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Development and quantification of Ethyl-p-toluene sulfonate and Isopropyl-p-toluene sulfonate at genotoxic level in tenofovir disoproxil fumarate by using advanced technique

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ABSTRACT

A sensitive GC-MS method was developed and validated for the content of Ethyl-p-toluene sulfonate and Isopropyl-p-toluene sulfonate in Tenofovir Disoproxil Fumarate by GCMS. RXI-1 ms column was selected with electron impact ionization technique, Quadrupole mass analyzer. Method is sensitive, precise, accurate and linear as per the parameters conducted for the validation activity. Calibration curve showed good linearity over the concentration range LOQ to 150% of the evaluation level. Limit of detection and Limit of Quantification for Ethyl-p-toluene sulfonate is 0.15µg/g (0.006µg/mL) and 0.45µg/g (0.019µg/mL) respectively. Limit of detection and Limit of Quantification for Isopropyl-p-toluene sulfonate is 0.21µg/g (0.008µg/mL) and 0.65µg/g (0.026µg/mL) respectively. Also method is evaluated for accuracy at 50 to 150% of the evaluation limit.

KEYWORDS

Development;
Validation;
Tenofovir;
Ethyl-p-Toluene sulfonate;
Isopropyl-p-Toluene sulfonate;
Gas chromatography - Mass spectrometer.

INTRODUCTION

Tenofovir Disoproxil Fumarate belongs to a class of antiretroviral drugs as nucleotide analogue transcriptase inhibitors (NRTIs), which block reverse transcriptase, an enzyme crucial to viral production. Tenofovir Disoproxil Fumarate is a pro drug form of Tenofovir and is available in fixed dose combination with Emtricitabine for once in a day dosing. Also it is also available in combination of Tenofovir, Emtricitabine and Efavirenz, which is single dose daily treatment for HIV. Tenofovir is approved for the treatment of HIV as well as for the treatment of chronic hepatitis B.

During the manufacture of Tenofovir, use of P-Toluene sulfonic acid in early stages and further use of alcohols like Isopropyl alcohol and Ethyl alcohol leads to the requirement to check the presence of Isopropyl-p-Toluene sulfonate (IPTS) and Ethyl-p-Toluene sulfonate (ETS). IPTS as well as ETS are the potential genotoxic impurities as they are known to induce genetic mutations and chromosomal abbreviations. As per the EMEA guideline, maximum daily exposure kept for these impurities is 1.5µg per day[1]. Based on the maximum daily dose the limit for these mentioned impurities is decided as 5µg/g for individual impurity and at the same time sum of both has to be less than 5µg/g in Tenofovir API. Also the parameters to be studied for establishing method parameters are decided based on

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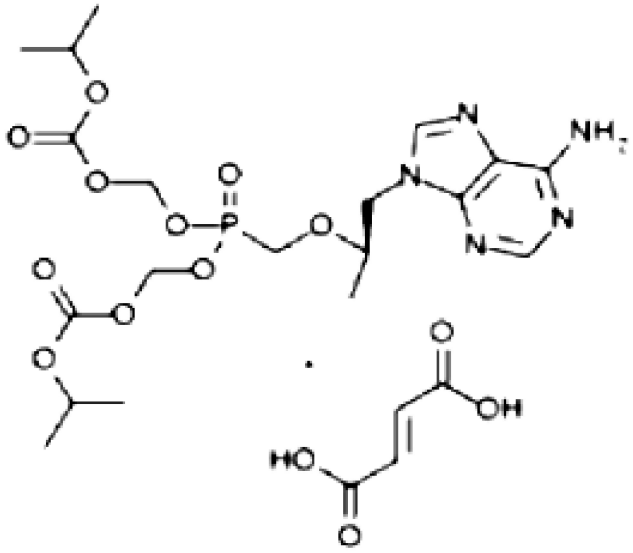
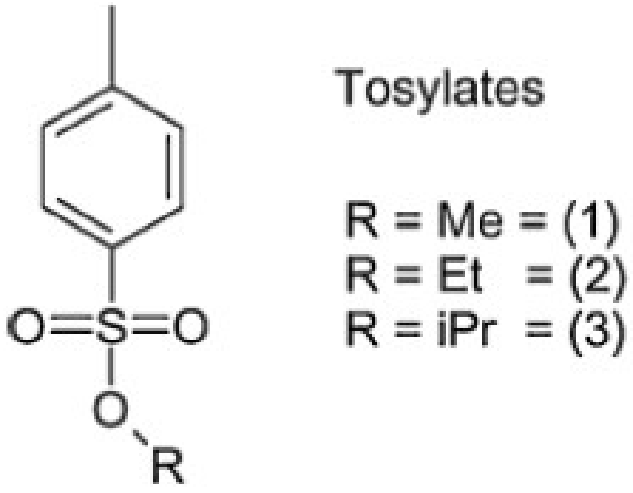
the International conference on Harmonisation guidelines for validation[2,3].

