How to Cite:
Gupta, K., & Shrivastava, P. (2022). Development and validation of UV spectrophotometric method for trimethoprim in pure and marketed formulation. *International Journal of Health Sciences*, *6*(S2), 10829–10839. https://doi.org/10.53730/ijhs.v6nS2.7860

Development and validation of UV spectrophotometric method for trimethoprim in pure and marketed formulation

Khushboo Gupta
M.Pharmacy, Department of Pharmaceutical Analysis and Quality Assurance, Kalinga University - Faculty of Pharmacy, Kotni near Mantralay New Raipur Chhattisgarh, 492101, India
Corresponding author email: khushboo.gupta@kalingauniversity.ac.in

Pranjul Shrivastava
M.Pharmacy, Department of Pharmaceutics, Kalinga University - Faculty of Pharmacy, Kotni near, Mantralay New Raipur Chhattisgarh, 492101, India
Email: pranjul.shrivastava@kalingauniversity.ac.in

.

***Abstract*---**Trimethoprim drug is an aminopyrimidine antibiotic with a methylene bridge linking the pyrimidine 2,4-diamine and 1,2,3-trimethoxybenzene functional groups.It is helpful in the treatment of UTI (Urinary Tract infection). A rapid and sensitive method was develop for the analysis of Trimethoprim in both pure and tablet dosage form. The Methanolic solution of trimethoprim pure drug at room temperature (25 ± 10C) was absorbed at a maximum of 285 nm. Under optimal and validated test conditions, Beer's rule was obeyed in the 10 – 60 µg / ml concentration range. he experiment was conducted for linearity, accuracy, precision(Intra day & interday precision), Range, detection limits, quantification limits, robustness, and ruggedness. The trimethoprim was also analysed by preformulation parameters with respect to Melting point, solubility,determination of λmax, calibration curve. The assay of marketed formulation was also performed.

***Keywords*---**trimethoprim, validation, photometer, analytical.

Introduction

Trimethoprim is an antibacterial agent with chemical structure named as 2,4diamino-5-(3',4',5-trimethoxybenzyl) pyrimidine. The scientist named Bushby and Hitchings synthesized Trimethoprim for first time. This molecule Trimethoprim was developed for the treatment of urinary tract infection.(1-

2).Trimethoprim is available in a single dose tablet as well as in combined dosage form i.e. Trimethoprim and sulfadiazine tablet. Its molar mass is 290.32g/mol $g.mol^{-1}$ .Its solubility is in Methanol, Ethanol , n-butanol.(3,4).This drug stops the function of enzyme dihydrofolate reductase for the formation of folic acid which is responsible for the structured DNA.(5,6) It can also be used to combat pathogenic bacteria .(7) Trimethoprim is only recommended for the treatment of uncomplicated, symptomatic urinary incontinence in the first instance. (8) The combination of Trimethoprim and Sulfadiazine can be effective against Acinetobacter, Aeromonas hydrophilla, Bartonella henselae, Burkholderia Pseudomallei , Brucella, Moraxella Catarrhalis , Mycobacterium tuberculosis (9-10)

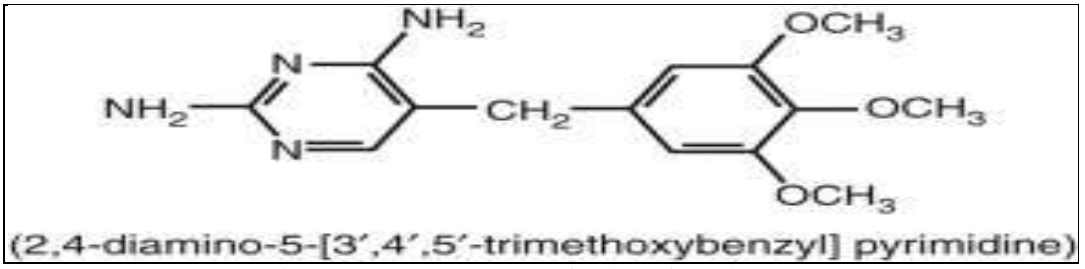

Figure -01 Structure of Trimethoprim (11)

The evidence shows that only a few selected spectrophotometer, HPLC-LC-MS and HPLC techniques for the analysis of Trimethoprim and sulfadiazine were recorded in conjunction with other drugs n bulk biological samples.(12-17)

Experimental

Chemicals and reagents

Methanol of analytical grade was used as a solvent in the whole experiment and all other chemicals and reagents used were AR grade. Trimethoprim pure drug was obtained from Indo safe pharmaceuticals from Jaipur. Purchased marketed tablets of Trimethoprim 70mg.

