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Spectrophotometric Determination of Amoxicillin by Coupling with Diazotized m – Nitroaniline

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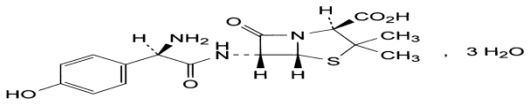
ABSTRACT

A sensitive spectrophotometric method for the assay of micro amount of amoxicillin trihydrate (AmoxT) in aqueous solution has been worked out. The method is based on the coupling of AmoxT with diazotized m–nitroaniline (DmNA) in basic medium. The azo dye formed is water – soluble, stable, and shows maximum absorption at 436 nm, Beer's law is obeyed over the range (3-30) µg.ml^{-1} with a molar absorptivity of 2.6006×10^4 l.mol^{-1}.cm^{-1} and Sandell's sensitivity index of 0.0161 µg.cm^{-2} , a relative error of -1.3 to - 0.1% and a relative standard deviation of ±0.2 to ±1.3% depending on the concentration level. The method has been successfully applied to the determination of AmoxT in its pharmaceutical preparations.

Key words: amoxicillin trihydrate, m– nitroaniline, diazo-coupling, spectrophotometric determination.

INTRODUCTION

Amoxicillin trihydrate is chemically (2S,5R,6R)-6-[[(2R)-2-amino-2-(4- hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid and has the following structure: (1)



Amoxicillin trihydrate ($C_{16}H_{19}N_3O_5S,3H_2O$) M.wt =419.4 g/ml

Amoxicillin, semi-synthetic drug belongs to a class of antibiotics called as Penicillin. It is used in treatment a wide range of infections caused by Gram-positive and Gram-negative bacteria , it is used to treat infections of the middle ear (otitis media), urinary tract ,throat, larynx (laryngitis), pharynx (pharyngitis), brosnchi (bronchitis), lungs (pneumonia) (2-4).

The reports found in the literature for amoxicillin determination concentrate on chromatographic methods (5-13), also hyphenated techniques have been used (14-21). Various spectrophotometric methods have been utilized for the determination of amoxicillin, these methods include diazo coupling reaction with different diazotized (22-26),oxidation-reduction reaction(27-31),ion pair complex(32),also uv(33) and derivative spectrophotometric(34-37),other methods included flow injection technique(38-40),volumetric(41,42) and gravimetric(43,44)methods have been utilized.

The purpose of the present study was to evaluate a sensitive and an accurate method for the determination of amoxicillin in the bulk and pharmaceutical dosage forms. The present method involves the diazo coupling reaction of amoxicillin with diazotized m-nitroaniline in alkaline medium to form a highly colored azo dye that has been proved successfully for the assay of amoxicillin in pharmaceutical preparations.

Experimental

Apparatus: **All measurements are performed using CECELL Recording Spectrophotometric, with 1 cm quartz cells.**

Reagents: All chemical used are of the highest purity available.

Working AmoxT 100 μg. ml^{-1}: This solution is prepared by diluting 0.0100 gm AmoxT in100 ml with distilled water in a volumetric flask.

Sodium hydroxide solution, 1M.: This solution is prepared by appropriate dilution of the concentrated volumetric (Fluka) solution with distilled water and then transferred to plastic bottle.

DmNA reagent solution, 5 mM. A 0.0685g of a m-nitroaniline (Fluka) is dissolved in about 90 ml distilled water. Then 4 ml of con..HCl is added and the solution is heated, the clear mixture is then transferred to a 100 ml volumetric flask and is cooled to(0 – 5)°C in an ice-bath. A 0.0345g NaNO2 is added and the mixture is stirred vigorously. After 5 minutes, the solution is made up to volume in 100 ml volumetric flask with cold distilled water. The solution is kept in a brown bottle in a refrigerator and is stable for 3 days at least.

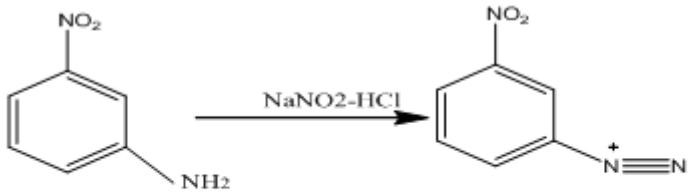
RESULTS AND DISCUSSION

The effect of various parameters on the absorption intensity of the colored dye is investigated and the reaction conditions have been optimized.