Previously different co-workers have worked on determination of benzene sulfonates and alky benzene sulfonates at trace level using different analytical and sample preparation techniques. Wollein U et.al and co-workers have worked on for the analysis of alkyl mesylates and alkyl besylates using GC-MS with direct injection technique[4]. G. E. Taylor and co-workers have worked on determination of trace level quantification of alkyl benzene sulfonates and benzene sulfonates with LC-MS technique[5]. Also literature can be seen on the quantification of Ethyl and Methyl p-Toluene sulfonates in active pharmaceutical ingredients using HPLC-UV technique[6]. D. P. Elder and co-workers have reviewed the different approaches and techniques used in analyzing the potential genotoxic impurities using GC and HPLC techniques with different detection techniques[7]. Interesting approach used for the analysis of these alkylating impurities is in situ derivatisation using Penta fluoro thio phenol as derivatisation reagent[8]. Quantification of tenofovir by HPLC[9-16], tenofovir by UV Spectrophotometric methods[17]. In this approach different diluent composition are studied with Headspace GC and MS as detection technique.



Tenofovir disoproxil fumarate



General structure of tosylate compounds

Figure 1 : Chemical structure of Tenofovir Disoproxil fumarate and related tosylates impurities proposed for analysis

EXPERIMENTAL

Materials

Reagents used during the development and validation activity are of the analytical grade and with quality testing certificate. Batches used for development and validation activity are of the commercial grade. n-Hexane is used as diluent for the preparation of all the standard solutions as well as sample preparation.

GC-MS operating conditions

Complete analytical development and validation activity is carried out with Shimadzu GCMS QP-2010 with Quadrupole mass analyzer and software control used is GCMS solution version 2.61. Rxi-1ms column is used for the activity with length 60 meter. Column oven programme used is as initial temperature 130°C and initial holding time of 5 min, then the temperature is raised to 250°C at the rate 10°C/minute and the final temperature hold is kept for 3 minutes. Injector port temperature is maintained at 220°C. Ion source temperature and Interface temperature are programmed at 200°C and 220°C respectively. Helium is used as the carrier gas with constant linear velocity 26.6 cm/sec. Ionization energy used for the optimum ionization is 70 eV.

For better sensitivity and specificity SIM mode is used for the analysis. Based on Mass spectra of both the components m/z values screened for SIM mode are 91, 155, 172, 200 and 214.

Preparation of solutions for analysis

n-Hexane is used as blank and is also used as diluent for the preparation of standards as well as sample solutions.

