International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

Novel HPLC Method for Determination of Process Related Impurities of Dasatinib Drug Substance

Sunil V. Lanke[1], Dhramveer Singh Shekhawat[2], Nishikumar Niture, Radhiah Che Rusli[3], Sajid Syed Shahnawaz[4]

Oncogen Pharma (Malaysia) Sdn Bhd, No.3, Jalan Jururancang U1/21,Hicom Glenmarie Industrial Park,40150 Shah Alam,Selangor, Malaysia

Abstract: ***A simple and sensitive reverse phase liquid chromatography (RP-HPLC) method has been developed for the determination of process related impurities of Dasatinib drug substance. Separation was achieved with a Inertsil ODS 3V, (Make: GL Sciences); 150mm x 4.6mm; particle size 5µmcolumn and buffer was prepared by dissolving 1.36g of potassium dihydrogen phosphate and 1.0g of sodium-1-octane sulphonic acid into 1000ml water, dissolve and adjust pH 6.0 with dilute potassium hydroxide solution.The flow rate was set 1ml/minute and the column temperature was 50°C. UV detection was performed at 315nm and injection volume 20µL with ambient sample temperature. The method is simple, rapid, and specific.Themethod is suitable for the determination of almost all process related impurities of Dasatinib drug substance. The method is useful for the determination of following impurities. A) KSM-01(Key starting material: 2-Amino-N-(2-chloro-6-methylphenyl)thiazole-5-carboxamide) b) DAS-01(N-(2-Chloro-6-methylphenyl)-2-[(6-chloro-2-methyl-4-pyrimidinyl)amino]-5 thiazole carboxamide) c) Dimer of DAS-01 d) N-Oxide dasatinib e) N-Deshydroxyethyl dasatinib f)Dimer of dasatinib drug substance***

Keywords: RP-HPLC, KSM (key starting material) and DAS-01 (Dasatinib intermediate)

1. Introduction

Dasatinib is an inhibitor of multiple tyrosine kinases. Dasatinib inhibited the growth of chronic myeloid leukemia (CML) and acute lymphoblastic leukemia (ALL) cell lines overexpressing BCR-ABL. The chemical name for Dasatinib is N-(2-Chloro-6-methylphenyl)-2-[[6-[4-(2-hydroxyethyl)-1-piperazinyl] -2-methyl-4 -pyrimidinyl]amino]-5 –thiazolecarboxamide. The molecular formula is $C_{22}H_{26}ClN_7O_2S.H2O$, which corresponds to a formula weight of 506.02 (monohydrate). The anhydrous free base has a molecular weight of 488.01. Dasatinib is a white to off-white powder. The drug substance is soluble in dimethylsulphoxide and practically insoluble in waterand slightly soluble in ethanol and methanol. Dasatinib is available in market with different strengths like 20 mg, 50 mg, 70 mg, 80 mg, 100 mg, and 140 mg.

Literature survey reveals that lot of analytical methods such as HPLC [1-2], LC-MS[3-4] and UPLC[5]methods have been reported for the determination of dasatinib drug product and drug substance, there are very few methods reported for the determination of related substances of Dasatinib drug substance by high-performance liquid chromatograph (HPLC)[6, 7, 9 &10] but these methods are not suitable for the evaluation all process impurities[a-f].Therefore, the present study aims to develop a novel,simple,sensitive, RP-HPLC method for determination of process related impuritiesin Dasatinib drug substance.In this paper, we describe method development for six process related impuritiesof Dasatinibdrug substance as per International Conference on Harmonization(ICH) recommendations. The method can be used for routine analysis and stability evaluation of Dasatinib drug substance stored in different packing and under different storage conditions and to quantify the process related impurities (Impurities a - f) at low concentrations [8].

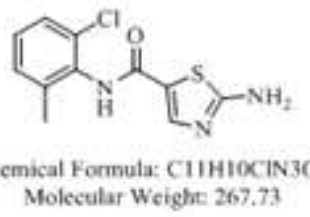


Figure 1: 2-Amino-N-(2-chloro-6-methyl phenyl) thizaole-5-carboxamide (KSM-01)

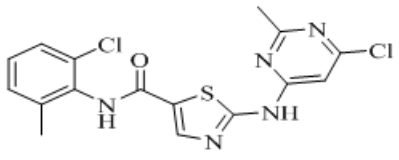


Figure 2: N-(2-Chloro-6-methylphenyl)-2-[(6-chloro-2-methyl-4-pyrimidinyl) amino]- 5-thiazolecarboxamide (DAS-01)