Principle of the method

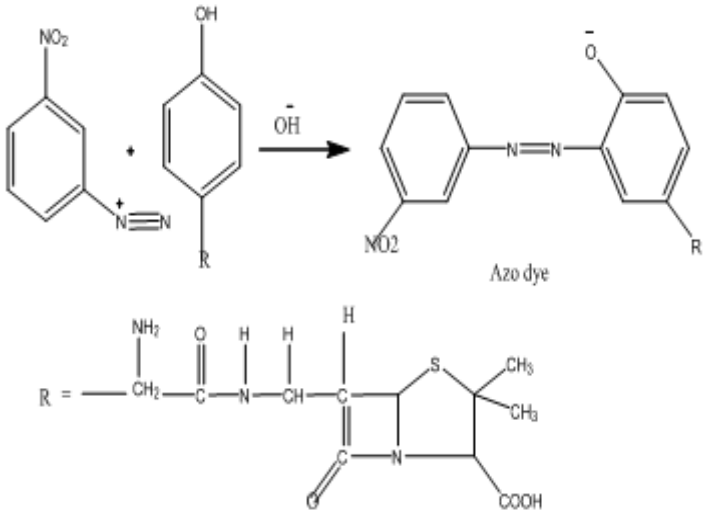
The method involves the coupling of the determinant AmoxT with DmNA to form in basic medium an intensely – colored dye. The method involves two steps:

1- Preparation of DmNA:



m-Nitroaniline Diazotised-m-Nitroaniline

2- React the DmNA with AmoxT:



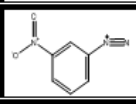
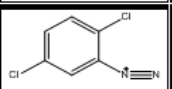
Study of the Optimum Reaction Conditions

For the subsequent experiments, 1ml AmoxT (100μg) is taken in final volume 10 ml and absorbance measurements are performed at 436 nm.

Choice of diazotised agent

Several aromatic diazotised agents have been tested for optimum conditions. The results in Table (1) show that DmNA give the most sensitive reaction ($\varepsilon = 2.037 \times 10^4 l.mol^{-1}.cm^{-1}$) in alkaline medium. Therefore, it has been selected for subsequent experiments.

Table 1: The selection of diazotized agent.

Diazotized (5mM)	Structure	Absorbance	λ max/ nm	ε (l.mol^{-1}.cm^{-1})
m-Nitro aniline		0.485	436	2.037×10^4
Sulphacetamide		0.387	451.5	1.626×10^4
2,5-Dichloroaniline		0.432	421.5	1.815×10^4

Effect of DmNA reagent amount

The effect of the amount of the DmNA reagent on the maximum absorbance of the dye formed, has been investigated and the results show that (1 ml) of DmNA (5 mM) reagent solution gives the highest intensity with a correlation coefficient (r =0.993) over a range of determined concentration of 1 – 20 µg.ml^{-1}, and (1 ml) of reagent is therefore recommended for the subsequent experiment.

Table2: Effect of DmNA on absorbance.

5mM(DmNA) soln.	Absorbance of different amounts of AmoxT(µg.ml^{-1})				r
	5	10	15	20	
0.8	0.183	0.291	0.319	0.535	0.948835
1.0	0.282	0.498	0.631	0.893	0.992893
1.5	0.227	0.462	0.537	0.876	0.972571

Effect of base

The preliminary experiments have shown that AmoxT can give colored dye with DmNA only in basic medium. Different bases (strong and weak) have been used. The results in table 3 indicate that the colored dye need a strong basic medium (pH =12).

Table (3): The effect of base on absorbance and color contrast.

Base used, soln.)1M(	λ_{max}(nm)	Δλ(nm)*	Absorbance	Final pH
NaOH	436	142.5	0.488	12.01
Na_2CO_3	462	88.5	0.212	10.23
$NaHCO_3$			No color contrast	

*$\Delta\lambda\ (nm) = \lambda_{max}^{S} - \lambda_{max}^{B}$ where S = Sample, B = Blank.

Table 4 shows that 0.6 ml of 1MNaOH was recommended in the subsequent experiment due to the highest intensity which was obtained for the formed azo dye.

Table4: The optimum volume of sodium hydroxide.

mL of NaOH (1M)	0.4	0.6	0.7	0.9
Absorbance	0.325	0.488	0.423	0.355

Effect of time on absorbance

The effect of time on the development and stability period of the colored dye is investigated under optimum experimental conditions described before. The formation of colored dye being complete after dilution and the absorbance of the colored species remained constant for, at least 2 hours(Table 5).

