https://doi.org/10.48047/AFJBS.6.10.2024.5896-5906

African Journal of Biological Sciences

A Novel Derivatised Method For The Determination Of Residual Ethylenediamine Content By Using Gc-Fid

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Article History Volume 6,Issue 10, 2024
Received:24 May 2024
Accepted : 02 Jun 2024
doi:10.48047/AFJBS.6.10.2024.5896-5906

ABSTRACT

The aim of the research work is to develop and validate a sensitive GC-FID (Gas Chromatography-Flame ionization Detector) method for the quantification of residual Ethylenediamine (Quantified as N,N'-Ditrifluoroacetyl-1,2-Ethandimine*)* in SOT-II (4-(4-aminophenoxy)-N-methyl-pyridine-2-carboxamide), which is the one of the intermediate of Sorafenib tosylate drug substance to fulfill the current regulatory requirements. A novel derivatised method was developed and validated according to ICH guidelines. Using a fused silica capillary column Rtx-35, 30m length, 0.53mm internal diameter, coated with 35% diphenyl and 65% dimethyl polysiloxane stationary phase of 1.0µm film thickness and Helium gas used as carrier gas. Chromatographic parameters i.e. Column flow rate: 5.0 ml/min, Injection volume: 2.0µL, Split ratio: 5:1 and Run time: 40 min. The method was calibrated with a linearity range from LOQ to 150% concentration with respect to the specification level and achieved a limit of detection (LOD) and limit of quantification (LOQ) were 11.5 ppm and 3.8 ppm, respectively, for Ethylenediamine with a correlation coefficient of 0.9992. The average recovery was found to be 100.0%. The method validation parameters strongly support and it was discovered to be specific, accurate, linear, sensitive, robust, and stable. This method is suitable for the detetermination of Ethylenediamine in the regular analysis of SOT-II intermediate.

Keywords: Ethylenediamine, Sorafenib tosylate, Gas chromatography, Method development and Method Validation.

INTRODUCTION

Sorafenib tosylate is chemically (SOT) is 4-[4-({[4-chloro-3-(trifluoromethyl) phenyl] carbamoyl} amino) phenoxy]-N-methyl pyridine-2-carboxamide. Its molecular formula is $C_{21}H_{16}ClF_3N_4O_3$. The chemical structure of Sorafenib tosylate is shown in Fig. 1. Sorafenib (Nexavar), a diarylurea derivative containing pyridine-2-carboxamide moiety, is a multi-kinase inhibitor used in colorectal cancer and hepatocellular carcinoma [1]. As monotherapy or in combination with other antitumor agents, sorafenib has displayed significant anticancer activity against different tumor types. In various preclinical and clinical studies, sorafenib has been proved to hinder cell proliferation, reduce cancer growth and angiogenesis, in addition to induction of apoptosis in tumor cells [2-4]. Initially, it has been developed as Raf1-kinase pathway inhibitor, however, it also binds with receptor tyrosinekinase such as FLt-3, c-KIT, VEGFR-2 and VEGFR-3 [5,6]. The clinical studies on imatinib,

sorafenib and nilotinib have shown an increased therapeutic potential, especially on castrate resistant prostate cancer and suggested that combination of two or three tyrosine-kinase inhibitors are safe and efficacious.[7,8]

The different raw materials were used in the synthesis of various drug substances. These raw materials and its impurities may be carryover upto final stage and present in the final drug substances as impurities. An important task of drug development is the control of impurities in active pharmaceutical ingredients (API) and its proven the product quality and minimizing safety risks.

For impurities known to be unusually potent or to produce toxic or unexpected pharmacological effects, the quantization/detection limit of the analytical procedures should be commensurate with the level at which the impurities should be controlled. Various types of impurities may be observed throughout the development lifecycle of an API. Some impurities having chemically reactive nature and may have unwanted toxicities including genotoxicity and carcinogenicity and these impurities are to be controlled based on the maximum daily dose [9]. Otherwise, these unwanted impurities are nongenotoxic, as per ICH guidelines on impurities, any impurity other than active drug substance is to be controlled with appropriate limit in the drug substance irrespective of harmful nature.