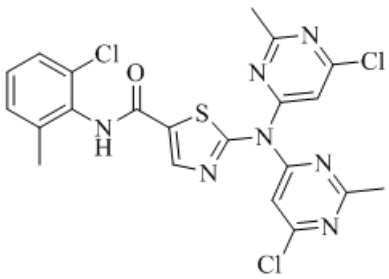
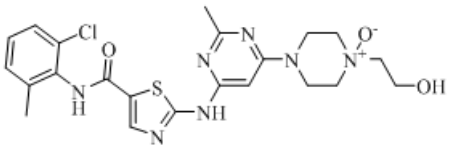
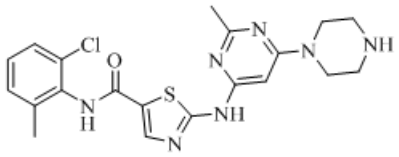


Figure 3: Dimer of DAS-01



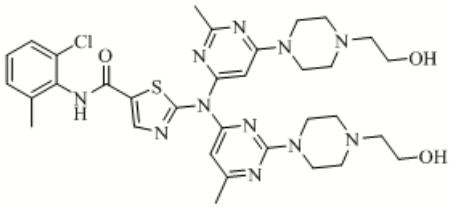
Chemical Formula: C22H26ClN7O3S
Molecular Weight: 504.01

Figure 4: Dasatinib N-Oxide



Chemical Formula: $C_{20}H_{22}ClN_7OS$
Molecular Weight: 443.95

Figure 5: N-Deshydroxyethyl Dasatinib



Chemical Formula: $C_{33}H_{42}ClN_{11}O_3S$
Molecular Weight: 708.28

Figure 6: Dimer of Dasatinib drug substance

Description of Brief Synthetic process of Dasatinib

The dasatinib has been synthesized by using key raw material 2-Amino-N-(2-chloro-6-methyl phenyl) thizaole-5-carboxamide (Carboxamide); it reacts with 4, 6-dichloro-2-methyl-pyrimidine followed by the addition of 2-piperazine-1-yl-ethanol formsDasatinib and upon purification yield pure Dasatinib.

2. Materials and Method

Materials and Reagents

HPLC grade acetonitrile and methanol were procured from Merck (Malaysia). AR grade of potassium dihydrogen phosphate, sodium-1-octane sulphonic acid, potassium hydroxide, dimethylformamide and hydrogen peroxide were procured from Merck, (Malaysia). HPLC grade water obtained from Millipore system (Millipore Inc., USA) was used for the analysis. The investigated sample of Dasatinib drug substance and its potential process related impurities (Impurities a-f, Figure 1- 6) were synthesized and received from synthetic laboratory of Oncogen Pharma (Malaysia) Sdn Bhd.

Instrumentation and Operating Conditions

Waters HPLC (USA) equipped with Alliance 2695 separations module and 2996 photodiodearray detector was used. Inertsil ODS 3V, (150mm×4.6 mm, 5 μm) column thermostated at 50°Cwasused for the separation. Solvent-A was prepared a solution of 1.36 g of Potassium dihydrogen phosphate and 1.0g of sodium-1-octane sulphonic acid into 1000 ml water, dissolved and adjusted pH 6.0 with dilute potassium hydroxide solution,and acetonitrile was used as solvent B. The flowrate and injection volume was 1.0mLmin−1 and 20 μL, respectively. The analysis was carried out under gradient conditions as in table -1

Table 1: Gradient time program

Time (min)	Solvent-A (%)	Solvent-B (%)
0	80	20
25	60	40
30	60	40
35	25	75
40	25	75
45	80	20
50	80	20

Preparation of Solutions

HPLC grademethanol used as diluent. The sample solution concentration of 500μgmL−1 was prepared for the determination of process related impurities. Standard Stock solution:mixture of all six impurities(KSM-01, DAS-01, dimer of DAS-01, N-oxide Dasatinib, N- deshydroxyethyl Dasatinib, dimer of dasatiniband dasatinib drug substance) at concentration about 100μgmL−1 prepared in diluent (methanol). The resultant solution was prepared at concentration about 0.5μgmL−1(0.1 % w.r.to sample concentration as per ICH guideline) by standard stock solution in diluent.