Table 5: Stability of azo dye.

µg **AmoxT**	**Absorbance /minute**									
	A.D*	10	20	30	40	50	60	90	120	O.N**
50	0.281	0.280	0.283	0.283	0.284	0.283	0.283	0.283	0.282	0.278
100	0.484	0.494	0.494	0.494	0.484	0.494	0.494	0.494	0.484	0.402
200	0.891	0.891	0.890	0.889	0.891	0.891	0.889	0.885	0.885	0.864

*After dilution **Over night

Final Absorption Spectra

Absorption spectra of the colored dye formed from coupling AmoxT with DmNA in basic medium, against its corresponding reagent blank show maximum absorption at 436 nm in contrast to reagent blank which shows absorbance (0.025) at the above λ_{max}. Fig 1.

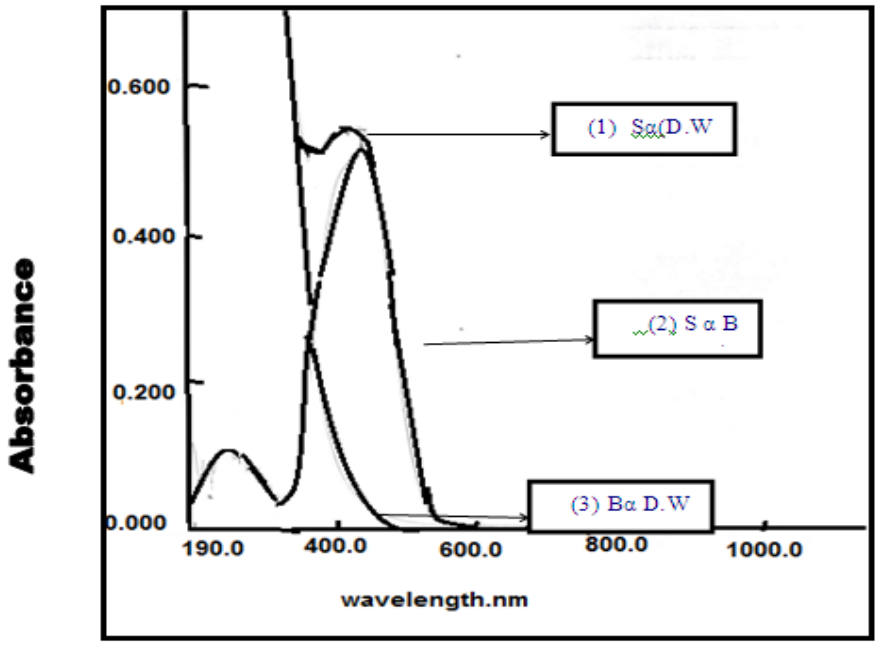


Fig. 1: Absorption spectra of 100µg AmoxT treated according to the recommended procedure and measured against (1) blank (2) distilled water and (3) blank measured against distilled water.

The wavelength of maximum absorption at 436 nm has been selected for the subsequent experiments.

Recommended Procedure and Calibration Graph

To a series of 10 ml volumetric flasks, aliquots covering the range of (30– 300) µg/10ml AmoxT are transferred,1 ml of DmNA (5 mM), then 0.6 ml of 1M NaOH solution added, then the volumes are made to the mark with distilled water. absorbances are measured at 436 nm against the reagent blank(Fig. 2).

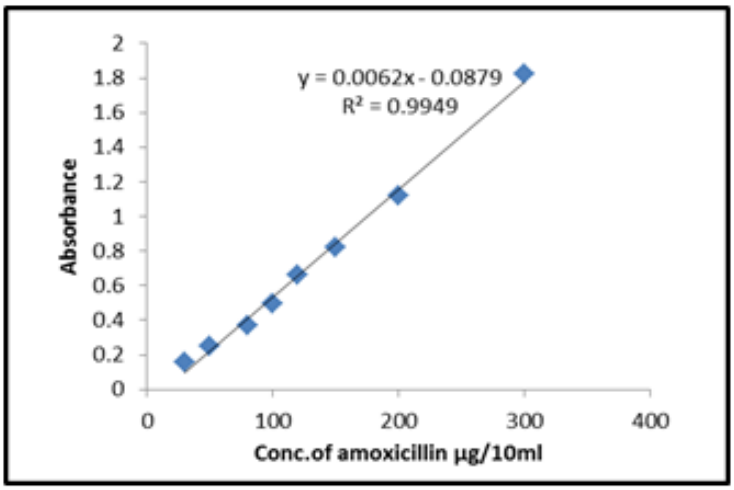


Fig. 2: Calibration graph for AmoxT determination using DmNA.

