Estimation of Emtricitabine and Tenofovir by HPTLC Method

Vijaykumar Morea[1], Abhay Bondgeb[2] and Kalimoddin Momin[3]

[1]Dept. of Chemistry, Kai. Rasika Mahavidyalaya Deoni, Dist-Latur, India
[2]Dept. of Chemistry, Shivneri Mahavidyalaya Shirur Anantpal, Dist-LaturIndia
[3]Dept. of Chemistry, RajarshiShahu Mahavidyalaya, Latur, Dist-Latur, India
Email- [1]vijaymore@gmail.com,

ABSTRACT:-

The HPTLC procedure was optimized for simultaneous determination of Emtricitabine and Tenofovir. The mobile phase Methanol: Toluene: Ethyl acetate: Ammonia (1.5:5.5:1.5:0.1 v/v/v/v) resulted in good resolution, and sharp and symmetrical peaks were obtained. It was observed that prewashing of HPTLC plates with methanol (followed by drying and activation) and pre saturation of HPTLC chamber with mobile phase for 20 min (optimum chamber saturation time) ensured good reproducibility and peak shape of three drugs.

KEY WORDS: *Mobile phase, environment-friendly solvents.*

1. INTRODUCTION:

High Performance Thin Layer Chromatography (HPTLC) is a powerful method equally suitable for qualitative and quantitative analytical tasks HPTLC has been reported to provide excellent separation, qualitative and quantitative analysis of a wide range of compounds, such as herbal and botanical dietary supplements, nutraceuticals, traditional western medicines, traditional Chinese medicines and Ayurvedic (Indian) medicines and determination of radio labeled substances in chemical, biochemical, biological, pharmaceutical, and medicinal samples. It includes the ability to analyze crude samples containing multi-components, application of large number of sample and a series of standards using the spray-on technique, choice of solvents for the HPTLC development is wide as the mobile phases are fully evaporated before the detection step, processing of standards and samples identically on the same plate leading to better accuracy and precision of quantification, different and universal selective detection methods, and in situ spectra recording in sequence to obtain positive identification of fractions, storage of total sample on layer without time constrains[1-3].

HPTLC is the most advanced form of modern TLC. It uses HPTLC plates featuring small particles with a narrow size distribution which results in homogenous layers with a smooth surface to be obtained. HPTLC uses smaller plates (10 × 10 or 10 × 20 cm). HPTLC plates provide improved resolution, higher detection sensitivity, and improved *in-situ* quantification and are used for industrial pharmaceutical densitometry quantitative analysis. Normal phase adsorption TLC on silica gel with a less polar mobile phase, such as chloroform– methanol, has been used for more than 90% of reported analysis of pharmaceuticals and drugs[4].Simple and precise HPTLC methods were developed for the simultaneous estimation of two anti-inflammatory drugs (curcuminand galangin). The method was tailored to analyze both drugs in their commercial dosage form (capsules) with no interference from ingredients. Chromatographic separation was performed over precoated TLC plates (60 F254, 20 cm× 10 cm, 250µm thickness, Merck, Darmstadt, Germany) via a linear ascending technique using n-hexane, ethyl acetate, acetic acid, and methanol as the mobile phase. Detection and quantification was achieved at 404 nm through spectrodensitometricanalysis[5].

The selection of mobile phase is based on adsorbent material used as stationary phase and physical and chemical properties of analyte. The mobile-phase systems are used based on their diverse selectivity properties are diethyl ether, methylene chloride, and chloroform combined individually or together with hexane as the strength adjusting solvent for normal-phase TLC and methanol, acetonitrile, and tetrahydrofuran mixed with water for strength adjustment in reversed-phase TLC. Separations byion pairing on C-18 layers are done with a mobile phase such as methanol–0.1 M acetate buffer (pH 3.5) containing 25 mM sodium pentanesulfonate (15.5:4.5).

A new high-performance thin-layer chromatographic (HPTLC) method has been established for determination of minocycline in human plasma. Chromatography was performed on aluminium plates coated with silica gel 60F254; the mobile phase was methanol: acetonitrile : isopropanol: water 5:4:0.5:0.5 (v/v)[6].