2-picolinic acid (Fig.2(c)) used as a starting material in the manufacturing process of Sorafenib tosylate. For the preparation of 2-picolinic acid, Ethylenediaminetetraacetic acid (ETDA) (Fig. 2(b)) used as a reagent. EDTA is mainly synthesised from Ethylenediamine (EDA) (Fig. 2(a)). EDA is chemical reagent / catalyst and has not been listed in residual solvents category in any of the regulatory guidelines and pharmacopoeias [10, 11]. However, EDA impurity is a non genotoxic and need to be controlled in Sorafenib tosylate drug substance or any intermediate stage.

None of the method was described for the quantification of low level Ethylenediamine in Sorafenib tosylate drug substance in throughout review of the literature. For the determination of Ethylenediamine in SOR-II, which is the one of the intermediate of Sorafenib tosylate drug substance, a novel, simple and sensitive derivative procedure by gas chromatography method has been developed and validated. The chemical structures of Ethylenediamine, Ethylenediaminetetraacetic acid, 2-Picolinic acid and SOT-II are shown in Fig. 2a to 2d.

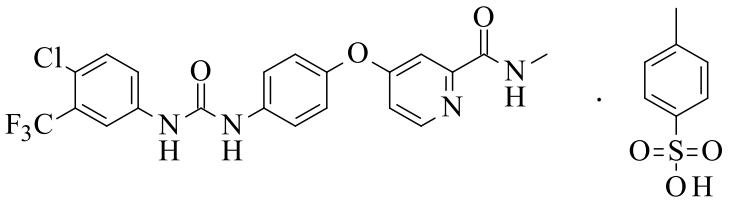


Fig. 1: Sorafenib Tosylate (SOT)

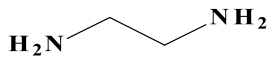


Fig. 2a: Ethylenediamine

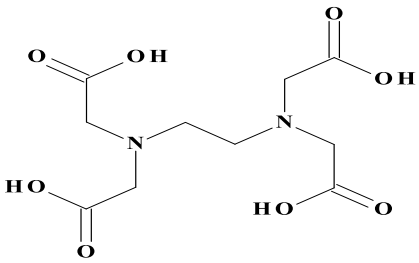


Fig. 2b: Ethylenediaminetetraacetic acid (ETDA)

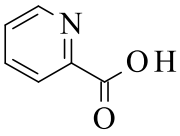


Fig. 2c: 2-Picolinic acid

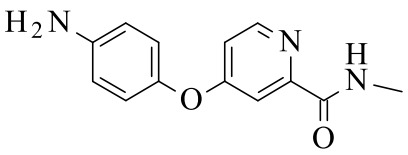


Fig. 2d: 4-(4-aminophenoxy)-N-methyl-pyridine-2-carboxamide (SOT-II)

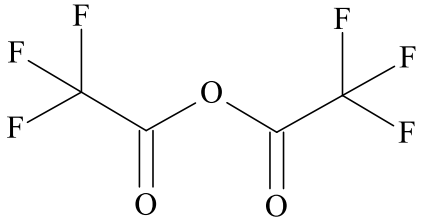


Fig. 2e: Trifluoroacetic anhydride

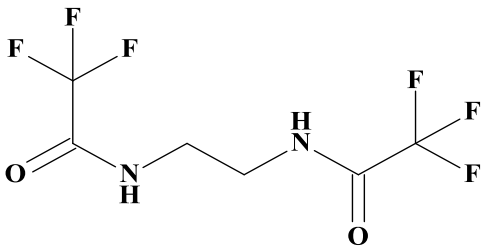


Fig. 2f: N,N'-Ditrifluoroacetyl-1,2-Ethandimine

EXPERIMENTAL

Chemicals, reagents, and samples

Ethylenediamine, Trifluoroacetic anhydrate, Methanol, Acetone, Toluene, N,N-Dimethylformamide, Acetonitrile were procured from Sigma Aldrich, Steinheim, Germany (All are GC grade with 99.5% purity). The investigated sample SOT-II was gifted from APL Research Centre-II (A Division of Aurobindo Pharma Ltd.) located in Hyderabad.

