World Journal of Analytical Chemistry, 2013, Vol. 1, No. 2, 18-22
Available online at http://pubs.sciepub.com/wjac/1/2/1

DOI:10.12691/wjac-1-2-1

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

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Received December 10, 2012; Revised April 01, 2013; Accepted April 19, 2013

Abstract A sensitive and simple spectrophotometric method has been proposed for the determination of D-Penicillamine (PA) in pharmaceutical formulations. 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 468nm. Under optimized reaction condition, the method was linear in the concentration range 1-15 μg mL^{-1}. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.11 μg mL^{-1}, 0.38 μg mL^{-1}, respectively. The method was applied successfully to the determination of PA in pharmaceutical dosage form. A proposal of the reaction pathway has been postulated. The results were in a good agreement with those obtained with the official USP method. The method is useful for routine analysis of PA in quality control laboratories.

Keywords: *D-Penicillamine, spectrophotometric, pharmaceutical formulation, method validation, NBD-CL*

1. Introduction

D-penicillamine (PA) (2-Amino-3-mercapto-3-methylbutanoic acid) is a naturally occurring sulfur – containing amino acid that belongs to the amino-thiol family. It is the main product of the decomposition of penicillin antibiotics [1]. PA is a thiol drug used in treatment of cystinuria, certain forms of metal intoxication and progressive systemic sclerosis [2] and Wilson's disease by acting as a chelating agent which is used to aid the elimination of copper [3], it is also effective for the treatment of several disorders including rheumatoid arthritis, primary biliary cirrhosis, scleroderma, fibroticlung diseases, heavy element poisoning and progressive systemic sclerosis [4]. The pure form D (or S) form of the drug is used, because the L(or R) form and DL (RS) racemic mixture are much more toxic as shown by severe adverse reaction such as neuritis in patients treated with the DL-PA [2]. Several methods have been reported for the analysis of D-PA in both pharmaceutical preparations and biological samples. These methods include chemiluminescence [1], spectrophotometry [3,4,5,6], spectrofluorometric [7], high performance liquid chromatography (HPLC) [8] and capillary electrophoresis (CE) [2,4] and. Although these methods have been successfully employed, some of them suffer from interference from the pharmaceutical or biological matrix, and the others are time-consuming or require expensive equipment and consequently are not suitable for routine analysis in common laboratories.

For these reasons, the development of new sensitive and simple spectrophotometric method that overcomes the drawbacks of the existing methodologies was very essential.

2-Chloro-7-nitrobenzo-2-oxa-1,3-diazol (NBD-CL) has been proved to be a useful and sensitive analytical derivatizing agent for spectrophotometric analysis of pharmaceuticals bearing a primary or secondary amino group [9-21]. The applications of NBD-CL for determination of pharmaceutical bearing amine group have recently been reviewed by Elbashir *et al.* [22,23].

The use of NBD-CL for spectrophotometric determination of PA has not been reported yet. Therefore in this work a sensitive and simple spectrophotometric method for determination of PA in pharmaceutical formulations has been developed.

2. Experimental

2.1. Apparatus

A Shimadzu 1800UV 1800 Spectrophotometer, with 1cm quartz cells was used to record the spectrophotometric data. Mi 150 PH/Temperature Bench meter was used to adjust pH of the buffered solutions.

2.2. Reagent and Solution

D-Penicillamine (PA) was kindly provided by ALFARES Pharmaceutical (Damascus-Syria), containing

250mg per capsules was obtained from local pharmacy. NBD-CL solution was freshly prepared in methanol at 0.3% (w/v) concentration. Buffer solution was prepared by adding 22.6mL of 0.1mol L^{-1} NaOH to 100mL 0.025mol L^{-1} $Na_2B_4O_7.10H_2O$ (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 solution of 100 µg mL^{-1}.

2.3. Procedure

2.3.1. Calibration curve

An aliquot of 0.10-1.5mL 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 468nm 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 41.7mg 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 1000 µgmL^{-1} concentration, 5.0mL was transferred to 50.0mL volumetric flask to obtain 100 µg mL^{-1} 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.