Analytical Procedure

20.0μL of blank (diluent), six replicate injections of standard solution and testsample solution were separately chromatographed. A resolution of not less than 2 between Dasatinib and dimer of Dasatinib was set as a system suitability requirement. The relative standard deviation (RSD) of not morethan 5.0% for all six process related impurities and Dasatinib peak areas obtained from six replicate injections of standard solution was used to verify the system precision.All the known process related impurities in test sample were determined againstmean area of respective impurities obtained from replicateinjections of standard solution. The peak elution order was shown in table-02; relative retention time (RRT) was calculated of each impurity against peak retention time (RT) of Dasatinib drug substance.

Table 2: Peak elution order

Components	RT (min)	(~) RRT
Dasatinib KSM-01	9.48	0.43
N-Oxide Dasatinib	14.12	0.64
N-Deshydroxyethyl Dasatinib	20.27	0.91
Dasatinib	22.18	1.00
Dimer of Dasatinib	23.70	1.07
DAS-01	29.22	1.32
Dimer of DAS-01	38.72	1.75

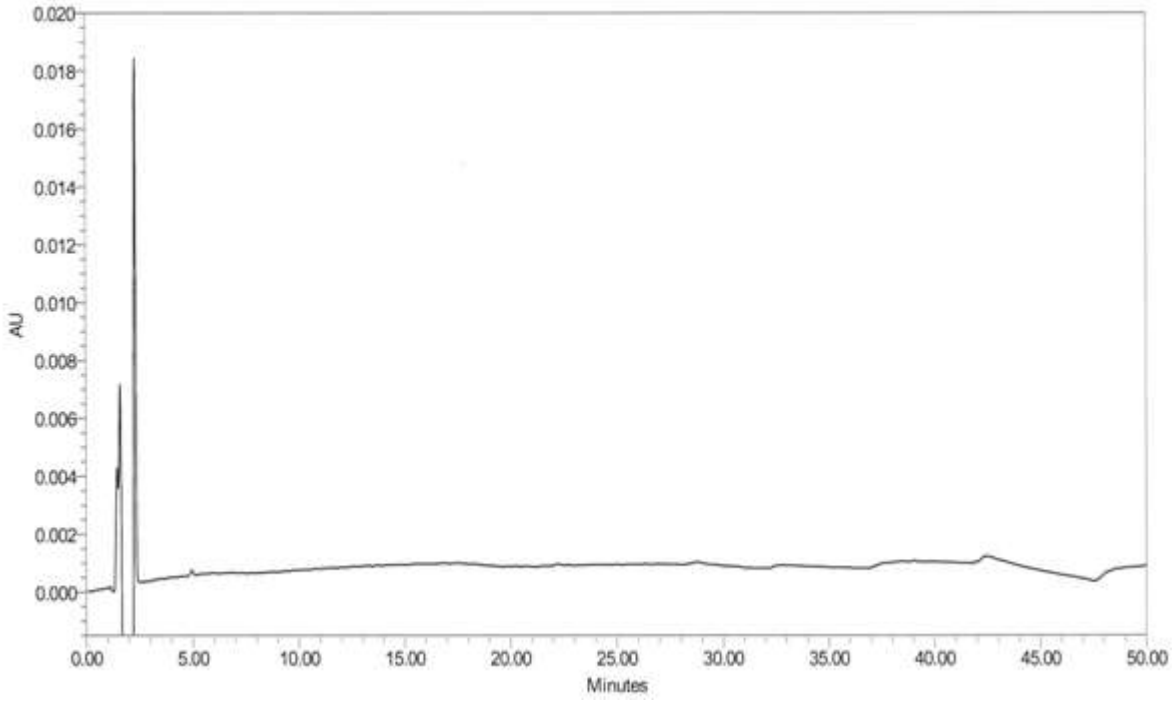


Figure 7: HPLC Chromatogram of blank (Diluent)