2. RESULT AND DISCUSSION:

Emtricitabine is 4-amino-5-fluoro-1-[(2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-2-(1*H*)-pyrimidon (Figure I)[7-8]. Emtricitabine is a nucleoside reverse transcriptase inhibitor (NRTI) for the treatment of HIV infection in adults. Emtricitabine is structurally related with Lamivudine. The drug works by inhibiting reverse transcriptase, the enzyme that copies HIV RNA into new viral DNA. Tenofovir is [{(1R)-2-(6-amino-9H-purin-9-yl) -1-methylethoxy}methyl] phosphonic acid (Figure II)[9]. Tenofovirbelongs to a class of antiretroviral drugs known as nucleotide analogue reverse transcriptase inhibitors (nRTIs), which block reversetranscriptase, an enzyme crucial to viral production in HIV-infected people. Literature review revealed that UV[10-14],HPLC[15-23]and HPTLC[19-22]methods have

been reported for analysis of Emtricitabine and Tenofovir as a single form and in combination with other drugs. To date there have been no published reports on simultaneous quantitation of Emtricitabine and Tenofovir by HPTLC in bulk drug and in tablet dosage form. This present study reports for the first time the simultaneous quantitation of Emtricitabine and Tenofovir by HPTLC in bulk drug and in tablet dosage form. The proposed method is validated as per ICH Guidelines[23].

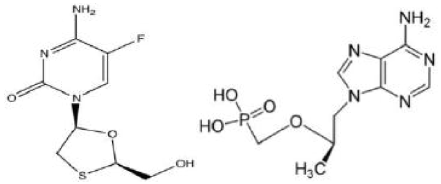

Figure I: Structure of Emtricitabine**Figure II:** Structure of Tenofovir

3. METHOD DEVELOPMENT:

The HPTLC procedure was optimized for simultaneous determination of Emtricitabine and Tenofovir. The mobile phase Methanol: Toluene: Ethyl acetate: Ammonia (1.5:5.5:1.5:0.1 v/v/v/v) resulted in good resolution, and sharp and symmetrical peaks were obtained at Rf 0.29 ± 0.02, 0.41 ± 0.02 for Emtricitabine and Tenofovir respectively. It was observed that prewashing of HPTLC plates with methanol (followed by drying and activation) and pre saturation of HPTLC chamber with mobile phase for 20 min (optimum chamber saturation time) ensured good reproducibility and peak shape of these drugs.

VALIDATION OF THE METHOD:-

LINEARITY-

Linear regression data for the calibration plots revealed good linear relationships between response and concentration over the ranges 320-1120 ng per spot Emtricitabine and 480-1680 ng per spot Tenofovir. Each concentration was applied in triplicate on the HPTLC plate (Table I).

Parameter	Emtricitabine	Tenofovir
Linearity range	320-1120 ng/spot	480-1680 ng/spot
correlation coefficient (r^2)	0.998	0.999
Slope	8.02	2.52
Intercept	178.8	50.14

Table I: Linear regression data for drugs

LOD AND LOQ: -

The LOD & LOQ were determined from slope of the lowest part of the calibration plot.LOD and LOQ of respected drug shown in table (II).

Parameter	Emtricitabine	Tenofovir
LOD	30.24	51.90
LOQ	91.64	157.29

Table II: LOD &LOQ for drugs

PRECISION:

The precision of the method was expressed as relative standard deviation (RSD, %). The results listed in Table (III)reveal the high precision of the method.

Drug	Conc.(ng/band)	Intra day			Inter day		
		*%mean	*SD	*%RSD	*%mean	*SD	*%RSD
Emtricitabine	800	99.90	0.73	0.73	99.96	0.90	0.90
Tenofovir	1200	99.98	0.72	0.72	99.73	0.82	0.82

Table III: Statistical evaluation of precision of developed method (n=3)

*Mean of three determinations, SD: Standard Deviation, R.S.D: Relative Standard Deviation

RECOVERY STUDIES:

When the method was used for extraction and subsequent analysis of these drugs from the pharmaceutical dosage forms and the extract was over applied with 100 and 120% of additional drug. As shown in the Table (IV) good recoveries of the Emtricitabine and Tenofovir in the range from 98.00 to 102.00 % were obtained at various added concentrations. The average recoveries of three levels (nine determinations) were 99.16±0.40 and 99.71±0.20 % for Emtricitabine and Tenofovir respectively.

Drug	Level of % recovery	%mean	*S.D.	*%R.S.D.
Emtricitabine	80%	99.59	0.13	0.13
	100%	99.23	0.32	0.32
	120%	98.68	0.65	0.65
Tenofovir	80%	98.59	0.60	0.60
	100%	99.93	0.24	0.24
	120%	100.63	1.64	1.64

Table IV: Recovery study Data

*Mean of three determinations, SD: Standard Deviation, R.S.D: Relative Standard Deviation

ROBUSTNESS:

The standard deviations of peak areas were calculated for the aforementioned four parameters (variation in composition of the mobile phase, amount of mobile phase, Time from spotting to chromatography, Time from chromatography to scanning) and coefficients of variation were found to be less than 2% in all cases as shown in Table (V).