Instrumentation:

In this research work, a GC model 7980A equipped with flame ionization detector with auto sampler (Make: Agilent Technologies, Santa Clora, CA, USA). The data acquisition and processing using Empower 3 Software Build 3471 were used.

Gas Chromatographic conditions and methodology:

After lot of trails and lot of GC columns were used for the quantification of EDA at low levels. In this all columns, proper peak was not getting for EDA. Among them, the Rtx-35 (30 m length × 0.32 mm × 0.25 µm film thickness) and DB-17 (30 m × 0.53 mm × 1.0 µm) columns showed credible

peak shape and retention times. Finally, good chromatographic separation, stable baseline, sensitivity and resolution obtained in Rtx-35 capillary column (30 m × 0.53 mm × 1.0 µm). Helium used as carrier gas and remaining method parameters like injection temperature: 220 °C; Column oven temperature: 80°C, hold 3 min, ramping: 20°C/min up to 250°C, held 28.5min; flow rate: 5.0 mL/min; split ratio 5:1; Run time:40 minutes and Injection volume: 2µL.

Preparation of standard solution

Diluent: (3% Trifluoroacetic anhydride in Acetonitrile solution v/v ratio)

Transfer 3 mL of Trifluoroacetic anhydride into a 100 mL volumetric flask half-filled with acetonitrile, dilute to volume with acetonitrile and mix well.

Standard stock solution-I

Weigh accurately about 50 mg of Ethylene0diamine standard into a 50 mL of volumetric flask half-filled with diluent and makeup to volume with diluent and mix well.

Standard stock solution-II

Transfer 5.0 mL of Standard stock solution-I into 50 mL volumetric flask and dilute to volume with diluent.

Standard solution:

Transfer 2.0 mL of Standard stock solution-II into a 20 mL of volumetric flask half-filled with diluent and dilute to volume with diluent, and mix well.

The prepared standard solution consists of approximately 100 ppm of Ethylenediamine with respect to the test concentration.

In this methodology, Ethylenediamine will be converted and monitored as Trifluoroacetic anhydride derivative of Ethylenediamine (i.e. N,N'-Ditrifluoroacetyl-1,2-Ethandimine)

Blank solution:

Introduce 1.0 mL of diluent into a 2 mL GC vial, close the vial with butyl rubber septa and mix well.

Test solution:

Weigh accurately about 100 mg of the test sample into a 2 mL GC vial, add 1 mL of diluent and close the vial with butyl rubber septa.

RESULTS AND DISCUSSION

Method development and optimization

The objective of this work is to quantification of Ethylenediamine (EDA) in SOT-II at trace level, which is the one of the intermediate of Sorafenib tosylate drug substance. EDA boiling point is 116°C and it's having volatile nature and has no chromophore. Gas chromatography (GC) is a suitable technique for analysis of such ultra-violet (UV) inactive volatile compounds like EDA and mostly available at all laboratories and easy to handle. Initially we are tried in GC with flame ionization detector but not achieved target limit.

Based on the SOT-II and EDA solubility data method development trails were carried out. Initially, we tried in DB-1 column (30m long with 0.53mm ID, 3.0µm ID) with 100% dimethyl polysiloxane as stationary phase. By setting the following method parameters at constant Flow 3.0 mL/min. Column oven temperature: 60°C, hold 5 min, ramping: 20°C/min up to 220°C, held 17min; split ratio 10:1 and Acetonitrile has been chosen as diluent in direct injection technique. In the above method parameters, with respect to test concentration, EDA solution 100ppm has been prepared and injected into GC. Interference observed at EDA retention time in as such sample in this trail, EDA peak shape was tailing observed and EDA response also very low. Various solvents were tried to avoid interference and response issues in direct injection technique in during method optimization

and even extraction procedure was also tried with formic acid and methylene chloride. However, in this all trails satisfactory results were not achieved.