The calibration graph show in Fig. 2 is linear over the range 3 – 30 µg.ml^{-1}. Higher concentration show negative deviation from Beer's law. The apparent molar absorptivity referred to AmoxT, has been found to be 2.6006×10^{4} l.mol^{-1}.cm^{-1}.

Application of the Method

To test the application of the present method, it has been used to determine AmoxT in its pharmaceutical preparations (Table 6).

Table 6: Determination of AmoxT in different pharmaceutical preparations

Drug	Amount taken µg/10ml	Recovery%	RSD%
JulphAmox- capsul, 500mg (Julphar,com.)	50	98.7	±1.3
	100	99	±2.3
Almox- capsul, 500mg ,(ALKEM-U.A.E)	50	98.8	±1.7
	100	101.1	±1.1
GlomoX-capsul, 500mg (globalpharma-U.A.E)	50	102.6	±1.5
	100	99.1	±2.5
AugmentinTm capsul-125mg m,(GlaxoSmithkline-gSK)	50	97.6	±2.2
	100	99.3	±2.9
Augmentin solution- 250mg/5ml	50	100.9	±1.2
	100	97.6	±0.4
AmoxyLin-capsul,500mg,(IRAQ-H.D.I)	50	96.8	± 3.2
	100	97.9	±1.7

The results above indicate that good recovery of determinant for different company.

CONCLUSION

The proposed method was a simple and has a good sensitivity. The proposed method has advantageous over some of the reported visible spectrophotometric methods with respect to, reproducibility, precision, accuracy and stability of the colored dye. The proposed method is suitable for the determination of Amox in pure form and in its formulation without excipients interference.