Instrumentation

A shimadzu UV-Spectrophotometer (UV-1800) with a pair of quartz cell were used in experiment.

Experimental work

Melting point

The drug's melting point was calculated using the Thieles tube process, In a capillary tube with one end sealed, a small quantity of drug substances was taken, using a Bunsen Burner. About 0.5 gm of completely dried and finely powdered compound was filled in capillary tube and was closed. The capillary tube was attached towards lower end of the thermometer. The thermometer was placed with the capillary tube in a thieles tube having liquid paraffin. The tube

was dipped in thieles tube and the temperature at which the drug melts was noted.(18)

Solubility Study

The solubility was determined by dissolving the drug in the different solvents. The excess amount of 100mg drug was weighed and dissolved in 5ml of methanol till to receive saturated solution. Further the saturated solution was filtered and the supernatant was analysed by UV spectrophotometer. The solubility study was also performed thrice for water, 0.1N HCl ,NaOH , Ethanol ,Methanol , Chloroform and water.(19)

Determination of λmax

A solution of Trimethoprim of concentration 10 µg/ml prepared in methanol and UV spectrum was taken. The solution analyzed in the range of 200-400nm.(20)

Standard calibration curve

100mg drug was taken and dissolved in Methanol to gives 1000 µg/ml in volumetric flask. The stock solution was diluted with Methanol to get 10 to 50 µg/ml of Trimethoprim. Methanol was used to determine the absorbance as blank at 285 nm using UV visvible spectrophotometer. The graph was plotted between concentration and absorbance in order to draw calibration curve. (21-22)

Standard solution Preparation

By dissolving 100 mg of Trimethoprim pure drug in 100ml of Methanol in a 100ml volumetric flask, the standard stock solution was prepared and diluted with methanol up to the mark to prepare a stock solution of 100µg / ml power.(23)

Sample solution Preparation

100mg of trimethoprim (tablet 70mg) was dissolved in 100ml volumetric flask and diluted to 100ml via methanol so that solution will be of 100µg / ml.(24)

Analytical Validation

System validation of the planned method is validation process established under the International Guideline Harmonization (ICH) conference under section Q2 (R1)

Linearity

Linearity is the experimental technique to produce results that linearity and concentrations are directly proportionate to each other within a given range of analytes in the sample. Fresh aliquots were prepared ranging from 10-60 µg / ml of standard stock solution and the absorbance of aliquots was recorded at 285 nm. Methanol as the reference solution for this process was used. The calibration curve was plotted between Absorbance and Concentration. The

correlation coefficient with the regression line of the Trimethoprim equation was determined.(25-26)

Precision

This parameter was performed in order with the same day i.e intraday precision by analyzing the 6 independent aliquots ranging from 10-60g/ml thrice a day with interval and absorbance was recorded at 284 nm. And the same procedure was carried out in different days i.e Interday precision for 6 aliquots and absorbance was recorded at 285nm. At last % Relative Standard Deviation for inter and intra precision was calculated. (27)

Accuracy

Accuracy of proposed system verified by performing triplicate restoration studies at three separate concentration ranges of 80%, 100%,120% each. It was obvious from the recovery analysis that the procedure for quantitative analysis of the tablet is very reliable, as the statistical findings were within the recommended limits.(28)

Limit of Detection - The detection limit for Trimethoprim by developed system was evaluated using calibration graphs. Limit of detection were analysed by formula
LOD = 3.3 × Standard Deviation\Slope

LOQ - The limit quantification for Trimethoprim by developed system was evaluated using calibration graphs. Limit of detection were analysed by formula

LOQ = 10 × Standard deviation\Slope
(29)

Robustness - The method's robustness carried out by conducting the observation at different wavelength range . The relevant absorbance were taken and outcome showed through %Relative Standard Deviation.