In almost all GC columns like DB-1, DB-5, DB-624 and DB-CAM, EDA peak shape was tailing observed. However, by GC for the quantification of EDA is very difficult in as such form. Hence, derivatization procedure has been chosen for the quantification of EDA by GC. For derivatization, in GC technique lot of reagents like Trimethylchlorosilane (TMCS), Trimethylsilylimidazole (TMSI), Bistrimethylsilylacetamide (BSA), Bistrimethylsilyltrifluoro acetamide (BSTFA), Trifluoroacetic anhydride (TFAA) and N-methyl-N-t-butyldimethylsilyl- trifluoroacetamide (MTBSTFA) etc. were available. Trifluoroacetic anhydride is an acylation reagent and protection reagent. TFAA is reactive towards amines, alcohols and phenol functional groups. Based on the reactivity of TFAA (Fig.2e) with amines, TFAA was selected as derivatization reagent. EDA reacts with TFAA to give N,N'-Ditrifluoroacetyl-1,2-Ethandimine (Fig.2f). Ethylenediamine converts into N,N'-Ditrifluoroacetyl-1,2-Ethandimine in the presence of Trifluoroacetic anhydride (Fig.3). Therefore, Ethylenediamine is quantified as N,N'-Ditrifluoroacetyl-1,2-Ethandimine in this derivatized method. The obtained derivatized peak was also confirmed by GC-MS as N,N'-Ditrifluoroacetyl-1,2-Ethandimine peak (Fig.4). In this present work, 100ppm was considered as specification level concentration. Further, in detailed experiment studies were discussed.

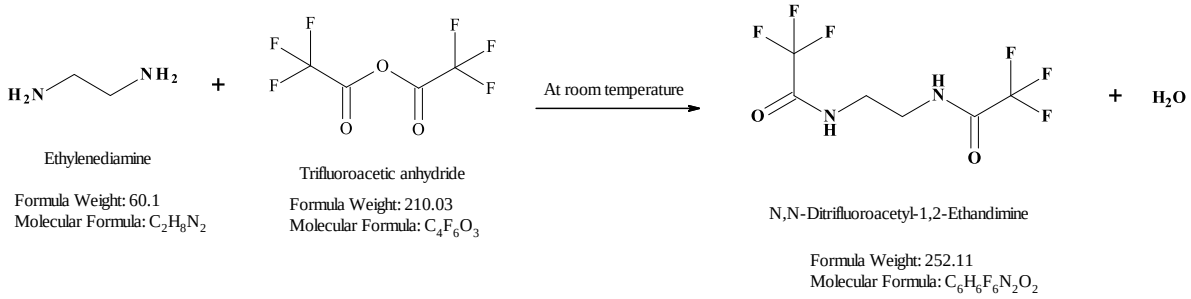


Fig. 3: Reaction mechanism of Ethylenediamine converts in to N,N'-Ditrifluoroacetyl-1,2-Ethandimine in presence of Trifluoroacetic anhydride