Parameters	**% RSD for Emtricitabine***	**% RSD for Tenofovir***
Mobile phase composition (± 0.1 ml)	99.05	98.95
Amount of mobile phase (± 1.0 %)	99.08	98.55
Time from spotting to chromatography (5 min)	98.86	99.14
Time from chromatography to scanning(10 min)	98.94	98.90

Table V: Results of Robustness

*Mean of three determinations, R.S.D: Relative Standard Deviation

FORCED DEGRADATION STUDIES:

HPTLC studies of the samples obtained during the stress testing of Emtricitabine and Tenofovir under different conditions. Different degradations peak as shown in figures 2-10.The mass balance is a process of adding together the assay value and the levels of degradation products to see how closely these add up to 100% of initial value with due consideration of the margin of analytical error. The amount of drug recovered after degradation studies and the Rf of the degradation products are given in table (VI).

a) ACID INDUCED DEGRADATION: The drugs were degraded in the acidic condition and shows different degradation products at Rf 0.15, 0.24 for Emtricitabine and 0.14, 0.29, 0.79 for Tenofovir as shows in the fig. III-IV.

b) BASE INDUCED DEGRADATION: The drugs were degraded in the alkaline condition and shows different degradation products at Rf 0.25 for Emtricitabine and 0.02 for Tenofovir as shows in the fig. V-VI.

c) HYDROGEN PEROXIDE INDUCED DEGRADATION: The drugs were degraded in hydrogen peroxide (3%) at room temperature shows different degradation products at Rf 0.57, 0.37 for Emtricitabine and 0.58 for Tenofovir as shows in the fig.VII-VIII.

Stress condition	Drug	Mass balance (%assay of recovered + %impurities + % degradents)	Rf values of degradation Products
Acid hydrolysis (0.1N HCl)	Emtricitabine	99.99	0.15,0.24
	Tenofovir	99.12	0.14,0.29,0.79
Alkali hydrolysis (0.1N NaOH)	Emtricitabine	100.10	0.25
	Tenofovir	98.96	0.02

Oxidation ($3\%H_2O_2$)	Emtricitabine	99.96	0.57
	Tenofovir	100.02	0.37,0.58

Table VI: Results of Forced Degradation studies

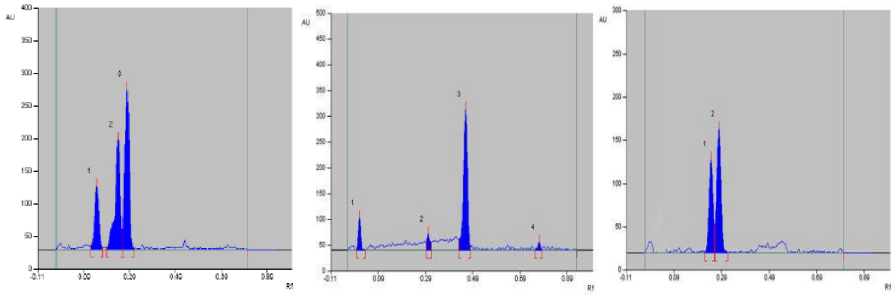

Fig. III: Densitogram of acid hydrolysisFig. IV: Densitogram of acid Fig. V: Densitogram of alkali
Of Emtricitabine hydrolysis of Tenofovirhydrolysis ofEmtricitabine

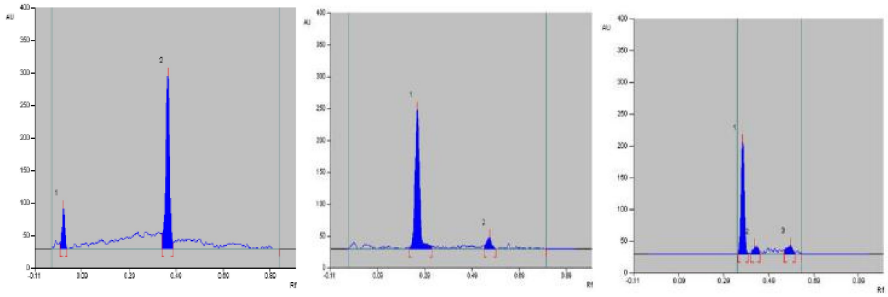

Fig. VI: Densitogram of alkaliFig. VII: Densitogram of oxidative Fig. VIII: Densitogram of oxidative
HydrolysisofTenofovirdegradation of Emtricitabinedegradation of Tenofovir

4. CONCLUSION:-

The proposed method based on the HPTLC was developed and validated as per ICH guidelines. The standard deviation and % RSD calculated for the proposed method is low, indicating high degree of precision of the method. The results of the recovery studies performed show the high degree of accuracy for the proposed method. Hence, it can be concluded that the developed chromatographic method is accurate, precise and selective and can be employed successfully for the estimation of Emtricitabine and Tenofovir in bulk and formulation.