3. Results and Discussion

3.1. Absorption Spectra

The absorption spectrum of D-PA was recorded against water (Figure 1), it was found that D-PA exhibits a maximum absorption peak (λ_{max}) at 198nm. Because of highly blue shifted λ_{max} of D-PA, its determination in the dosage form based on the direct measurement of its absorption for ultraviolet is susceptible to potential interferences from the common excipients. Therefore, derivatization of D-PA red–shifted light-absorbing derivative was necessary. The reaction between D-PA and NBD-CL was performed, and the absorption spectrum of the product was recorded against reagent blank (Figure 1). It was found that the product is brown colored exhibiting λ_{max} at 468nm, and the λ_{max} of NBD-CL was 342nm. The λ_{max} of D-PA- NBD-CL derivative was red-shifted, eliminating any potential interference. Therefore, the measurements were carried out at 468nm.

3.2. Optimization of Reaction Conditions

The optimum conditions for the development of method were established by varying the parameters one at a time while keeping the others fixed and observing the effect produced on the absorbance of the colored product. In order to establish experimental conditions, the effect of various parameters such as, pH, concentration of NBD-CL temperature, and time of heating were studied.

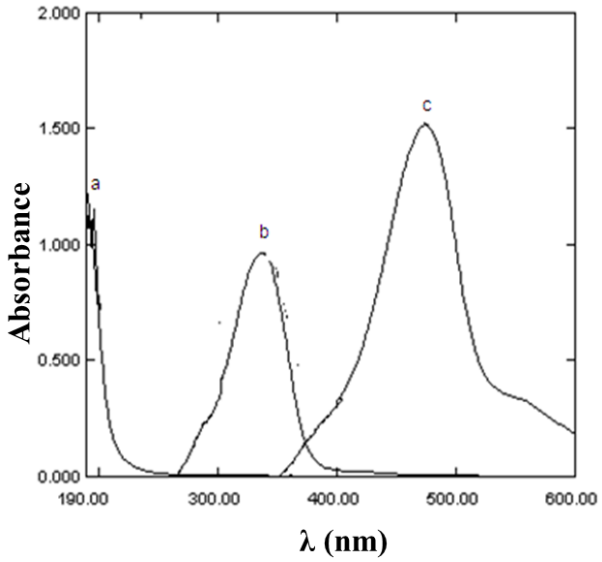


Figure 1. Absorption spectrum of D-PA (a), NBD-CL and reaction product of D-PA with NBD-CL (c) against blank. Conditions: D-PA (15 µg ml^{-1}), NBD-CL (0.3 %, w/v) and pH 10.5

The influence of pH on the absorbance of product I was investigated in the range of 7.0-12.0, the absorbance of the solution increases rapidly up to pH 10.5 and then decrease (Figure 2). At pH 10.5, the absorbance reaches its maximum; in other words, the degree of the nucleophilic substitution reaction is also maximal. At pH > 10.5, the absorbance of solution decreases sharply. Presumably it may be that the increase of hydroxide ion holds back the nucleophilic substitution reaction between D-PA and the chromogenic reagent. Consequently, the absorbance of the solution reduces. In order to keep the high sensitivity for the determination of D-PA, pH 10.5 was selected for optimal experimental conditions.

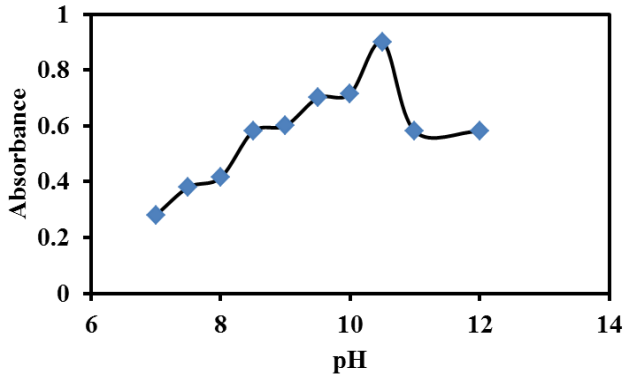


Figure 2. Effect of pH on the reaction of D-PA with NBD-CL. D-PA (15 µg/ml): 1mL; buffer ; 1.0ml; temperature 25 ℃; reaction time: 25min

The effect of NBD-CL concentration was studied over the range 0.05–0.6% (w/v) as shown in Figure 3. Increasing the concentration of NBD-CL results in more products up to an amount of 0.3%, after which the absorbance remained almost constant. Therefore a concentration of 0.3% NBD-CL was considered optimum.