Ruggedness – This parameter was analyzed by different analyst(two) and the resulted absorption has been noted. Findings were shown by percentage Relative Standard Deviation.(30)

Analysis of Marketed tablet formulation of Trimethoprim tablet

Took twenty tablets and powdered them. The 10 mg powder has been accurately weighed and transferred to a 10ml of volumetric flask, where it was sonicated to fully dissolve the drug. The volume was then increased to 10 mL with methanol to obtain a concentration of 1 mg/ml standard solutions. Pipette 1.0 ml of the above standard solutions into a 10 ml of volumetric flask and dilute to the desired concentration. The absorbance of the sample read at 285 nm, and the amount of drug retrieved has been calculated. (31-33)

Result and Discussion

Melting point

Melting point of Trimethoprim has been recorded between 198-200°C.

Table 1 Melting point of Trimethoprim drug

S.No	Melting point °C	Average melting point °C
1	198	198
2	200	
3	199	

Solubility

The solubility was observed only by visual inspection . we can conclude that trimethoprim showed solubility in methanol, water, ethanol and chloroform.

Table 2 Solubility observation of Trimethoprim drug

S.no	Solvent	Observation
1	Methanol	Freely Soluble
2	Ethanol	Found Soluble
3	Water	Found soluble
4	Chloroform	Found Soluble

Standard calibration curve

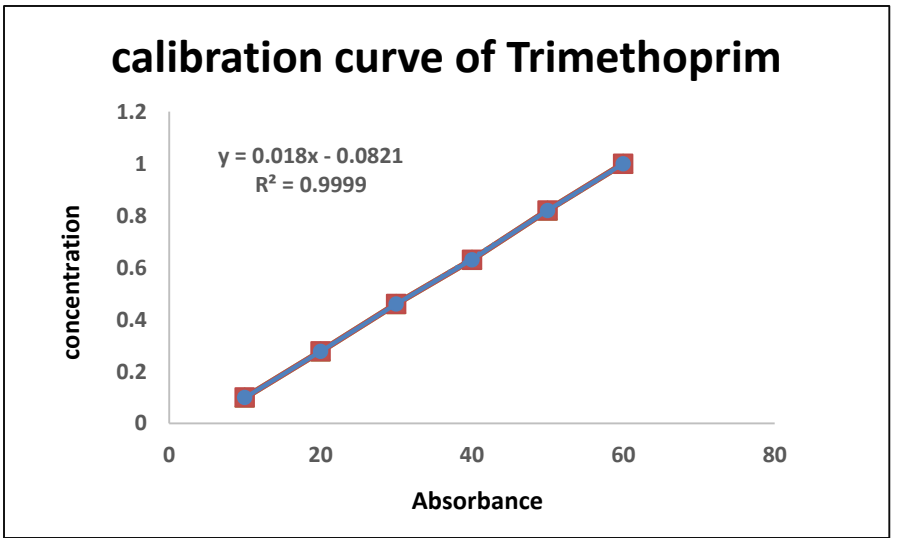

Fig 2 Calibration Curve of Trimethoprim

Table 3 Trimethoprim Absorbance

Concentration (µg/ml)	Absorbance
10	0.12
20	0.27
30	0.46
40	0.63
50	0.82
60	1.01

Linearity

The linearity of Trimethoprim has been recorded from 10(µg/ml) to 60 (µg/ml) with absorbance 0.23 ,0.33, 0.45, 0.58, 0.67 & 0.79 respectively. It followed Beer's law. The correlation coefficient was found to be 0.9983.The equation found was y = 0.0113x + 0.1133.The observation data is shown in table 4.