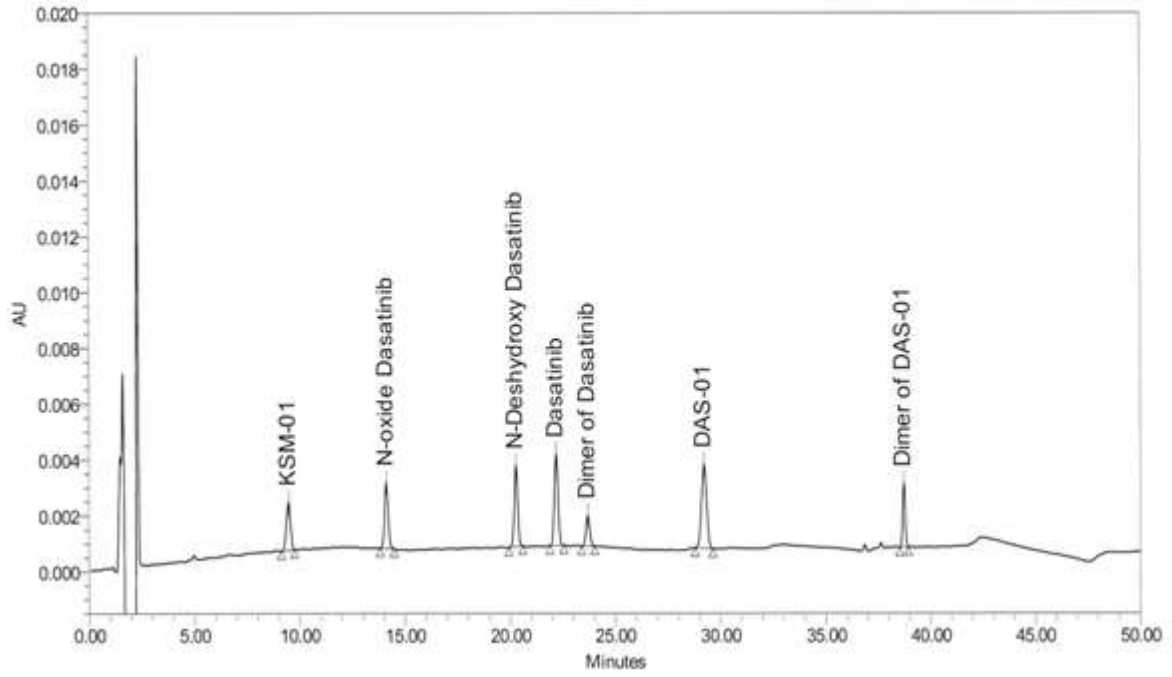


Figure 8: HPLC Chromatogram of Standard solution (Mixture of impurities)