REFERENCES

[1] Briish Pharmacopeia,(2007) Briish Pharmacopeia Commission, London SW 5NQ.
[2] Wilson O.C. , Ole G. and Delgado N.J. (2004).Textbook of Organic Medicinal and Pharmaceutical Chemistry. 11th Edn., Lippincott Williams and Wilkins , 351 West Camden Street, Baltimore.MD21201", 310-317.
[3] Brogden RN. , Heel RC. , Speight TM. and Avery GS. (1979).Amoxicillin injectable: a review of its antibacterial spectrum.pharmacokinetics and therapeutics use. Drugs, **18**(3),169-184.
[4] Kagan B.,(1977) . Ampicillin rash.Western J. of Medicine , **126**(4) , 333-335.
[5] Rele R.V. and Mali R.N. (2013).Simultaneous determination of amoxicillin trihydrate and bromhexine hydrochloride in pharmaceutical dosage by reverse phase high performance liquid chromatography. Der Pharma. Chemica. **5** (1) , 273–278
[6] Tippa D. M. R. and Singh N.(2010). Development and validation of stability indicating HPIC method for simultaneous estimation of amoxicillin and clavulanic acid in injection. **AJAC**,**1**, 95 – 101 .
[7] Pires LR. ,Ortiz RM., Decastro SC. and Pedrazzoli JR.(2003) .HPLC determination of amoxicillin comparative bioavailability in healthy volunteers after a single dose administration . J. Pharm. Sci .,**6** (2) : 223 – 30 .
[8] Bojaraju V. , Sireesha D. , Prassad V.V. and Diwan P.V.(2012). Reverse phase high performance liquid chromatography method for the simu- ltaneous estimation of amoxicillin trihydrate , and tinidazole in the tablet dosage form. World J. of Chemistry , **7** (2) , 47-52 .
[9] Giang D.T. and Hoang V.D.(2010). Comparative study of RP-HPLC and U.V spectrophotometric techniques for the simultaneous determination of amoxicillin and cloxacillin in capsules.J. Young Pharm, **2** (2) , 190 – 195 .
[10] Ahmed M., Babu S. and Shetty S.K.(2011).Development and validation of amoxicillin by RP- HPLC method in bulk drug and pharmaceutical dosage forms. Int.J.Chem.Tech. Res. , **3** (3) , 1037 -1041 .
[11] Pei Q. , Yang G.P., Z.J.Li, Peng X.D.(2011). Simultaneous analysis of amoxicillin and sulbactam in human plasma by HPLC - DAD for assessment of bioequivalence. J. of Chromatogr. B , **879** (21) , 2000 – 2004.
[12] Injac R. , Kocevar N. and Strukelj B.(2009) Optimized method for determination of amoxicillin , ampicillin , sulfamethoxazole and sulfacetamide in animal feed by micellar electrokinetic capillary chromatography and comparison with high - performance liquid chromatography. J.Croat.Chem.Acta ,**82**(3), 685-694 .
[13] Dhoka M.V. , Gawande V.T. and Joshi P.P.(2010).HPLC determination of amoxicillin trihydrate and bromohexine hydrochloride in oral solid dosage forms. J.of Pharma. Sci. and Res. , **2** (8) , 477 - 483 .
[14] Delis G. , Batzias G. , Kounenis G. and Papadopoulou K.(2009). Application and validation of a LC / Fluorescence method for the determination of amoxicillin in sheep serum and tissue cage Fluid. J . of Pharma. and Biomed.Anal.,**49**(2),375-380.
[15] Krasucka D. , Kowalski C.J. and Osypiuk M.(2011).Determination of amoxicillin in pig plasma by liquid chromatography with fluorecence detection. Bull Vet Inst Pulawy,**55**,771-775.
[16] Wen A. , Hany T. , Chen S. and Wang Z.(2008). Simultaneous determination of amoxicillin and ambroxol in human plasma by LC-MS/MS validation and application to pharmacokinetic study. J.of Pharma. and Biomed.Anal.,**48**(3) , 829-834
[17] Li H., Xia X., Xue Y.,and Tang S.(2012).Simultaneous determination of amoxicillin and prednisolone in bovine milk using ulta -high performance liquid chromatography tandem mass spectrometry.J .of Chromatogr. B**,900**(15700232) , 59-63
[18] Lugoboni B., Gazzotti T., Zironi E., Barbarossa A.(2011).Development and validation of a liquid chromatography / tandem mass spectrometry method for quantitative determination of amoxicillin in bovine muscle. J . Chromatogr.B , **879** (21), (1980 – 1986) .
[19] Freitas A., Barbosa J. and Ramose F.(2014).Multi-residue and multi-class method for the determination of antibiotics in bovine muscle by ultra-high-performance liquid chromatography-tandem mass spectrometry. Meat Sci., 1-17.
[20] Bogialli S., Capitolino V. , Curini R., Corcia A.D., Nazzari M. and Sergi M.(2004).Simple and rapid liquid chromatography –tandem mass spectrometry confirmatory assay for determining amoxicillin and ampicillin in bovin tissues and milk. J.Agric. Food Chem.,**52**(11),3286-3291 .
[21] Heller D.N., Nochetto C.B., Rummel N.G. and Thomas M.H. (2006).Development of multiclass methods for drug residues in eggs : hydrophilic solid – phase extraction clean up and liquid chromatography /tandem mass spectrometry analysis of tetracycline , fluoroquinolone , sulfonamide , and B – lactam residues. J. Agric . Food Chem. , **54** (15) , 5267 - 5278 .
[22] Nagaraja P. and Shrestha A. K.(2010).Spectrophotometric method for the determination of drugs containing phenol group by using 2,4 - dinitrophenylhydrazine . E – J. of Chem. ,**7** (2) , 395 – 402 .
[23] Al-Uzri W.A.(2012).Spectrophotometric determination of amoxicillin in pharmaceutical preparations through diazotization and coupling reaction . Iraqi J. of Sci.,**53**(4),713-723 .
[24] Sueny K.B. and Dasilva V. L.(2011). Amulti commuted flow analysis method for the photometric determination of amoxicillin in pharmaceutical formulation using a diazo coupling reaction. J.Braz.Chem.Soc., **22**(2) ,279-285.
[25] El– Ashry S.M., Belal F. , EL-Kerdawy M.M.and EL Wasseef D.R.(2000).Spectrophotmetric determination of some phenolic antibiotics in dosage forms. J.Microchimica acta ,**135** (3-4) , 191 – 196.
[26] Al-Uzri W. A.(2012).Spectrophotometric determination of amoxicillin in pharmaceutical preparations through diazotization and coupling reaction. Iraqi J. of Sci. ,**53**(4.), 713-723.
[27] Pasha I.S., Mohammed T., Kumar S.A. and Nkhila V.(2012).New visible spectrophotometric methods for the determination of amoxicillin trihydrate in bulk drug and their formulations . Int.J.of Pharma. and Ind. Res. ,**2**(2) ,106-109.
[28] Saleh G.(1996). Two selective spectrophotometric method for The determination of amoxicillin and cefadroxil . Analyst , **121** , 641 – 645 .
[29] Revanasiddappa H.D. and Veena M.A.(2008).A sensitive spectrophotometric determination of ritodrine , pentazocine , isoxsuprine hydrochlorides and amoxicillin in pure and pharmaceutical samples. E– J.of Chem., **5** (1), 100 – 106 .
[30] Haidar O. I.(2015).Simple indirect spectrophotometric determination of amoxicillin in pharmaceutical preparations . J. of Nat.Sci. Res.,**5**(16), ,142-147.
[31] Othman N. S and AL-Saffar R. S. (2015).Spectrophotometric determination of amoxicillin in pharmaceutical preparations . Int. J.of Enh. Res. in Sci. Tech. and Eng., **4** (6), ,167-173.
[32] Mrudul R. K. and Ravin M. J,(2014)" A new spectrophotometric method for determination of amoxicillin using bromocresol green. World J.of Pharmacy. and Pharma. Sci., **3**, 2, 1340-1348.