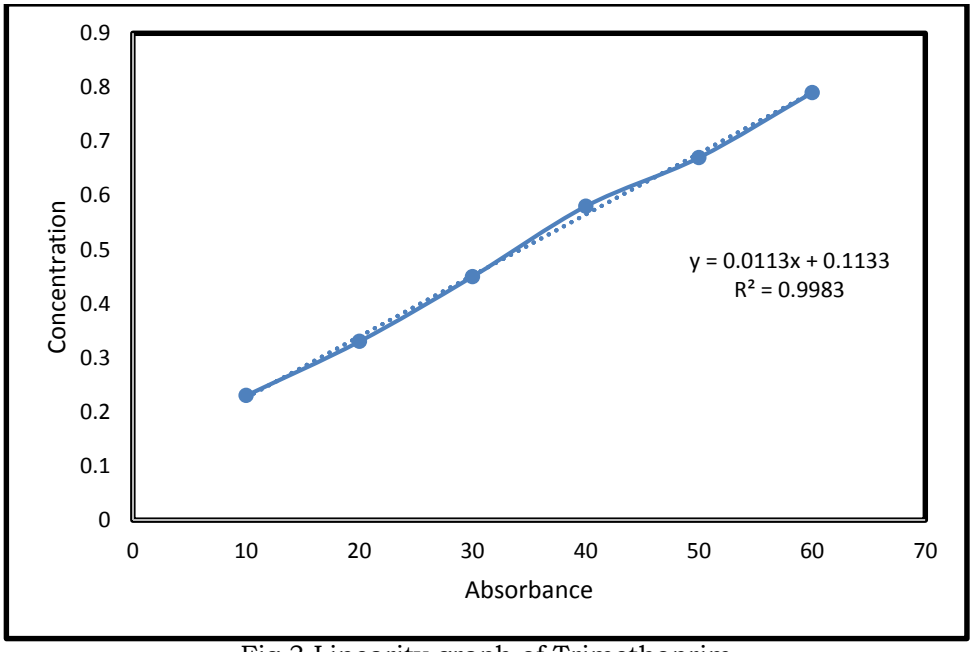

Fig 3 Linearity graph of Trimethoprim

Table 4 Linearity of Trimethoprim

Concentration(µg/ml)	Absorbance
10	0.23
20	0.33
30	0.45
40	0.58
50	0.67

60	0.79

Precision

The method was validated by intraday and interday precision. The result of interday and intraday prescision are shown in table 5 and table 6.Standard deviation of interday precision ranges from 0.011 - 0.891 and % RSD found to be in the range 1.69% to 6.97%. The % RSD was found in the range 1.00% to 4.15%. The result obtained showed minimum deviation and are with in a limit.

Table 5 Result of interday precision

Result of interday precision		
conc	abs mean ± SD (n=3)	%RSD
10	0.087 ± 0.011	2.64%
20	0.759 ± 0.104	4.45%
30	1.106 ± 0.154	1.92%
40	2.021 ± 0.118	2.53%
50	5.386 ±0.891	1.69%
60	8.385 ± 0.754	6.97%

Table 6 Result of intraday precision

Result of intraday precision		
Conc	abs mean ± SD (n=3)	%RSD
10	0.463 ± 0.077	1.00%
20	1.016 ± 0.031	2.17%
30	1.365 ± 0.077	1.92%
40	1.65 ± 0.109	1.81%
50	1.959 ± 0.223	4.15%
60	2.424 ± 0.300	2.12%

Limit of detection and Limit of quantification

Table 7 Result of LOD

LOD = 3.3 × σ \ S
3.3 × 0.2588\0 .018 =0.446 µg/ml

Table 8 Result of LOQ

LOQ = 10 × σ \ S
10 × 0.2588\0 .018 = 0.346 µg/ml

Accuracy

The accuracy was determined by % recovery method. The % mean recovery calculated ranges between 99.12±0.05 to 98.99±0.28.

Table 9 Determination of Accuracy

Accuracy (Recovery) Study			
Conc. (µg/ml)	LOA (%)	Amount (µg) Mean	% Mean Recovery
10	80	8.6	98.99±0.28
	100	11.6	
	120	12.63	
20	80	9.3	99.32±0.23
	100	13.6	
	120	14.49	
30	80	9.3	99.12±0.05
	100	13.6	
	120	14.49	

Robustness & Ruggedness

The robustness and ruggedness determination data is shown in table 10 & table 11. The robustness of the method was recorded 2.82% , 3.10% and 2.22% at wavelength 280nm , 284nm and 288nm respectively.

Table 10 Results of Robustness study

Robustness								
At 280			At 285			At 289		
Abs	X ± SD	% RSD	Abs	X ± SD	% RSD	Abs	X ± SD	% RSD
0.82			0.81			0.91		
0.85	0.85 ±		0.85	0.84 ±		0.94	0.93 ±	
0.88	0.024	2.82%	0.87	0.043	3.10%	0.95	0.03	2.22%

Table 11 Results of Ruggedness study

Concentration (µg/ml)	Absorbance at 285nm	
	Analyst 1	Analyst 2
10	0.38	0.38
20	0.47	0.46
30	0.68	0.62
40	0.72	0.72
50	0.84	0.81
60	0.92	0.91

Analysis of marketed table

The percent recovery of the drug in formulation ranged from 98.42 to 99.21 percent in commercially available Trimethoprim tablets. The findings show that the percent drug content in marketed formulations is almost identical to that of pure drug.