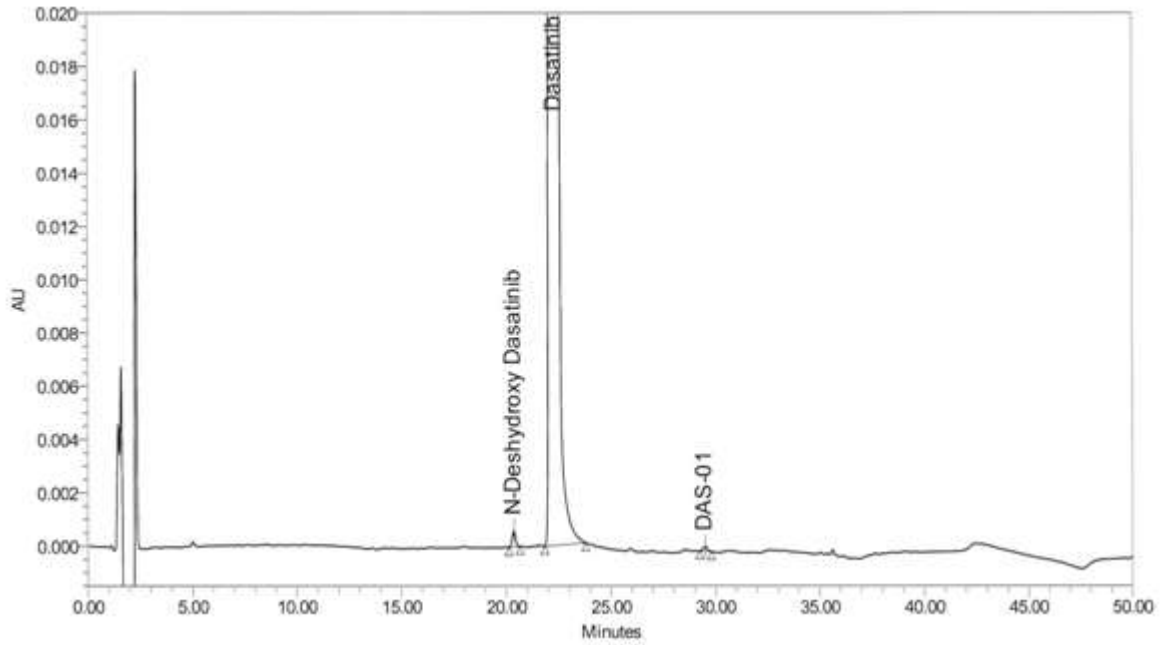


Figure 9: HPLC Chromatogram of Dasatinib drug substance

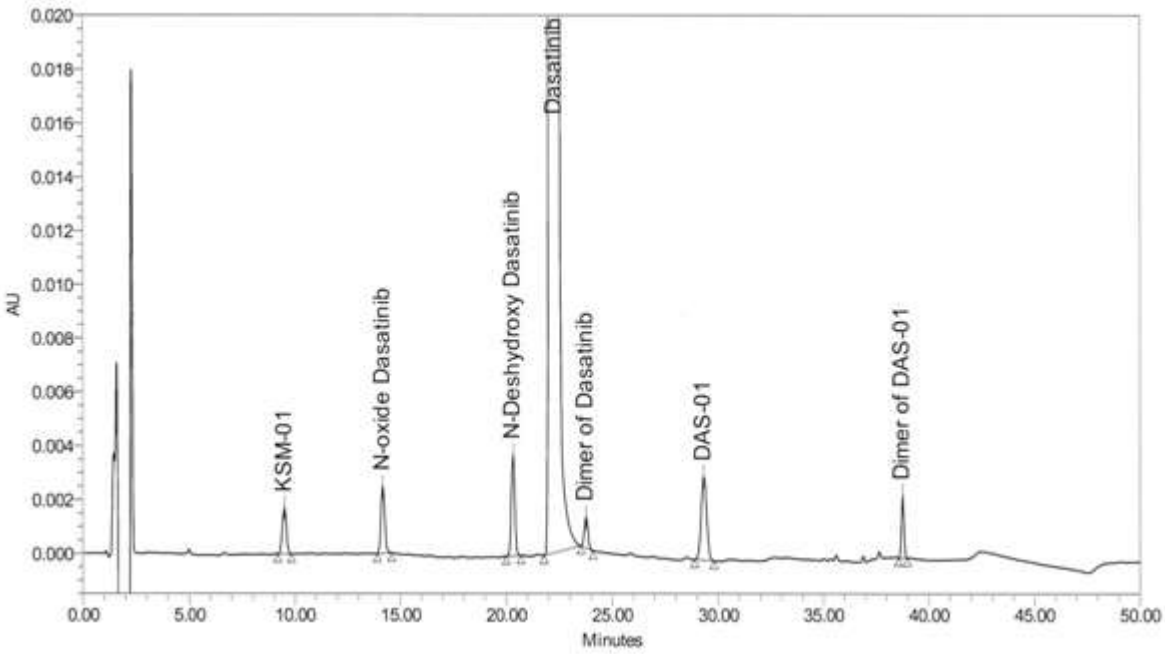


Figure 10: HPLC Chromatogram of Dasatinib drug substance with Spiked impurity mixture

Procedure for Forced Degradation Study

Forced degradation study was conducted on bulk drug substance to prove the stability-indicating property and selectivity of the method. Forced degradation of Dasatinib drug substance was carried out under acid/base hydrolytic, oxidative, thermolytic, and photolytic stress conditions. Solutions of drug substances were prepared in diluent and then treated with concentrated 2N hydrochloric acid (heated at 90°C for 24h), aqueous 2N sodium hydroxide (heated at 90°C for 24h),and aqueous 3% hydrogen peroxide (3% H_2O_2 / kept for 1h at RT).After the degradation, these solutions were diluted withdiluent and analyzed by the proposed method. For thermalstress, sample of drug substance was placed in oven withcontrolled temperature of 105◦C for 48h. For photolyticstress, the sample was exposed under UV light in photo stability chamber of wavelength 365 nm to produce 200-watt hours /square meter exposure into 100 ml volumetric flask and exposed in fluorescent tube light in photo stability chamber to provide illumination and produce visible light measured in LUX for 1.2 million LUX hours exposure into 50 ml volumetric flask. After the exposure to the above stress conditions, solutions of these samples were preparedby dissolving respective samples in diluent and diluted tothe desired concentration and further subjected to analysisusing the proposed method and chromatograms are given in figures 11 – 17. Photodiode array detector was employed to check and ensurethe homogeneity and purity of Dasatinib peak in all the stressed sample solutions. Peak purity data as shown in table-3