Standard solution is prepared by weighing and dilut-

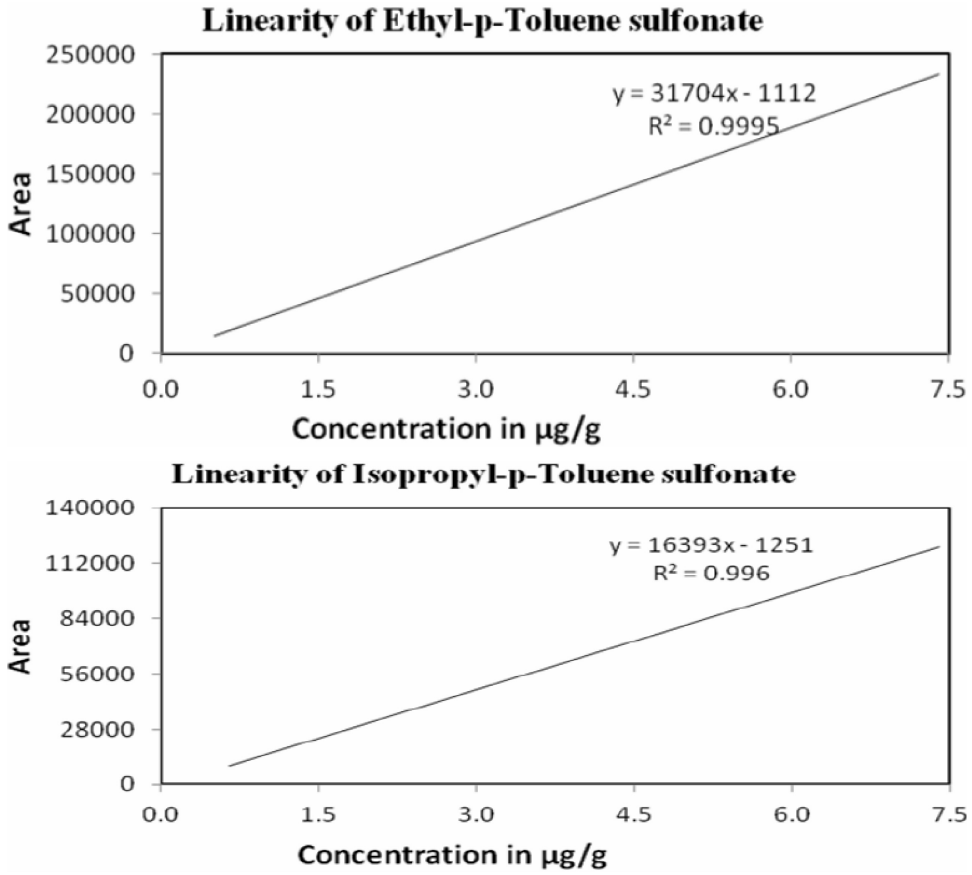


Figure 2

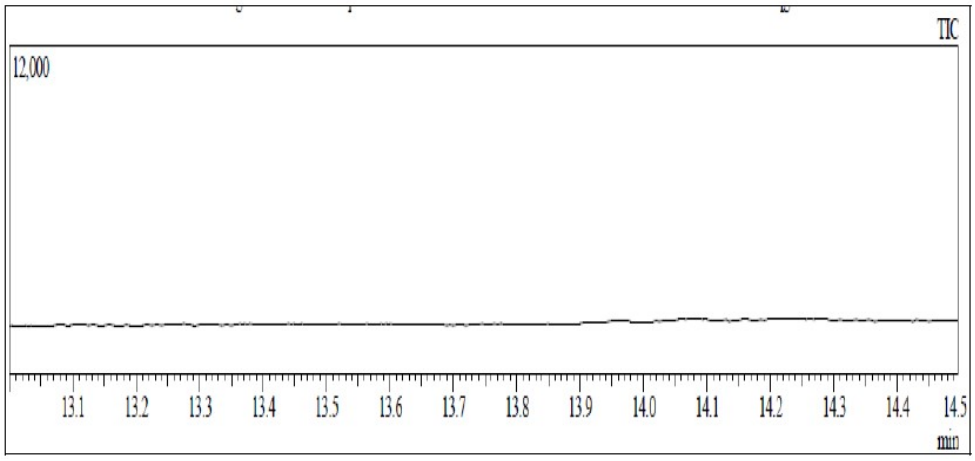


Figure 3 : Chromatogram of blank solution

ing 25mg of each i.e. Ethyl-p-Toluene sulfonate and Isopropyl-p-Toluene sulfonate to 50mL with n-Hexane. Further the 1.0mL of resulting solution is diluted to 50mL and is used as Standard stock solution. Standard stock solution is further diluted 50 times with n-Hexane and is used as final standard solution. Concentration of final standard solution is 5.0µg/g i.e. 0.2µg/mL.

Tenofovir sample solution is prepared by weighing and diluting 0.200g of sample to 5mL with n-Hexane and sonicated the resulting solution for 5 minutes and supernatant solution is used for the analysis.