Keeping pH at 10.5, the effect of amount of buffer solution on the absorbance of product I was also studied. It shows that the absorbance of product I enhances rapidly with the rise of amount of buffer solution, and becomes maximal when the amount of buffer solution is 1.5ml. Therefore, the amount of 1.5ml buffer solution was

selected to ensure the highest absorbance of product I, as shown in Figure 4.

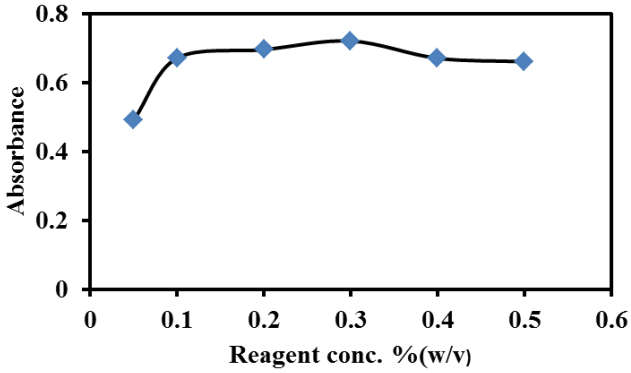


Figure 3. Effect of NBD-CL concentration on the reaction of D-PA with NBD-CL. D-PA (15 μg/ml): 1ml; buffer solution (pH 10.5); 1.0ml; temperature 25 ℃; reaction time: 25min

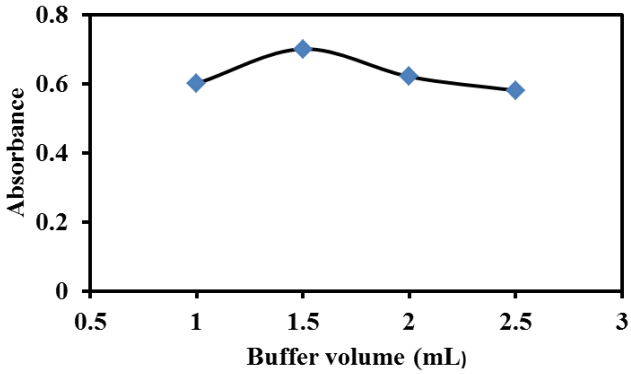


Figure 4. Effect of the volume of the buffer on the reaction of D-PA with NBD-CL. D-PA (15 μg/ml): 1mL; buffer solution (pH 10.5); temperature 25 ℃; reaction time: 25min

The effect of temperature on the reaction was studied by carrying out the reaction at different temperatures (25–80 ℃). It was found that the reaction of D-PA with NBD-CL was not affected by increasing the temperature, and the reaction at room temperature (25 ℃) went to completion in 30 minutes, and longer reaction time up to 40 minutes did not affect the reaction (data not shown).Therefore, further experiments involving NBD-CL reagent were carried out at room temperature (25 ℃) for 30 minutes.

From the above experiments, the optimized conditions used for the assay were: pH 10.5, NBD-CL concentration 0.3% w/v, volume of the buffer 1.5, reaction time 30min and temperature 25 ℃.

Furthermore, the molar ratio of NBD-CL to D-PA in the reaction mixture was studied according to Job's method of continuous variation [24]. A 7.50×10^{-4} mol L^{-1} standard solution of D-PA and solution of NBD-CL were used. The reaction stoichiometry was found to be a good approximation 1:2 ratio(drug/ reagent), confirming that one molecule of D-PA reacts with two molecule of NBD-CL, Figure 5. Based on the observation molar ratio, the reaction pathway was postulated to proceed as shown in Scheme 1.

