/hexane solution
Materials on the EP list are analyzed by UV in a confirmation test.
Red line: UV-1800/resolution:1nm

Featuring the highest resolution in its class*, the UV-1800 easily satisfies the standards of wavelength resolution demanded by the European Pharmacopoeia. Additionally, using a spectroscope with a Czerny-Turner mounting results in a compact, bright optical system. Stray light, wavelength repeatability and baseline stability have also been advanced to meet user requirements.

*As of March 2007, according to Shimadzu research.

Space-saving design

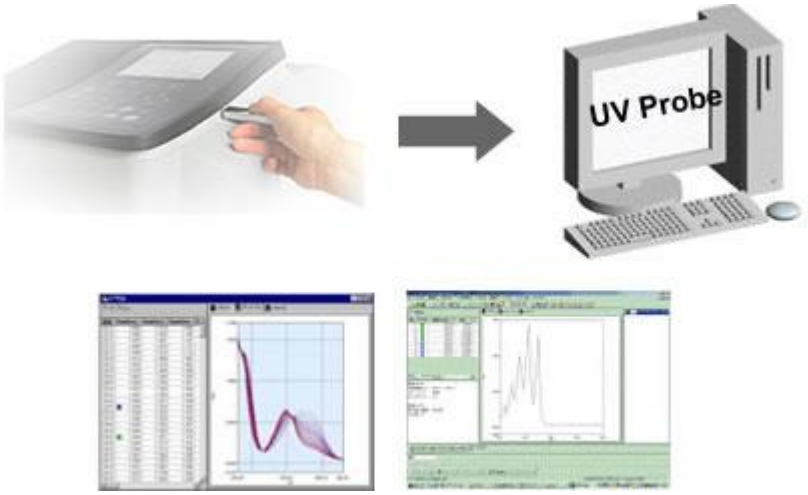


Only 450 mm wide, the UV-1800 is one of the most compact instruments in its class, allowing installation in tight spaces. In comparison to the UV-1700, the setting space has decreased by about 15%, and the width has narrowed by about 20%.

User friendly

USB memory can be connected directly to the UV-1800. Users can now analyze data on a PC using UV Probe software. In addition, data for spectra and time-course curves can be displayed and saved with commercial spreadsheet software. Printing is possible to printers that support PCL control codes.
E.g. HP Business Inkjet 1200
HP Photosmart D5160

Using UV Probe software, provided as standard, makes it possible to control the UV-1800 with a PC. (A USB cable is required for connection to the PC.)



Chapter two

Experimental section

2-1 Apparatus:

- A shimadzu 1800UV 1800 spectrophotometer, with 1cm quartz cells was used to record the spectrophotometric data.
- Mi 150pH/Temperature bench meter was used to adjust pH of the buffered solutions.
- Sensitive balance.

2-2 Reagent and Solution:

- D-Penicillamine (PA) was kindly provided by Al-basmalah pharmaceutical, containing 250mg per capsules was obtained from local pharmacy.
- NBD-CL solution was freshly prepared in methanol at 0.2% (W/V) concentration.
- Buffer solution was prepared by adding 22.6mL of 0.1mol/L NaOH to 100mL, 0.025mol/L $Na_2B_4O_7.H_2O$ (borax).
- All chemicals and reagent were of analytical-reagent grade.
- A stock solution of D-PA was prepared in distilled water and diluted further with the water to obtain standard of 100µg/mL.

2-3 Procedure:

2-3-1 Calibration curve:

An aliquot of 0.10-1.5 mL from standard solution was added to 1.0mL buffer solution in 10mL volumetric flask, 1.5mL of 0.3% NBD-CL was added to the later and the volume was brought to 10mL with water and mixed. The absorbance of the derivative was measured after half an hour at 465nm against a blank prepared similarly.

2-3-2 Determination of D-PA in dosage forms:

For preparation of sample solution, ten capsules were weighed and powdered then a quantity of powder equivalent to 41.7 mg of D-PA was transferred into a small conical flask. Filtered into 50mL volumetric flask and completed to the mark with distilled water to obtain 1000µg/mL concentration. 5.0mL was transferred to 50mL volumetric flask to obtain 100µg/mL concentrations. Aliquot volume was transferred into 10mL volumetric flask, and then the procedure was applied as described in calibration curve. The nominal content of the capsule was determined from regression equation.