RESULTS AND DISCUSSION

Method development

First of all the evaluation limits established based

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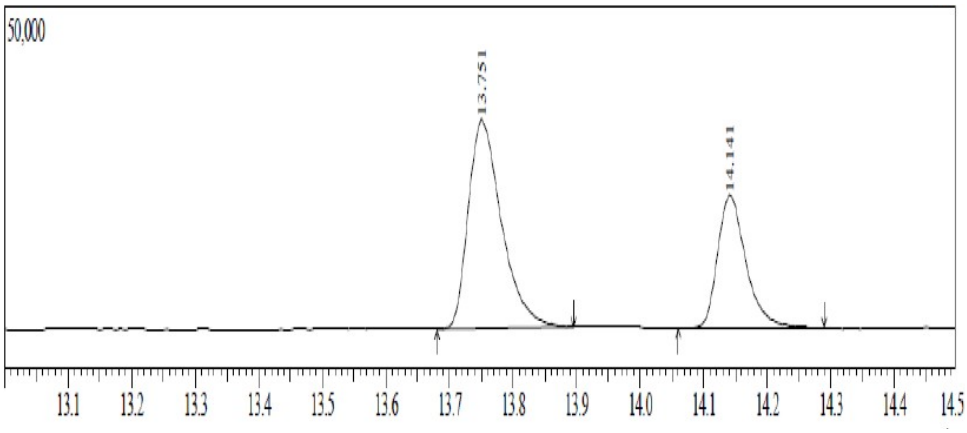

Figure 4 : Chromatogram of Standard solution at Specification concentration SIM mode (Retention time: 13.751min: ETS, 14.141 min: IPTS)

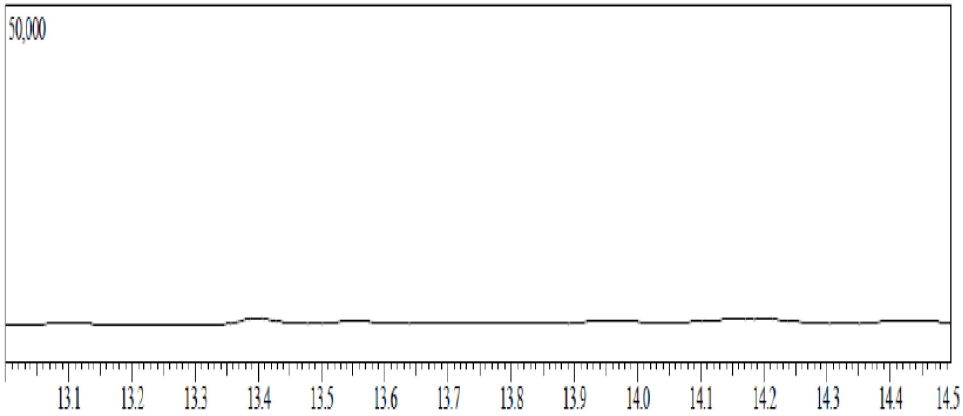

Figure 5 : Chromatogram of sample solution

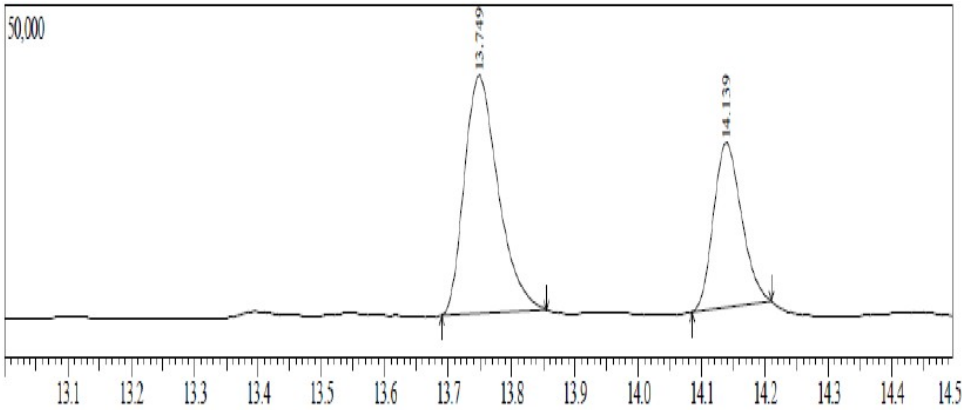

Figure 6 : Chromatogram of Spiked sample solution (Retention time: 13.749 min: ETS, 14.139 min: IPTS)