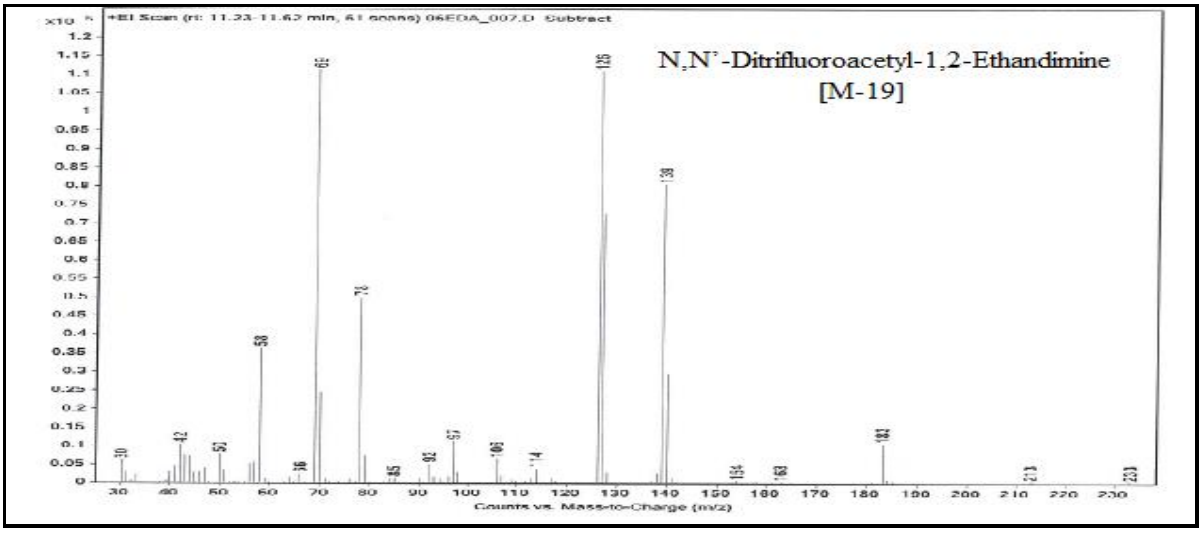


Fig. 4: Mass spectrum of derivative of Ethylenediamine peak i.e, N,N'-Ditrifluoroacetyl-1,2-Ethandimine

In DB-1 column, Sample interference was resolved but analyte peak shape is tailing observed. Finally, Rtx-35 column (30m, 0.53mm, 1.0µm) was used and programme was changed by keeping carrier gas (Helium) flow as 5.0mL/min constantly. Column oven temperature: 80°C, hold 3 min, ramping 20°C/min up to 250°C, hold 28.5min; split ratio 5:1; Run time: 40 minutes and Injection volume is 2.0µL.

EDA was completely converted to N,N'-Ditrifluoroacetyl-1,2-Ethandimine in this derivatization process . It was confirmed by injecting EDA as such. No peak was observed at EDA retention time. In methodology section, final optimized chromatographic conditions and sample preparations were mentioned.

METHOD VALIDATION

The validation of an analytical procedure is the process by which it is established through laboratory experiments (for the developed method) that the performance characteristics of the method meet the requirements for the intended analytical applications.

The process of validation of any analytical method entails a series of studies. The GC method was validated and performed as per the international conference on harmonization (ICH) guidelines Q2 (R1)[11] in the present study. Method validation conditions are system suitability, specificity, the limit of detection, Limit of quantification, Precision for Limit of quantification, linearity, range, method precision, accuracy, robustness, intermediate precision, and solution stability.

Specificity

The specificity investigation was carried out by injecting the solvents that were used during the synthesis process of Sorafenib tosylate. According to the findings of the experiment date, there was no interference in the retention time of TFAA derivative of Ethylenediamine. All solvents individual retention times and all spiked sample data are given Table 1 and Table 2 respectively. Typical GC chromatograms are shown in Fig 5.

Table 1: Summary of retention times of EDA and known solvents

Impurity / Solvent Name	Retention time (min)
Methanol	1.46
Acetone	1.60
Toluene	3.12
N,N-Dimethylformamide	4.16
TFAA derivative of Ethylenediamine	7.97

Table 2: All spiked sample (SOT-II spiked with EDA including all residual solvents)

Impurity / Solvent Name	Retention time (min)
Methanol	1.46
Acetone	1.60
Toluene	3.12
N,N-Dimethylformamide	4.19
TFAA derivative of Ethylenediamine	7.98