Chapter three

Results and discussions

3-1 Results:

λ_{max} =465nm.

3-1-1 Buffer solution absorption:

Buffer (pH)	Absorption
9.0	0.438
9.5	0.721
10.0	0.344
10.5	0.986
11.0	-0.334
11.5	0.015

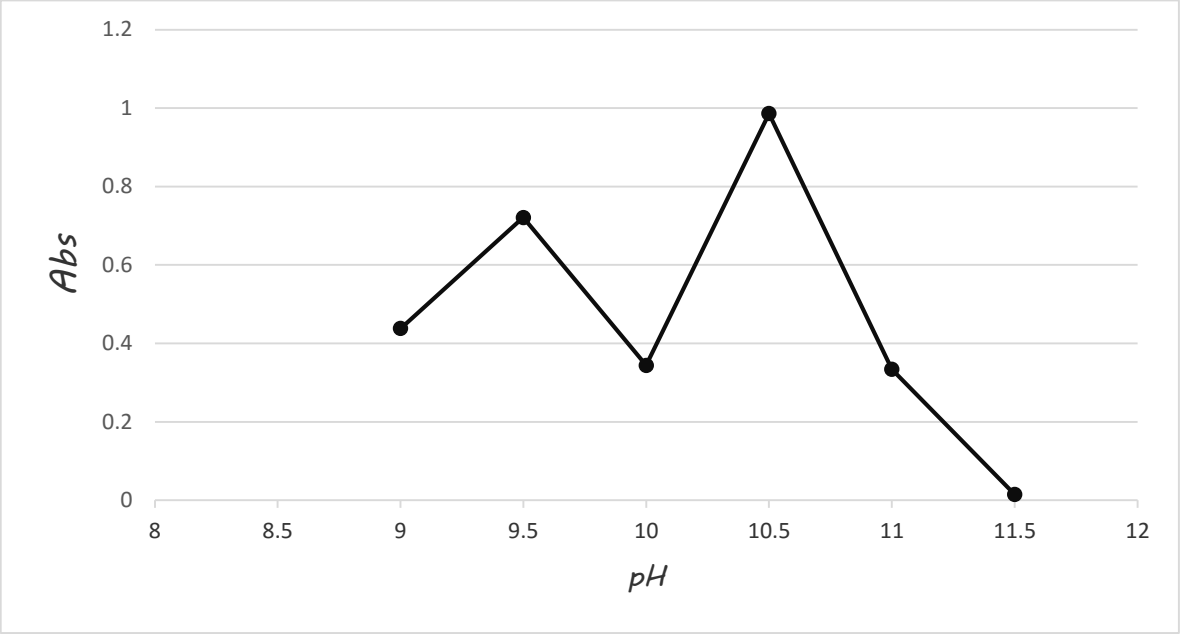


Figure1: effect of pH on the reaction of D-PA with NBD-Cl. reaction time: 30min**.**

3-1-2 Absorption of reagent:

Concentration mol/L	Absorption
0.01	0.050
0.02	0.087
0.03	0.041
0.04	0.036
0.05	0.034