Tablet	Label claim (mg)	Amount recovered	% drug recovered	Mean ± Standard Deviation	% RSD
Trimethoprim	70	69.9	98.42	69.48±0.28	0.34%
Trimethoprim	70	69.11	98.72		
Trimethoprim	70	69.45	99.21		

Result

The proposed UV visible Spectroscopic method has been recorded precise , selective, rapid and economical. Validated in terms of precision , linearity, accuracy ,LOD , LOQ and ruggedness. Trimethoprim exhibited maximum absorption at 285nm and obeyed Beer's law in the concentration range 10 - 60 µg/ml. The proposed method shoed linear regression y = 0.018x - 0.0821 with a correlation coefficient (R2) of 0.9999. The developed technique has been recorded to be precise as the % Relative Standard Deviation values for intra day and interday has been resulted to be 0.031 to 0.223 and 0.011 - 0.891 respectively. For each added concentration, good drug recoveries (98.99 percent to 99.12 percent) were obtained, indicating that the procedure was successful. The Limit Detection and Limit Quantification resulted 0.446 µg/ml and 0.346 µg/ml respectively. The technique resulted robust and rugged as indicated by the %Relative Standard Deviation values 2.82% at 280 nm , 3.10% at 284 nm and 2.22% at 288nm.

References

1. Gupta khushboo & gidwani bina, The trimethoprim study: mechanism, pharmacology, tolerance and determination using specific spectroscopic strategies, european journal of biomedical And pharmaceutical sciences Http://www.ejbps.com, *2020, volume 7, issue 11, 91-96.*
2. Asscher .4\V: Urinary tract infections, Br. bled. J. 1: 1332 (1977).
3. Gupta Khushboo, Shrivastava Pranjul , Prajapati Amarjeet ,"An orview on Chromatography ",International Journal of Creative research thoughts, Volume 8, Issue 4 April 2020 ISSN: 2320-2882
4. Bactrim, Bactrim DS (trimethoprim/sulfamethoxazole) dosing, indications, interaction, adverse effects and more.
5. Wormer,GP; Keusch,GT;Heel RC (December 1982)." Co-trimoxazole (trimethoprim-sulfamethoxazole) an update review of its antibacterial activity and clinical efficacy" drugs. 24 (6): 459-518.
6. Amina M.A Bass , Sahar et.al " Determination of trimethoprim by various analytical techniques A-Review" ISSN : 2456-6934.
7. Salim AM, Haseeb YSZ. Spectrophotometric determination of sulfadiazine via diazotization and coupling reaction-application to pharmaceutical preparations. Raf J Sci 2013;24:61-73.
8. Goran S, Elizabeta DS, Marija S, Romel V. Optimization, validation and application of UV-Vis spectrophotometric colourimetric methods for determination of trimethoprim in different medicinal products. Mac Vet Rev 2016;39:65-76