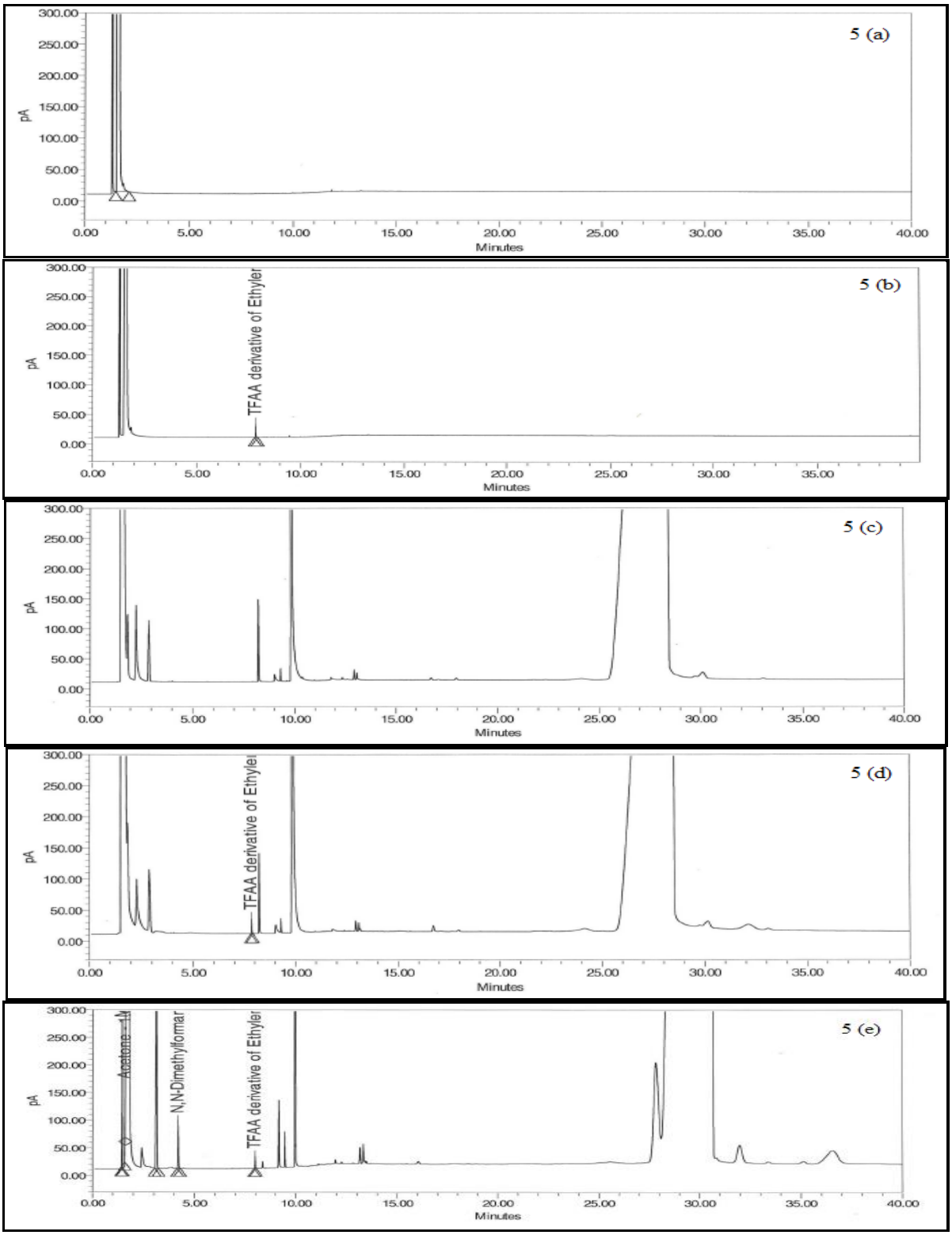


Figure 5: Typical GC Chromatograms of a) Blank solution, (b) Standard solution, (c) SOT-II (Control sample), (d) SOT-II spiked with EDA (Spiked sample) and (e) SOT-II spiked with EDA including all residual solvents (All spiked sample)

Limit of detection (LOD) and Limit of quantification (LOQ):

In this method, Specification level standard solution (100ppm concentration) of EDA was prepared and injected into GC. The LOD and LOQ values for EDA was determined by signal to noise ratio (s/n) method. The minimum concentration at 3:1 S/N was considered as LOD and the concentration at

10:1 S/N was established as LOQ. The predicted LOD and LOQ values obtained for EDA were 3.8 ppm and 11.5 ppm respectively with respect to sample concentration. Precision was verified by preparing the solutions at about LOD and LOQ concentrations and injected each solution six times in to GC and the achieved précised values are given in Table 3. The overlaid GC Chromatograms of LOQ solution and LOD solution are shown in Fig. 6(a) and Fig. 6(b).