Table 3: Peak purity data

Degradation Mechanism	Dasatinib (% area)	% Degradation	Peak Purity		Chromatogram
			Purity Angle	Threshold	
Controlled Sample	99.98	NA	2.88	3.49	Fig-11
Thermal stress	99.98	0.0	2.65	3.26	Fig-12
UV radiation	99.98	0.0	2.78	3.38	Fig-13
Fluorescent lamp	99.98	0.0	2.90	3.48	Fig-14
Peroxide treated	83.26	16.7	1.67	2.62	Fig-15
Base treated	99.63	0.36	2.18	3.19	Fig-16
Acid treated	98.81	1.17	2.07	3.03	Fig-17

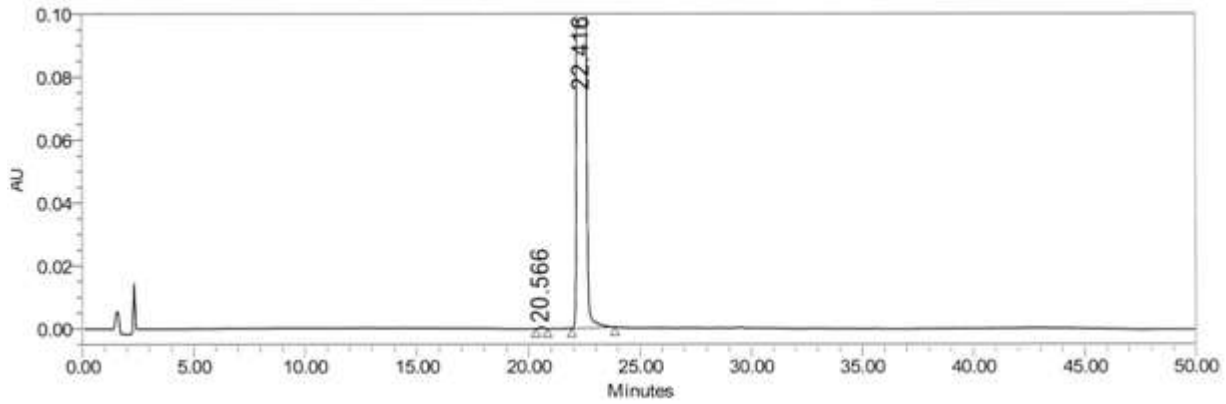


Figure 11: HPLC chromatogram of controlled sample

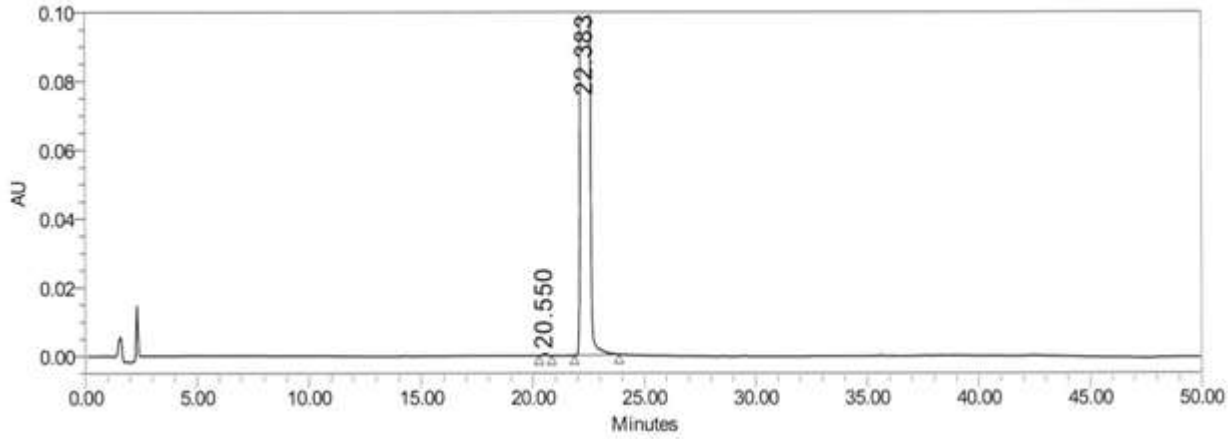


Figure 12: HPLC Chromatogram of Thermalstresssample

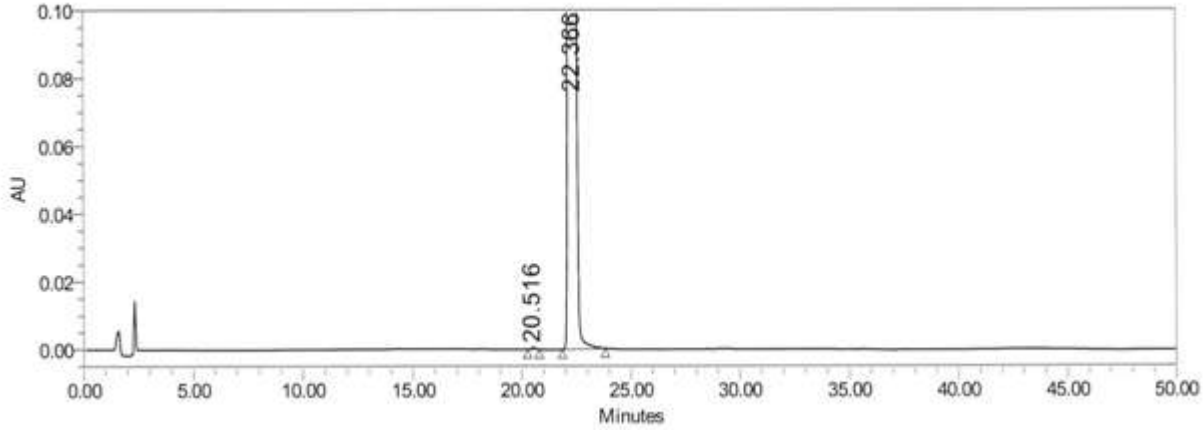


Figure 13: HPLC Chromatogram of UV radiationexposed Sample