3.3. Analytical Method Validation

Under the described experimental condition, linear relationship was found between the absorbance at λ_{max} 468 nm and the concentration of the drug. The regression equation was found to be as A=0.093C+0.018 (r^2 =0.997, n=7) (where A is the absorption, and c is the concentration of D-PA in μg ml^{-1})

The limits of detection (LOD) and limits of quantitation (LOQ) where determined using the formula:

$$\text{LOD or LOQ} = K\ S.D.\ a\ /\ b \qquad (1)$$

Where:

K=3 for LOD and 10 for LOQ, S. D. a is the standard deviation of the intercept, and b is the slope, the obtained results are summarized on Table 1.

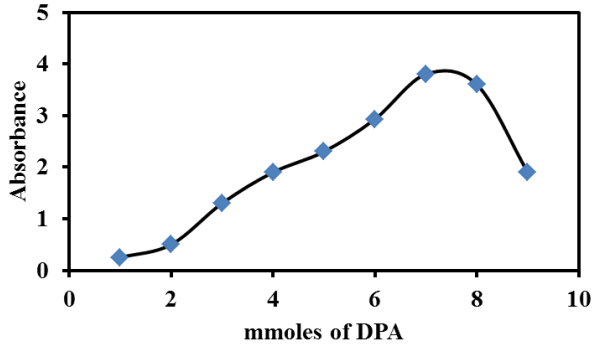
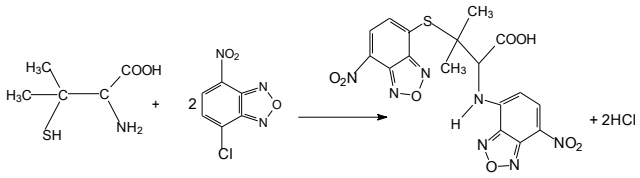


Figure 5. The continuous variation plot for the stoichiometry of the reaction of D-PA with NBD-CL



Scheme 1. Reaction pathway of D-PA with NBD-Cl

Table 1. Parameter for the performance of the proposed method

Parameter	value
Measurement wavelength	468nm
Linear range	1-15 μg mL^{-1}
Regression equation	Y= 0.093X + 0.019
Slope ±SD	0.093 ±0.000004
Intercept ±SD	0.019 ±0.00036
Correlation coefficient (r^2)	0.997
Limit of Detection (LOD)	0.11 μg ml^{-1}
Limit of Quantification (LOQ)	0.38 μg ml^{-1}
Molar absorptivity (L $mol^{-1}cm^{-1}$)	4.678×10^3

The accuracy of the proposed method was carried out by applying standard addition technique. A different amount of standard solution was added to a known concentration of the drug sample. The average percent recoveries obtained in range 99.4–100.1 (Table 2).

Table 2. Recovery studies for the determination of PA by proposed method

Sample No.	Sample content (μg mL^{-1})	PA amount added	Amount Found	Recovery
1	2	0.5	2.51	100.1%±0.60
2	"	8.0	9.80	98.0% ±1.57
3	"	15.5	17.40	99.43%±1.13

Robustness was examined by evaluating the influence of small variation in the method variables on its analytical performance. In these experiments, one parameter was changed whereas the others were kept unchanged, and the recovery percentage was calculated each time. It was found that variation in the NBD-CL concentration of 0.3 ± 0.02 % (w/v) and optimal experimental conditions of temperature (25 ± 2 ℃), time (30 ± 2min) and pH (10.5 ± 0.2), did not significantly affect the procedures and recovery values were 96.2–104.0 % and the RSD values did not exceed 0. 497 % (Table 3).

Table 3. The Influence of small variation in the assay conditions on the analytical performance of the proposed method for the determination of D-PA

Parameter	Recovery (%)
Recommended conditions	101.36 ±0.50
NBD-CL concentration (% 0.28) NBD-CL concentration (% 0.32)	96.20 ±0.17 99.20 ±0.13
Buffer pH 10.3 Buffer pH 10.7	99.50 ±0.17 97.60 ±o.19
Temperature (℃) 23 Temperature (℃) 27	103.00 ±0.23 100.80 ±0.29
Reaction time (min) 28 Reaction time (min) 32	100.56 ±0.16 102.00 ±0.36

3.4. Application to the Pharmaceutical Dosage Form

PA capsules were subjected to the analysis by the proposed method as well as with the official HPLC method (United States Pharmacopeia, 2006) and the results were statistically compared with each other. The label claim percentage was 102.8 ± 1.99 (Table 4). Statistical analysis of the results reveals that at the 95% level of confidence, the calculated t- and F-values indicate the proposed methods to be as precise and accurate as the standard methods.