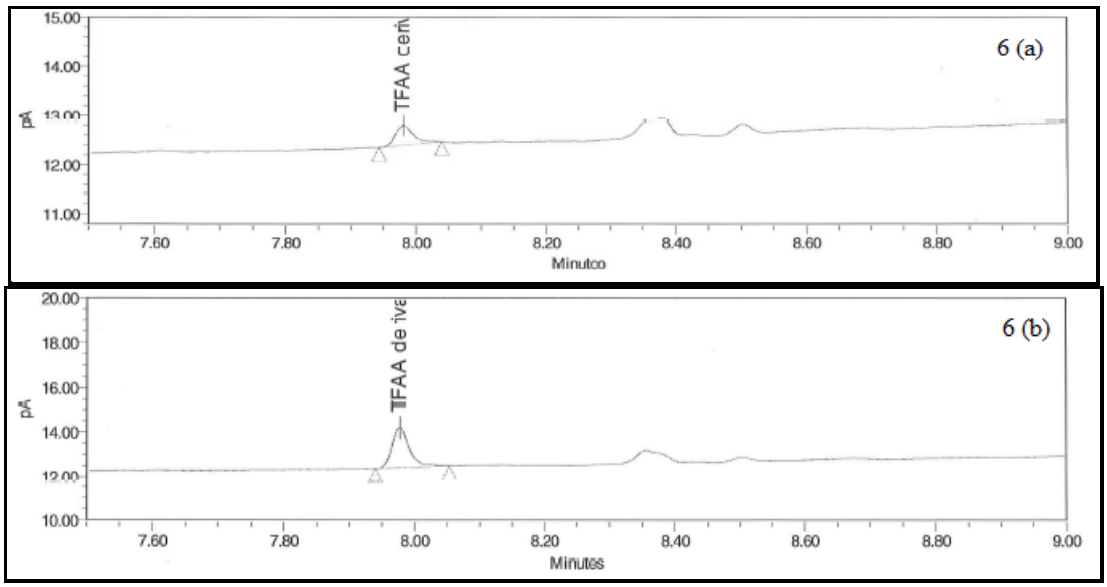


Figure No. 6: Typical GC Chromatograms of a) LOD solution, (b) LOQ solution

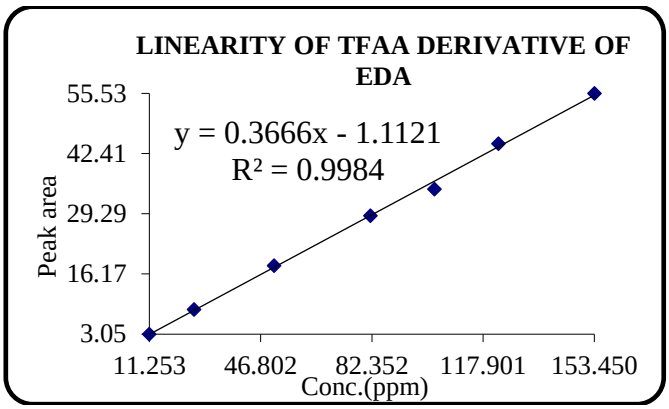
Linearity

The linearity was evaluated by the series of solutions were prepared using EDA at concentration levels from LOQ to 150% of specification level (100µg/g) and each solution was injected in triplicate into GC. Statistical data like slope, intercept, STEYX and Correlation Coefficient were established by using the peak area response versus concentration data. The derived correlation coefficient was 0.9992 indicating the best fitness of the linearity curve of the developed method. The calculated statistical results are shown in Table 3.

Table 3: LOD/LOQ and Linearity experiments results.

Statistical parameters	Results
Correlation coefficient	0.9992
Concentration range (ppm)	11.5- 150
Calibration points	7
Intercept	-1.1121
Slope (S)	0.3666
Limit of detection (ppm)	3.8
Limit of quantification (ppm)	11.5
Precision for Limit of Detection (%RSD)	2.1
Precision for Limit of Quantification (%RSD)	1.7

Linearity plot for TFAA derivative of EDA (Concentration *Vs* Area)



Accuracy

Accuracy of the method was verified through recovery experiments by spiking known amount of EDA at six levels i.e. LOQ level, 50%, 80%, 100%, 120% and 150% of specification level (100ppm) in to SOT-II (Spiked samples) and SOT-II sample solutions (Control sample) were prepared without spiking any EDA. Each preparation was analysed in triplicate and percent recovery was calculated. The obtained recovery results are tabulated in Table 4.