REFERENCES:-

1. CAMAG,(2010-2011). Basic equipment for modern thin layer chromatography. Switzerland: Camag. Available from:camag.com/downloads/free/brochures/CAMAG-basic-equipment-08.pdf
2. CAMAG, (2010-2011). Available rom:camag.com/downloads/free/brochures/CAMAG_TLC10-11_E.pdf
3. Patel, R.B. and Patel, M.R. and Patel, B.G. (2011) Shrivastava, M.M. HPTLC. *New York: Springer*, pp. 41- 54.
4. Shrivastava, M.M. (2011) In:Shrivastava, M.M. HPTLC. *New York: Springer*, pp. 3- 24.
5. Kharat. S, Namde. A, Mehta. P., (2016), *Journal of Taibah University for Science* xxx xxx–xxx, 1-7.
6. Jain, G. K., Jain, N., Iqbal, Z., Talegaonkar, S., Ahmad, F. J., and Khar, R. K., (2007).,(19), 197-205
7. www.drugbank.ca/drugs/DB08864
8. www.rxlist.com/edurant-drug.htm
9. Indian pharmacopoeia, (2007), Govt. of India, Ministry of Health and Family Welfare, *The Indian Pharmacopoeia Commission, Ghaziabad,* (vol- I) 1075-1076.
10. Mithiles. G. S., Jena. S, Dr.Banji. D., Roy S., (2012*), Asian Journal of Research in Chemistry*, 5(12).
11. .Nagaraju P.T, Channabasavaraj. K. P., Shantha Kumar P. T.(2011), *International Journal of ChemTech Research*, 3(01):23-28.
12. Madeesh Sk, Ismail Y .and Gunasekaran A. V., Narendra A, Deepikab. D. And Mathrusri A. M., (2013)
13. Soumya. B., Thimmaraju. M. (2012), *International Journal of Pharmacy and Pharmaceutical Science Research,*]2(01):9-15.
14. Anandakumar. K., Kannan. K. and Vetrichelvan. T. (2010)*Der Pharmacia Lettre*, 2(5): 221-228.

15. Kavitha K Y, Geetha G, Hariprasad R, Venkatnarayana R, (2013), Subramanian G..*International Journal of Comprehensive Pharmacy* 01 (07).
16. Venkata C. R., Devi. P. R., Mukkanti. K., (2012), *International Journal Of Pharmacy And Pharmaceutical Sciences,* 4(3): 595-599.
17. Murali P., Mamade. D. A., Jos H., Ann V. S., (2010), Erwin Adams. *Science Direct Talanta*, 125-128.
18. Syed S. H., Premnath S., Udupa, Krishna. M., (2013), *International Journal Of Pharmacy And Pharmaceutical Sciences,* 5(2):245-248.
19. Havele. S. and Dhaneshwar. S. R., (2012), *Songklanakarin Journal of Science and Technology*, 34(6):612-622.
20. Sentenac, S., Fernandez, C., Thuillier. A., Lechat. P., Aymard. G., (2003), *Journal of Chromatography B,* (793):317–324.
21. Mirna El B., Marie-Claude G., Jerome G., (2007*), Journal of Chromatography B*, (854) :192–197.
22. CH Venkat R., Devi. P. R., Mukkanti. K., Srinivasu. P., (2012 *International Journal Of Pharmaceutical And Phytopharmacological Research*, 2(1): 40-45.
23. Rathore. A. S., Sathiyanarayanan. L, and Mahadik. K. R., (2012), *International Scholarly Research NetworkISRN Chromatography.*

Microwave Assisted Synthesis of β-Amino-ketones in Ionic Liquid

A. S. Kure Patil[1], G. M. Patel,[1] S. K. Akuskar[1], A. M. Patil[2]

[1]Heterocyclic Research Lab, S.M.P. College, Murum, Tal. Omerga, Dist. Osmanabad, Maharashtra- 413605
[2]Department of Chemistry, SPHJ Science College, Chandwad. Dist. Nashik, Maharashtra-423101
Email: abhijeet_kure@rediffmail.com

ABSTRACT:

Microwave irradiation has been used to synthesize β-aminoketones smoothly with low priced and eco-friendly heterogeneous catalyst in solvent free ambient condition Polymer supported ionic liquid medium. A range ofβ-amino-ketones have been obtained in three component tandem reaction between acetophenone or cyclohexanone, Aldehyde, and aniline with a better efficiency (up to 96% yield) within 5 min.