on daily dose correlation and is proposed as 5µg/g. Diluents used for development activity are Dichloromethane, Methanol, Isopropanol, n-Hexane but based on the recovery and least interference observed at the retention time of analytes, n-Hexane is finalized as diluent. Column used for the development trials are DB-5, DB-1, DB-624 and Rtx-1301 with different dimensions. Finally based on peak performance and minimum baseline interference, Rxi-1 ms with length 60 meter, internal diameter 0.25 mm and 0.25 µm film thickness is chosen as suitable.

Solvent cut time is also proposed for the analysis as it will reduce the unnecessary exposure of MS to diluent components. Solvent cut time is kept up to 10.0 minutes from the start of acquisition. Also, MS acquisition is kept 'ON' between 13.0 to 14.5 minutes, this is the range in

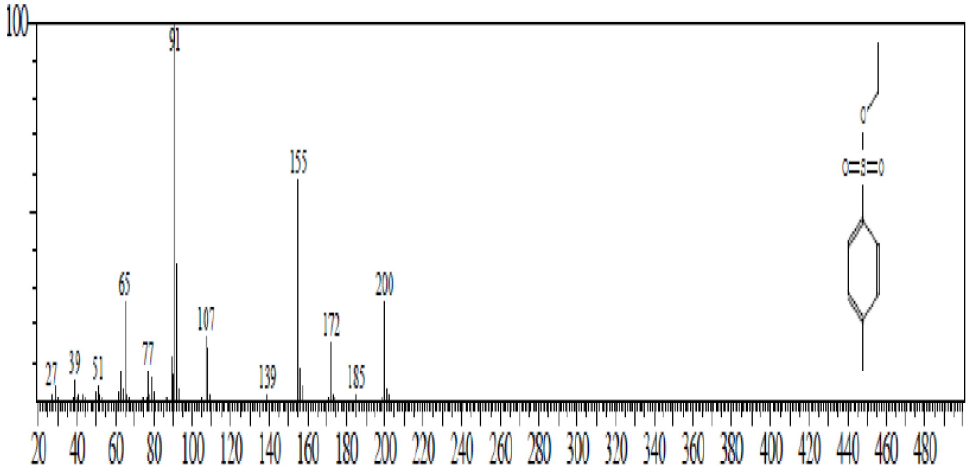

Figure 7 : Spectrum of Ethyl-p-toluene sulfonate

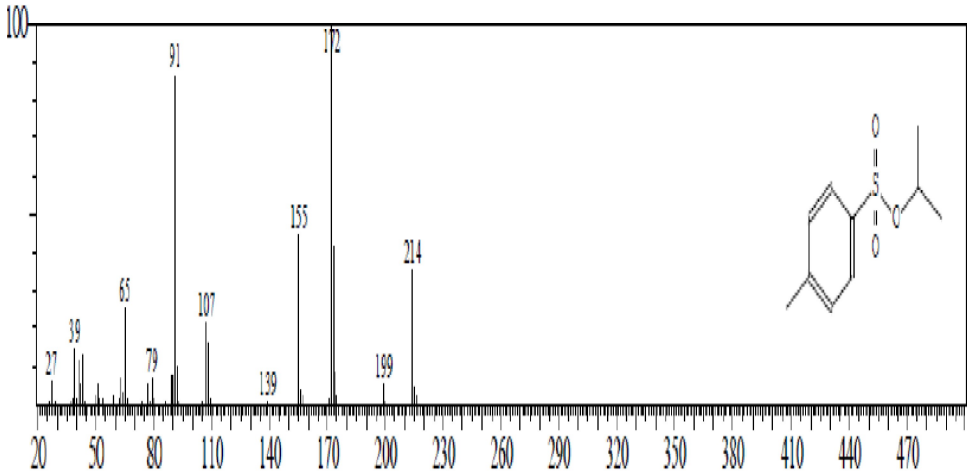

Figure 8 : Spectrum of Isopropyl-p-toluene sulfonate

which ETS and IPTS are eluted out of the column. Purpose behind proposing the MS acquisition range is to avoid the unnecessary exposure of MS to sample components which are not of our analysis interest.