Table 4: Accuracy experiment results

Sample Name	EDA (ppm)
Control sample	0.00

Level	Amount added (ppm)	Amount found (ppm)	%Recovery
LOQ level	12.16	13.35	109.8
50% Level	54.99	54.37	98.9
80% Level	88.01	87.79	99.8
100% Level	110.12	109.22	99.2
120% Level	132.02	127.78	96.8
150% Level	164.86	157.64	95.6

Precision

The precision was the study of the method using repeatability (Method precision) and reproducibility (Ruggedness). The performance of the method was evaluated with replicate injections of standard and sample solutions. Standard solutions (at 100% level and 150% level of specification limit) were analysed by injecting six times for checking the performance of the GC system under the test conditions on the day tested (System Precision).

The results achieved for the system precision (For 100% level and 150% level) experiment were reported in Table 5. Repeatability (Method Precision) experiment was performed by prepared six sample solutions were using single batch of SOT-II spiked with EDA about known concentration (100ppm) level and injected into GC.

The intermediate precision was the inter-day variation (Ruggedness) was defined as the degree of reproducibility obtained by following the same procedure as mentioned for method precision experiment. Ruggedness of the method was evaluated by preparing six individual sample preparations (same sample which was used in Method precision experiment) by spiking EDA to SOT-

II and injected into different column, different instrument and different analyst on different days. The obtained precision experiment results are shown in Table 5.

Table 5: Summary of %RSD for EDA from standard solution

Injection ID	System Precision (At 100% level)	System Precision (At 150% level)	Method Precision EDA content, ppm	Ruggedness EDA content, ppm
1	35.12	55.50	110.13	117.17
2	35.13	55.86	110.06	120.72
3	35.47	55.63	107.47	120.67
4	35.19	55.49	108.04	123.92
5	34.88	56.35	108.11	122.22
6	34.68	55.47	107.89	123.36
Mean	**35.08**	**55.72**	**108.62**	**121.34**
SD	**0.27**	**0.34**	**1.17**	**2.44**
%RSD	**0.8**	**0.6**	**1.1**	**2.0**

Robustness

Robustness was assessed by studying the impact of small variations in oven temperature, rate and flow rate on the peak area of Ethylenediamine at 100 ppm. The % RSD of derivative of Ethylenediamine peak areas is summarized in Table-6. The results show that the RSD is well within 2.0% and thus demonstrated the robustness of the suggested method. (Acceptance criteria: RSD of peak areas should be ≤ 15% for six injections).

Table 6: Robustness

EDA					
	As per the method's idle condition	Oven temperature (80°C) and Rate (20°C/min)		Flow rate (5.0 mL/min)	
		78°C and 18°C	82°C and 22°C	4.5	5.5
%RSD	1.1	1.0	0.4	0.5	0.6

Method Recommendation

Based on the outcome of the method validation studies, the recommendations of the standard test procedure of Ethylenediamine in SOT-II are summarized in Table 7.

Table-7: Method recommendations for Ethylenediamine		
LOD (ppm)	LOQ (ppm)	Retention time (min)
3.8	**11.5**	**7.97**

CONCLUSION

The developed GC method is rapid, simple, cost-effective, linear, precise, accurate and robust, and selective for the trace level determination of EDA content in SOT-II. The developed method LOD and LOQ values were satisfactory. The method is more suitable and more consistent. Additionally, as per current ICH Q2 (R1) and ICH M7 (R1) guidelines, this method was fully developed and validated for

identification and trace level quantification of Ethylenediamine content in SOT-II, intermediate of Sorafenib tosylate drug substance. The developed methods can be used for routine analysis, stability studies, and quality control in the pharmaceutical industry.

ACKNOWLEDGMENT

The authors are very thankful to the management of APL Research Centre-II (A Division of Aurobindo Pharma Ltd.) located in Hyderabad for their provision and motivation to carry out the research work and also very glad to the management of Andhra University, Visakhapatnam, Andhra Pradesh and India for their encouragement of this research work.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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