[33] Gujral R. S. and Haque S. M.(2010). Simultaneous determination of potassium clavulanate and amoxicillin tri-hydrate in bulk , pharmaceutical formulations and in human urine samples by UV spectrophotometry. Int. J . Biomed. Sci. , **6** (4) , 335 – 343.

[34] Vishal D.M. , Tukaram G.V. and Pramod J.P.(2011). Simulltanous estimation of amoxicillin trihydrate and bromhexine hydrochlorid in oral solid dosage forms by spectrophotometric method. IRJP, **2** (3) , 197 - 201.

[35] Ünal K., Palabiyik İ.M. , Karacan E. and Onur F.(2008).Spectrophotometric determination of amoxicillin in pharmaceutical formulations . Turk J . Pharma. Sci **.**, **5** , (1) ,1-16 .

[36] Dikran S.B.and Hussan M.J.M.(2009).First- and second- order derivative spectrophotometry for individual and simultaneous determination of amoxicillin and cephalexin . NJC.,**34** ,260-269.

[37] Al-Saidi K.H.and Abdulameer S.S.(2011).Simultaneous determination of amoxicillin and potassium clavulanate antibiotics in pharmaceutical sample using derivative spectrophotometric method. J.Biotech. Res.Center ,**5** (3) ,49.

[38] Jinghua Y., Wei W. F. and Jiadong D.(2009). Determination of amoxicillin with novel rhodanine ramification by flow injection chemiluminescence. 3rd International Conference on Bioinformatics and Biomedical Engineering , 1-4.

[39] AL-Abachi M.Q. and Subhi S.(2013).Flow injection –spectrophotometric determination of phenylephrine hydrochloride and amoxicillin trihydrate in pharmaceutical preparations. J.of Al-Nahrain Univ. , **16** (1) ,42-52..

[40] Al-Abachi M.Q. and Hadi H.(2009). Flow injection spectrophotometric determination of amoxicillin in pharmaceutical samples by coupling with diazotized p-nitroaniline. Iraqi. J . Mark . Rec . Cons . Protection , **1**(1), 105 – 119 .

[41] Ejele AE. , Anyawu NU. and Anunuso CL.(2011).Evaluation of drug quality II : Determination of amoxicillin can concentration in proprietary capsule. Inter. J. of Nat. and Appl. Sci. , **7** (1) , 109 – 114.

[42] Blazheyevskiy M.Y., Karpova S.P. and Kabachnyy I.V.(2013).Guantitative determination of some penicillin by iodometric method using potassium peroxomono sulphate. J.Chem. and Pharma. Res., **5**(11) , 637-643.

[43] Montes A. , Nunes A. , Gordillo M.D. , Pereyra C. , Duarte C.M.M. and Delassa E.J.M.(2013).Amoxicillin and ethyl cellulose precipitation by two supercritical ant-solvent processes. J.Chem.Technol.,665-667.

[44] Montes A., Tenorio A., Gordillo M.D., Pereyra C. and Delaassa E.J.M.(2010).Screening design of experiment to supercritical ant-solvent precipitation of amoxicillin :Exploring new miscible conditions. J. of Supercritical Fluids,**51**, 399 – 403 .