Table 4. The analysis of D-PA capsules by the recommended procedure and the official USP method

Recovery % + RSD (n=5)		t-value	f-value
Proposed	Official		
102.8 ±1.99	100.75±0.69	1.78	0.12

The tabulated values for t and F at 95% confidence limit are 2.78 and 6.26, respectively

4. Conclusion

The present paper described the evaluation of NBD-CL as analytical reagents in the development of simple, sensitive, and accurate spectrophotometric methods, for the determination of D-PA in bulk and pharmaceutical formulations. The proposed method is simple, reliable, specific, accurate, reproducible, and highly sensitive, for the determination of D-PA in commercially available dosage forms. The method is also cost effective and environmentally friendly; therefore the proposed method can be used advantageously as a routine method for the determination of D-PA in quality control and industry.

References

[1] Suliman, F.O., AL-Hinai, M.M., AL-Kindy, S.M.Z., and Salama, S.B, "Enhancement of the chemiluminescence of penicillamine and ephedrine after derivatization with aldehydes using tris(bipyridyl)ruthenium(II) peroxydisulfate system and its analytical application," *Talanta*, 74(5) 1256-1264. Feb.2008.

[2] Gotti, R., Pomponio, R., Anderson, V., and Cavrini, V, " Analytical study of penicillamine in pharmaceuticals by capillary zone electrophoresis," *J. Chromatogr*. A, 844(1-2).361-369. Jun.1999.

[3] AL-Majed, A.A, "Spectrophotometric estimation of D-penicillamine in bulk and dosage forms using 2,6-dichloroquinone-4-chlorimide, (DCQ)," *J Pharm Biomed Anal*. 21(4).827-833.Dec.1999.

[4] Yang, X., Yuan, H., Ch Wang, Su, X., Hu, L., and Xiao, D, "Determination of penicillamine in pharmaceuticals and human plasma by capillary electrophoresis with in-column fiber optics light-emitting diode induced fluorescence detection," *J. Pharm. Biomed. Anal*,45(2).362-366. Oct.2007.

[5] Rojanarata, T., Opanasopit, P., Ngawhirunpat, T., Saehuan, C, "Ninhydrin reaction on thiol-reactive solid and its potential for the quantitation of d-penicillamine,"*Talanta*, 82(2).444-449. Jul.2010.

[6] Elbashir,A.A., Awad, S. F. "A New Spectrophotometric Method for Determination of Penicillamine in Pharmaceutical Formulation Using 1, 2-naphthoquine-4-sulfonate (NQS)," J. Pharmacovigilance1, 1-5, March.2013

[7] AL-Majed, A.A, "Specific spectrofluorometric quantification of D-penicillamine in bulk and dosage forms after derivatization with 4-fluoro-7-nitrobenzo-2-oxa-1,3-diazole," *Anal Chim. Acta*, 408(1-2), 169-175. Mar.2000.

[8] Russell J., Mckeown, J.A., Hensman, C., Smith, WE., and Reglinski, J, "HPLC determination of biologically active thiols using pre-column derivatisation with 5,5'-dithio-(bis-2-nitrobenzoic acid)," *J. Pharm. Biomed. Anal*,15(11).1757-1763. Jul.1997.

[9] Taha, E.A., Salama, N.N., and Fattah, L, "Spectrofluorimetric and spectrophotometric stability-indicating methods for determination of some oxicams using 7-chloro-4-nitrobenz-2-oxa-1,3-diazole (NBD-CL)," *Chem Pharm Bull*.,54(5).653-658. May.2005.

[10] El-Enany, N., El-Sherbiny, D., and Belal, F, "Spectrophotometric, spectrofluorometric and HPLC determination of desloratadine in dosage forms and human plasma," *Chem Pharm Bull*., 55(12)1662-1670. Dec.2007.