Further based on the full scan of ETS and IPTS in the m/z range 10-500 amu; SIM mode m/z is finalized. For analysis purpose the m/z values selected for SIM mode are 91, 155,172, 200 and 214. Spectra of the analytes are compared with reference spectra in the NIST library.

Method validation

Developed method is proposed for the complete validation to prove its use in the routine analysis. Validation activity is planned on the basis of International Conference on Harmonisation validation guideline.

System suitability

While starting every activity, six injections of standard solution were injected in the system as system suitability solution. System suitability criteria kept is as 15 % relative standard deviation for peak area responses of every component.

Specificity, Limit of detection and limit of quantification

Specificity is performed by injecting all the solvents in the process and concluded that no interference is observed at the retention time of analyte peaks.

Calibration curve method is used for establishment of LOD and LOQ. For LOD and LOQ prediction, standard solutions ranging between 0.1µg/g and 1.9µg/g were prepared and injected into the GC-MS. Further based on the calibration curve and resulting Slope and STEYX values LOD and LOQ values are obtained. LOD and LOQ values obtained for ETS are 0.15µg/g and 0.45µg/g respectively (0.006µg/mL and 0.018µg/mL). LOD and LOQ values obtained for IPTS are

Full Paper

0.21µg/g and 0.65µg/g respectively (0.008µg/mL and 0.026µg/mL). The RSD % obtained during LOQ precision for Ethyl-p-toluene sulfonate is 2.1 and Isopropyl-p- toluene sulfonate is 4.4

Linearity

Linearity of the method is proved on complete quantification range i.e. from LOQ to 150% of the Specified limit. Linearity curve is plotted with peak areas against the concentration of respective analyte. Linearity equation observed for Ethyl-p-Toluene sulfonate is (y = 31704x + 1112) and for Isopropyl-p-toluene sulfonate is (y = 16393x -1251). The correlation coefficient values observed are 0.99975 and 0.99822 for ETS and IPTS respectively which is very good at such a low level.

Precision and accuracy

System precision results observed are 2.8 and 4.2 respectively for ETS and IPTS. Method precision activity is completed by spiking the Tenofovir Disoproxil fumarate sample with standard solution at the specification limit as both analytes were not detected in the sample batches. Results of the precision observed are compared on the basis of % RSD, which is observed to be 1.3 and 2.1 respectively for ETS and IPTS.

The mean results obtained at three accuracy levels i.e 50%,100%and 150% are in the range of 97% to 102% for Ethyl-p-toluene sulfonate and Isopropyl –p-toluene sulfonate.

Mass spectral analysis

Retention time of ETS is 13.7 minutes and IPTS is 14.1 minutes. Based on the retention time of both the components MS acquisition is kept on between 13.0 minutes and 14.5 minutes. Ethyl-p-Toluene sulfonate mass spectra shows fragments at 200, 172, 155, 107, 91and 65. Similarly Isopropyl-p-Toluene sulfonate shows fragments at 214, 172, 155, 107, 91 and 65 respectively. Spectra of both the components is compared and matched with NIST spectrum library.

CONCLUSION

Method is developed and validated for the content of Ethyl-p-Toluene sulfonate and Isopropyl-p-Toluene sulfonate in Tenofovir Disoproxil Fumarate. Based on the parameters performed as per ICH guidelines and analysis of three commercial batches, it is concluded that the method is precise, Accurate, Linear, sensitive for the analysis purpose. Also the method is suitable for the analysis of regular batches as the results obtained are showing high level of consistency and repeatability.

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REFERENCES

[1] Guideline on the limits of Genotoxic impurities, EMEA/CHMP/QWP/251344/2006, **(2006)**.

[2] European Medicines Agency, ICH topic Q 2 (R1), Note for the Guidance on Validation of Analytical Procedures: Text and Methodology, CPMP/ICH/381/95, **(1995)**.

[3] The European Agency for the Evaluation of medicinal Products, ICH topics Q 2 B, Note for the Guidance on Validation of Analytical procedures: Text and methodology, CPMP/ICH/281/95, **(1997)**.