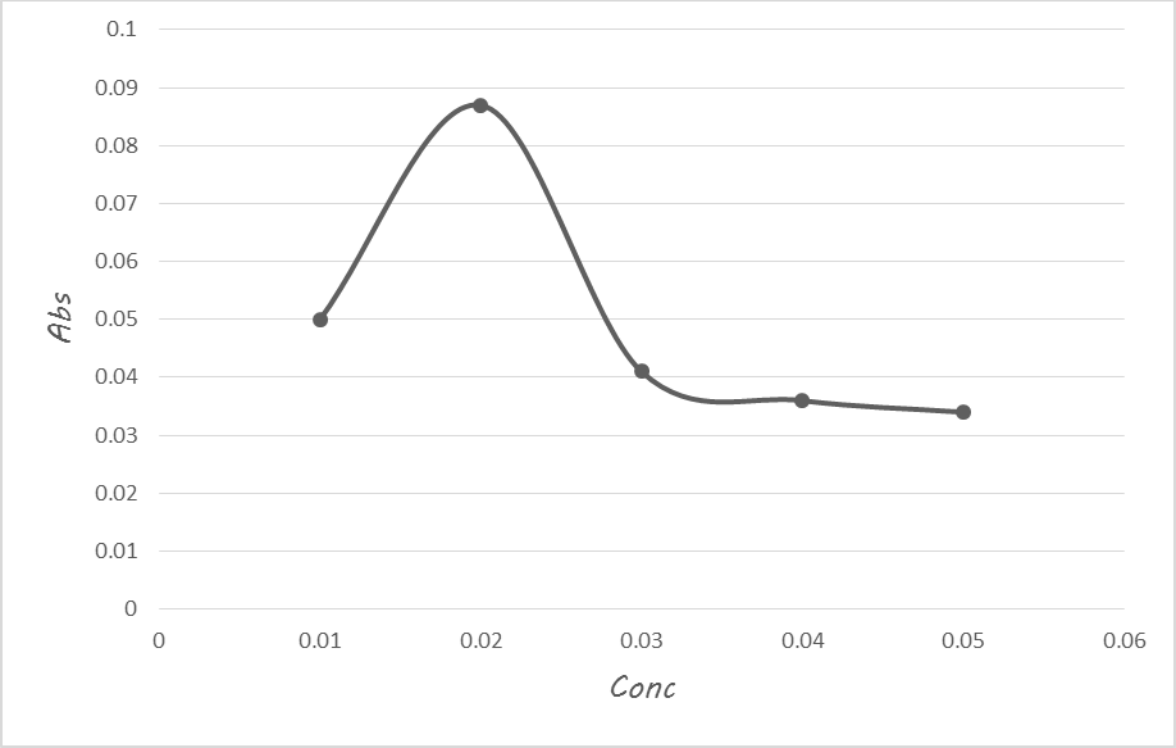
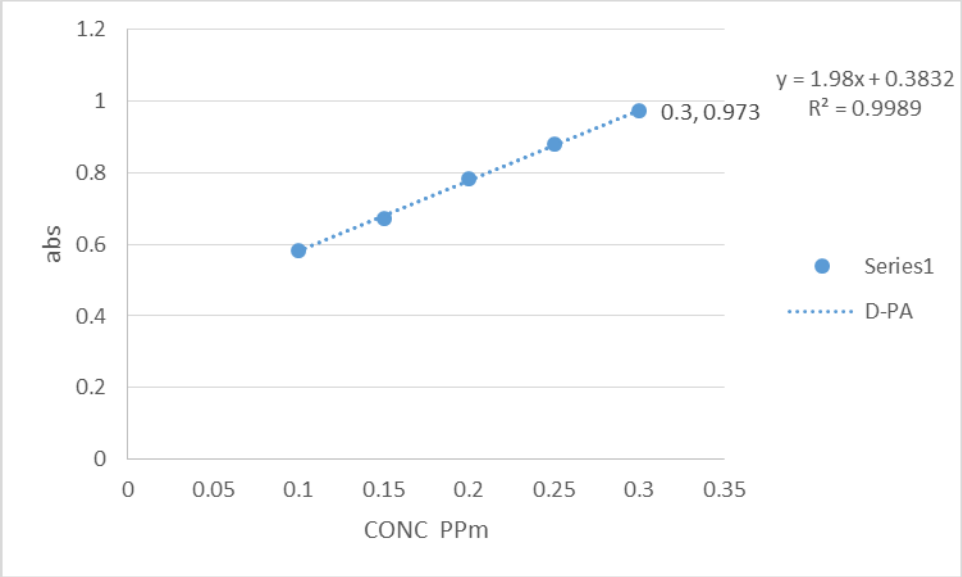


Figure2: effect of NBD-CL concentration on the reaction of D-PA with NBD-CL. Reaction time: 30min.