KEYWORDS: *Amino-ketones, Ionic liquid, Mannich, Microwave.*

1. INTRODUCTION:

One of the ultimate goals and challenges in chemistry is to develop the practical synthetic methods for the creation of functionalized molecules and in the journey of this pursuit, development of reusable novel heterogeneous catalysts also has an equally significant to match green chemistry principles in organic synthesis[1, 2].*β*-amino carbonyl compounds from simple and easily available starting materials is one of the such functionalized molecules which unceasingly fascinated to the organic chemists. The Mannich[3-14] type reaction is a classic method for the preparation of *β*-amino carbonyl compounds and therefore a very important carbon-carbon bond-forming reaction in organic synthesis. The versatility and potential to create both functional and structural diversity using this reaction have long stimulated the creativity of chemists[15-17].It has been employed numerous times successfully as a key step in natural product synthesis as well as in medicinal chemistry[18-21].The products of Mannich reaction, *β*-amino carbonyl compound, are synthetic intermediates of huge value. It is widely employed in the organic synthesis of natural compounds, medicine and biologically active compounds[22-26]. Today, it has shown broad applicability in other areas such as pesticides, explosives, dyes and paints. In case of catalyst several perspectives like reusability, sustainability, easy separations are endlessly adding the values in the organic synthesis of natural compounds and to get more and more biologically active products[22, 27] as well. Most Mannich reactions are catalyzed by Lewis acid or Brønsted acid[28-31]. In recent years, the researchers continued to study new synthetic methods and new catalysts including TMG[32], $Sm(OTf)_3$[33], $Fe(HSO_4)_3/SiO_2$[34], triphenylphosphine[35], nano-$Mn(HSO_4)_2$[36], Zeolite[37] were reported. Variety of asymmetric versions of Mannich reaction have been reported[38-43].

Various amino compounds such as amino ketones, amino aldehydes, amino acids and esters etc. can be obtained in a single step using the three component Mannich reaction. *β*-amino carbonyl compounds exhibit a wide range of biological activities. Particularly *anti*-tubercular[44], *anti*-HIV[45], *anti*-cancer[46], *anti*-fungal[47], *anti*-malarial[48] etc. Also, *β*-amino-ketones are the immediate precursors to the 1, 3-aminoalcohols. Although there are many excellent methods for the preparation of *β*-amino-ketones, most of them suffer from the common drawbacks use of strong acids, metal catalysts, long reaction hours, low diastereoselctivity, non-reusable solvents, poor yields etc. Encouraged by the all this researched literature along with pyridinium supported ionic liquid reagents and catalysts[49-52] and as a part of our continual endeavour to develop new heterogeneous catalyst, our team worked to synthesize metal free polymer supported ionic liquid an outstanding heterogeneous catalyst for the synthesis of *β*-amino carbonyl compounds from simple and easily available starting materials. In this manuscript, we have described an environment friendly, microwave assisted, multicomponent method for the synthesis of *β*-amino-ketones in an ionic liquid medium, as a better alternative.

2. RESULT AND DISCUSSION:

Initially we stirred the cyclohexanone **1a**, benzaldehyde **2a** and the aniline **3a** in ionic liquid (prepared from the polymer supported pyridine, 1,2-oxathiane 2,2-dioxide and the triflic acid) (Scheme 1) at 25 °C, over 10 hours, however, we got 30 % conversion to the corresponding *β*-aminoketone **4a**. Carrying out the same reaction at 50 °C for just 90 min. resulted in the formation of the *β*-aminoketone **4a** in 65% yield. Continuation of the same reaction for next 4 hours could not improve the yield further. The elevation in the reaction temperature from 50 °C to 70 °C could reduce the reaction time from 90 min. to 60 min. but could not improve the yield considerably 68%. Increasing the temperature up to 100 °C resulted in 65% of the product yield. Based upon the obtained results It was concluded that the elevation in temperature could offer the yield up to 68% and reduce the reaction time to from 10 h to 1 h (table 1)

Therefore, we thought to try the same reaction under microwave irradiation. Accordingly when the cyclohexanone 1a, benzaldehyde 2a and aniline 3a were subjected under the microwave irradiation (Samsung microwave 470 W) in the prepared ionic liquid, the reaction was complete within 5 min. (monitored using thin layer