[4] U.Wollein, N.Schramek; Simultaneous determination of alkyl mesilates and alkyl besilates in finished drug products by direct injection GC/MS: Eur.J. Pharm.Sci., **45(1-2)**, 201-4, Jan 23 **(2012)**.

[5] G.E.Taylor, Mark Gosling, Andrea pearce; Low level determination of p-Toluene Sulfonate and Benzene Sulfonate esters in drug substance by high performance liquid chromatography/mass spectrometry, Journal of Chromatography A, **1119**, 231-237 **(2006)**.

[6] A sensitive and simple HPLC-UV method for trace level quantification of Ethyl-p-Toluenesulfonate and Methyl-p-Toluenesulfonate, two potential genotoxins in active pharmaceutical ingradients, Amasa Nageswari, Kallam Venkata siva ram Krishna reddy, Khagga Mukkanti, Sci.Pharma., **79**, 865-876 **(2011)**.

[7] D.P.Elder, A.Teasdale, A.M.Lipczynski; Control and analysis of alkyl esters of alkyl and aryl sulfonic acids in novel active pharmaceutical ingredients (APIs), Journal of Pharmaceutical and Biomedical Analysis, **46(1)**, 1-8, 7 January **(2008)**.

[8] A generic approach for the determination of residues of alkylating agents in active pharmaceutical ingredients by in situ derivatisation-headspace-gas

chromatography-mass spectrometry, Journal of pharmaceutical and biomedical analysis, **45(3)**, 472-479 5 November **(2007)**.

[9] Prashant S.Devrukhakar, Roshan Borkar, Nalini Shastri, K.V.Surendranath; A Validated Stability-Indicating RP-HPLC Method for the Simultaneous Determination of Tenofovir, Emtricitabine, and a Efavirenz and Statistical Approach to Determine the Effect of Variables ISRN Chromatography, **2013**.

[10] P.B.Kandagal, D.H.Manjunatha, J.Seetharamappa, S.S.Kalanur; RP HPLC Method for the Determination of Tenofovir in Pharmaceutical Formulations and Spiked Human Plasma Analytical Letters, **41(4)**, **(2008)**.

[11] Anandakumar Karunakaran, *Kannan Kamarajan,* Vetrichelvan Thangarasu; A Validated RP - HPLC method for simulataneous estimation of Lamivudine and Tenofovir disoproxil fumarate in pure and in tablet dosage form, Eurasian Journal of Analytical Chemistry, **7(2)**, **(2012)**.

[12] K.Anandakumar, G.Abirami, S.Murugan, B.Ashok; RP-HPLC method for simultaneous estimation of lamivudine, tenofovir disoproxil fumarate and efavirenz in tablet formulation, Journal of Analytical Chemistry, **68(9)**, 815-821 September **(2013)**.

[13] Tripti Sharma, Neelam Mishra, Swapank Moitra, sudamc si, Dsnnsns G.Sankar; A validated RP-HPLC method for estimation of tenofovir disoproxil fumarate in bulk and pharmaceutical formulation, Asian.J.Pharm.Clin.Res., **5(Suppl 3)**, 108-110 **(2012)**.

[14] U.Seshachalam, B.Rajababu, B.Haribabu, K.B.Chandrasekhar; Enantiomeric Separation of Tenofovir on an Achiral C18 Column by HPLC Using L-Phenylalanine as a Chiral Mobile Phase Additive. Journal of Liquid Chromatography & Related Technologies, **31**, 410-420 **(2008)**.

[15] S.Sentenac, C.Fernandez, A.Thuillier, P.Lechat, G.Aymard; Sensitive determination of tenofovir in human plasma samples using reversed-phase liquid chromatography. Journal of Chromatography B, **793**, 317-324 **(2003)**.

[16] V.Jullien, J.M.Treluyer, G.Pons, E.Rey; Determination of tenofovir in human plasma by high-performance liquid chromatography with spectro-fluorimetric detection. Journal of Chromatography B, **785**, 377-381 **(2003)**.

[17] A.A.Shirkhedkar, C.H.Bhirud, S.J.Surana; Application of UV Spectrophotometric methods for estimation of tenofovir disoproxil fumarate in tablets. Pakistan Journal of Pharmaceutical Sciences, **22**, 27-29 **(2009)**.