[11] Saleh, H.M., EL-Henawee, M.M., Ragab, G.H., and El-Hay, S.S.A., "Utility of NBD-CL for the spectrophotometric determination of some skeletal muscle relaxant and antihistaminic drugs," *Spectrochim Acta A* ,67(5).1284-1289. Oct.2007.

[12] Shehata, M.A., El-Sayed, G.M., and Abdel-Fattah, L.E, "Utilization of 4-chloro-7-nitro-2,1,3-benzoxadiazole (NBD-CL) for kinetic spectrophotometric assay of befunolol hydrochloride in its pharmaceutical formulation," *J AOAC Int.*, 89(3).646-650. May-Jun.2006.

[13] Olojo, RO., Xia, R.H., and Abramson, J.J, "Spectrophotometric and fluorometric assay of superoxide ion using 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole," *Anal Biochem.* 339(2). 338-344. Apr. 2005.

[14] Olgun, N., Erturk, S., and Atmaca, S, "Spectrofluorimetric and spectrophotometric methods for the determination of vigabatrin in tablets," *J. Pharm. Biomed. Anal.*, 29(1-2).1-5. Jun.2002.

[15] Onal, A., Kepekci, S.E., and Oztunc, A, "Spectrophotometric methods for the determination of antidepressant drug paroxetine hydrochloride in tablets," *J AOAC Int.*, 88(2) 490-495. Mar-Apr.2005.

[16] El-Emam, A.A., Hansen, S.H., Moustafa, M.A., El- Ashry, S.M., and El-Sherbiny, D.T, "Determination of lisinopril in dosage forms and spiked human plasma through derivatization with 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-CL) followed by spectrophotometry or HPLC with fluorimetric detection," *J. Pharm.Biomed.Anal.*, 34(1).35-44. Jan.2004.

[17] Amin, A.S., Ragab, G.H., and Saleh, H, "Colorimetric determination of β-blockers in pharmaceutical formulations," *J. Pharm. and Biomed.Anal.*, 30(4).1347-1353. Nov.2002.

[18] Abdellatef, H.E, "Kinetic spectrophotometric determination of tramadol hydrochloride in pharmaceutical formulation," *J. Pharm. Biomed. Anal*., 29(5)835-842. Jul.2002.

[19] Darwish, I.A., Amer, S.M., Abdine, H.H., and AL-Rayes, L.A.I, " New Spectrophotometric and Fluorimetric Methods for Determination of Fluoxetine in Pharmaceutical Formulations," *Int J Anal Chem*. 257(3)1-9. May.2009.

[20] Önal, A., and Sagirli, O, "A Spectrophotometric and spectrofluorimetric methods for the determination of pregabalin in

bulk and pharmaceutical preparation," *Spectrochim Acta A* 72(1).68-71. Feb.2009.

[21] El-Shabrawy, Y., Belal, F., Sharaf, El-Din, M., Shalan, Sh, "Spectrophotometric determination of fenoterol hydrobromide in pure form and dosage forms," *IL Farmaco*,58(10)1033-1038.Oct. 2003.

[22] Elbashir, A.A., Suliman, F.O., Aboul-Enein, H.Y, "The application of 7-chloro-4-nitrobenzoxadiazole (NBD-CL) for the analysis of pharmaceutical-bearing amine group using spectrophotometry and spectrofluorimetry techniques," *Appl Spectros Rev.*, 46(3). 222-241. Mar.2011.

[23] Elbashir, A.A., Suliman, F.O., Aboul-Enein, H.Y, "The Application of 7-Chloro-4-Nitrobenzoxadiazole and 4-Fluoro-7-Nitro-2,1,3 Benzoxadiazole for The Analysis of Amines and Amino Acids Using High-Performance Liquid Chromatography," *GU J Sci* 24(4).679-697.Oct.2011.

[24] Job, P, "Advanced Physiochemical Experiments,2nd ed.,Oliner and Boyd, Edinburgh, 1963,p.54 Anal. Chem, 1928; 9, 113.