3-1-3 Absorption of Penicillamine:

Concentration	**Absorption**
0.1	0.535
0.15	0.701
0.2	0.795
0.25	0.852
0.3	0.973
Unknown	0.739



3-1-4 Parameters for the performance of the proposed method:

Parameter	***value***
Measurement wavelength	465nm
Linear range	1-3µg/mL
Regression equation	Y=1.98X+0.3832
Slope ± SD	1.98 ± 0.103
Intercept ± SD	0.3832 ± 0.023
Correlation coefficient	0.9989
Limit of detection (LOD)	0.0348µg/mL
Limit of quantification (LOQ)	0.1162µg/mL

the results above are obtained from the equation:

LOD or LOQ=K.S.D.a/b.

Where:

K=3 for LOD and 10 for LOQ, S.D.a is standard deviation of the intercept and b is the slope.

3-2 Conclusion:

Penicillamine (PA) was determined in a pharmaceutical formulation using 4-chloro-7-nitrobenzo-2-oxa-1, 3-diazol (NBD-CL) using spectrophotometric method.

3-3 Discussion:

The influence of pH on the absorbance of product. It was investigated in the range of 9.0 to 11.5. The absorbance of the solution increases rapidly up to pH 10.5, then decreases. At pH 10.5 the absorbance reaches its maximum (figure1). The effect of NBD-CL concentration was studied over the range 0.1 to 0.5% (w/v) as shown in figure2. Increasing the concentration of NBD-CL results in more products up to an amount of 0.2% (0.2% was optimum).

The regression equation was found to be as $Y=1.98X+0.3832$ ($r^2=0.9989$) (where Y is absorption, X is concentration of D-PA in µg/mL).

The limit of detection (LOD) and limit of quantification (LOQ) were determined using the formula:

LOD or LOQ=K.S.D.a/b.

Where:

K=3 for LOD and 10 for LOQ, S.D.a is standard deviation of the intercept and b is the slope.

References:

1. *Peisach, J.; Blumberg, W. E. (1969). "A mechanism for the action of Penicillamine in the treatment of Wilson's disease". Molecular pharmacology **5** (2): 200–209.*
2. *Aronson, J.K. (2010). Meyler's side effects of analgesics and anti-inflammatory drugs. Amsterdam: Elsevier Science.*
3. *Parker, C. W.; Shapiro, J.; Kern, M.; Eisen, H. N. (1962). "Hypersensitivity to penicillenic acid derivatives in human beings with penicillin allergy". The Journal of experimental medicine **115** (4): 821–838.*
4. *"WHO Model List of Essential Medicines" (PDF). World Health Organization. October 2013. Retrieved 22 April 2014.*
5. *Camp, A. V. (1977). "Penicillamine in the treatment of rheumatoid arthritis". Proceedings of the Royal Society of Medicine **70** (2): 67–69.*
6. *Rosenberg, L. E.; Hayslett, J. P. (1967). "Nephrotoxic Effects of Penicillamine in Cystinuria". JAMA: the Journal of the American Medical Association **201.***
7. *Steen, V. D.; Medsger Jr, T. A.; Rodnan, G. P. (1982). "D-Penicillamine therapy in progressive systemic sclerosis (scleroderma): A retrospective analysis". Annals of internal medicine **97** (5): 652–659.*
8. *Peterson, R. G.; Rumack, B. H. (1977). "D-Penicillamine therapy of acute arsenic poisoning". The Journal of Pediatrics **91** (4): 661–666.*
9. *Hall, A. H. (2002). "Chronic arsenic poisoning". Toxicology Letters **128** (1–3): 69–72.*
10. *Grasedyck, K. (1988). "D-Penicillamine--side effects, pathogenesis and decreasing the risks". Zeitschrift fur Rheumatologie. 47 Suppl 1: 17–19.*

11. *Fishel, B.; Tishler, M.; Caspi, D.; Yaron, M. (1989). "Fatal aplastic anaemia and liver toxicity caused by D-Penicillamine treatment of rheumatoid arthritis". Annals of the rheumatic diseases **48** (7): 609–610.*

12. Table 14-2 in: *Mitchell, Richard Sheppard; Kumar, Vinay; Abbas, Abul K.; Fausto, Nelson. Robbins Basic Pathology. Philadelphia: Saunders. ISBN 1-4160-2973-7.* 8th edition.