9. Gentleman MS, Burt HM, Kitts DD, McErlane KM. Highperformance liquid chromatographic determination of sulphadiazine and trimethoprim in chinook salmon muscle tissue. J Chromatogr A 1993;633:105–10.
10. Batzias GC, Botsoglou NA, Kotsaki-Kovatsi VP, Kounenis G. New simple liquid chromatographic method for the determination of trimethoprim, sulfadiazine and n4-acetylsulfadiazine in plasma of broilers. J Chromatogr B Anal Technol Biomed Life Sci 2002;769:253-9
11. Nachilobe P, Cassidy RM, Fesser ACE. Determination of trimethoprim in bovine serum high-performance liquid chromatography with confirmation by thermospray liquid chromathography-mass spectrometry. J Chromathogr 1993;616:243-52.
12. Nordholm L, Dalgard LA. Assay of trimethoprim in plasma and urine by high-performance liquid chromatography. J Chromatogr 1982;233:427-31.
13. Stephen H. Zinner, Kenneth H. Mayer, in Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases (Eighth Edition), 2015.
14. Brumfitt W, Pursell R. Trimethoprimsulfamethoxazole in the treatment of bacteriuria in women. J Infect Dis., 1973; 128(suppl): S657-S663.
15. Derek G. Waller BSc (HONS), DM, MBBS (HONS), FRCP, Anthony P. Sampson MA, PhD, FHEA, FBPhS, in Medical Pharmacology and Therapeutics (Fifth Edition), 2018.
16. Hamilton-Miller JMT. Mechanisms and distribution of bacterial resistance to diamino-pyrimidines and sulfonamides. J Antimicrob Chemother, 1979; 5(supple): 61 -73.
17. Calaca G.N., Pessoa Ch.A., WohnrathK., Nagata N., "Suimultaneous Determination of Sulfamethoxazole and Rtimethoprim in Pharmaceutical Formulations by Square Wave Voltammetry", International Journal of Pharmacy and Pharmaceutical Sciences, 2014; 6(9).
18. FDA approved drug products: sulfamethoxazole /Trimethoprim oral suspenson,www.accessdata.fda.gov
19. FDAapproved drug product:Bactrim oral tablets,www.accessdata.fda.gov 26. Reddy S.P., Vardhan S.V.M., Krishna K.B., Rambabu C.," Extractive spectrophotometric determination of trimethoprim in pharmaceutical formulations", ACAIJ, 2014; 14(11).
20. VenipokalaR., Kumari K., Bollikola H.B.," UV Spectrophotometry Method Development and Validation of Sulfadiazine and Trimethoprim in Combined Dosage Form", Int J Pharm Pharm Sci, 2018; 10(1): 103-107.
21. Stojković G., Dimitrieska-Stojković E., MarijaSoklevska M., RomelVelev R.," Optimization, Validation and Application of UVVISSpectrophotometric-Colorimetric Methods for Determination of Trimethoprim in Different Medicinal Products", Mac Vet Rev, 2016; 39(1): 65-76
22. Schwartz DE, Ziegler WH. Assay and pharmacokinetics of trimethoprim in man and animals. Postgrad Med J., 1969; 45(S~ppl): 32-33.
23. Derek G. Waller BSc (HONS), DM, MBBS (HONS), FRCP, Anthony P. Sampson MA, PhD, FHEA, FBPhS, in Medical Pharmacology and Therapeutics (Fifth Edition), 2018.
24. Stephen H. Zinner, Kenneth H. Mayer, in Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases (Eighth Edition), 2015.
25. Mark Kester PhD, ... Kent E. Vrana PhD, in Elsevier's Integrated Review Pharmacology (Second Edition), 2012.

26. Medina R., Miranda M., Hurtado M., DomínguezRamírez A.M., Octavio Reyes O., Ruiz-Segura J.C.," In vitro Evaluation of Trimethoprim and Sulfamethoxazole from Fixed-Dose Combination Generic Drugs using Spectrophotometry: Comparison of Flow-Through Cell and USP Paddle Methods", Trop J Pharm Res, 2015; November, 14(11): 2061-2069.
27. Lakkanatinaporn P., Matayatsuk Ch.," Simultaneous HPLC method for determination of sodium trimethoprim phenylpropanoldisulphonate and sodium sulfaquinoxaline in veterinary drugs ", Songklanakarin J. Sci. Technol., 2004; 26(6).
28. Welcome co. Burroughs, ”Trialkoxybenzylpyrimidines and method”, 1959.
29. Welcome co. Burroughs, ”Chemotherapeutic solutions containing a Sulphur and a salt of a 2,4- diamino-5-benzylpyrimidine”, 1976.
30. AktiengesellschaftHoechst, ”esters of 5-methyl10,11-dihydroprostaglandin-A1”, 1980. 35. Lion corporation, oral composition, 1987.
31. John P Curtis, ”Appettite Suppressant dentifrice”, 1990. 37. Rajkumar et al., ”Trimethoprim in Paediatric Urinary tract infection” Urol.1988-1989, 9: 77-81.
32. Flory ed, ”Analytical Profile of drug substances, 7: 445-475.
33. Marcus JC Long, Yue Pan, Hsin-Chieh Lin, Lizbeth Hedstrom, Bing Xu, ”Jornal of the American chrmical society, 2011; 133(26): 100006-10009.