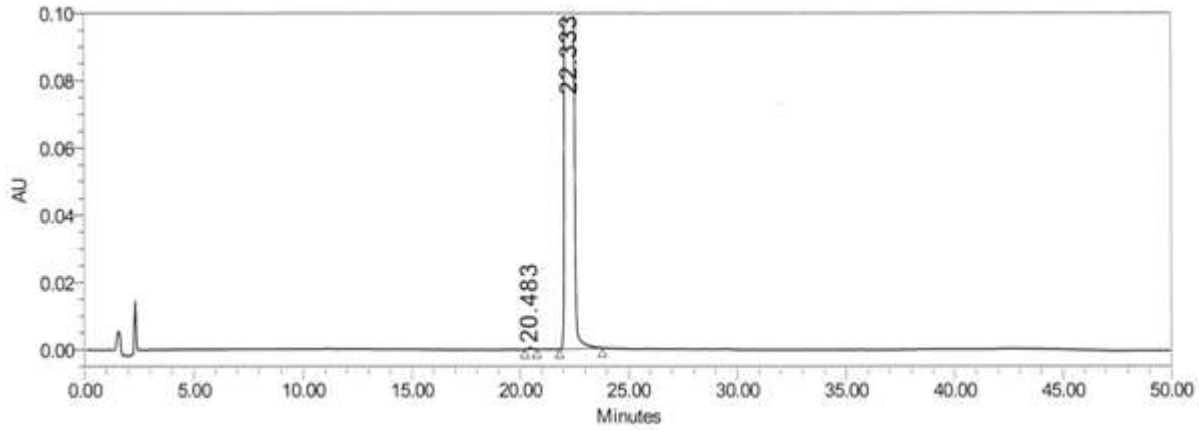


Figure 14: HPLC Chromatogram of Fluorescent lamp exposed Sample

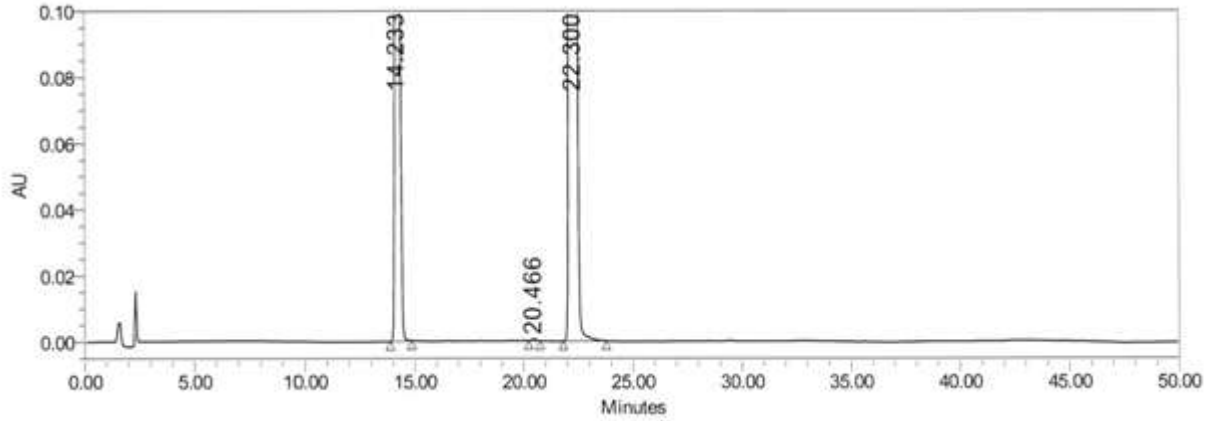


Figure 15: HPLC Chromatogram of Peroxide treated Sample

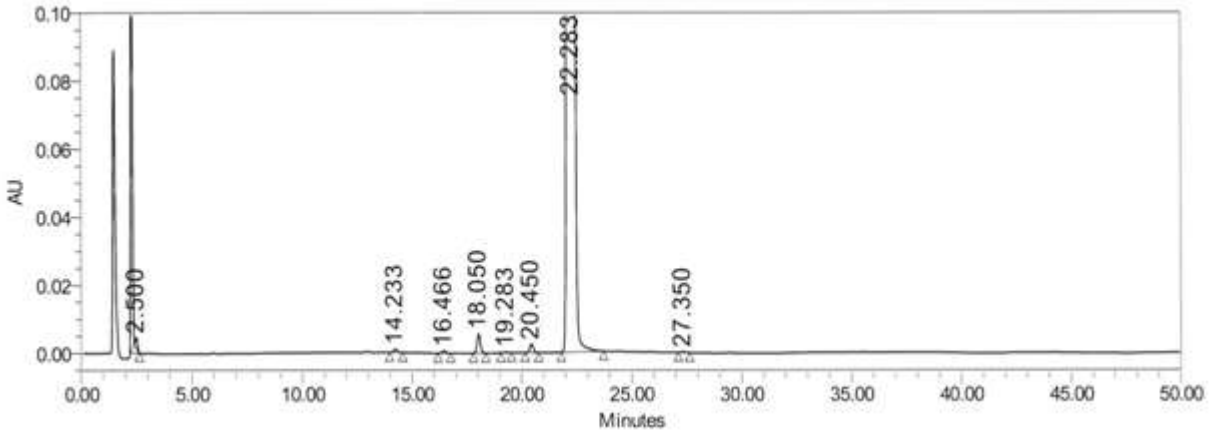


Figure 16: HPLC Chromatogram of base treated Sample

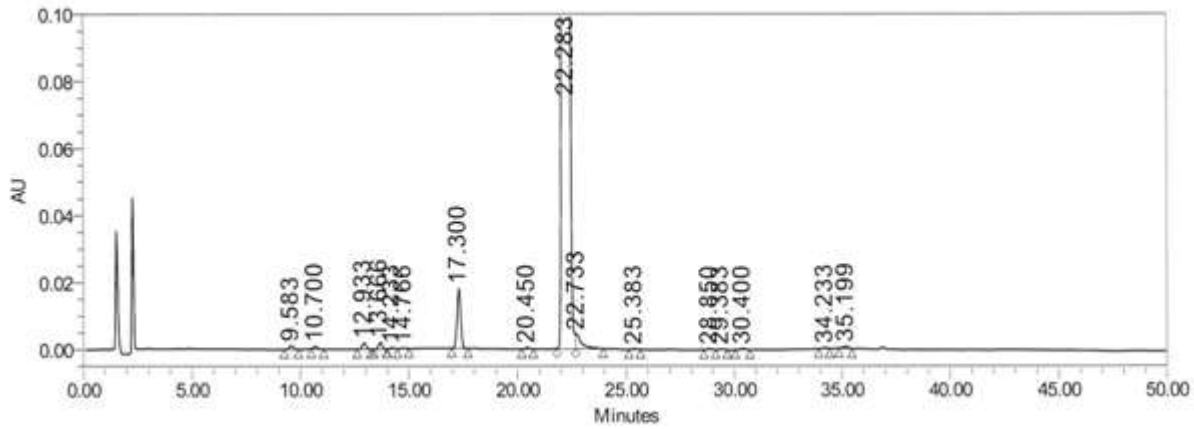
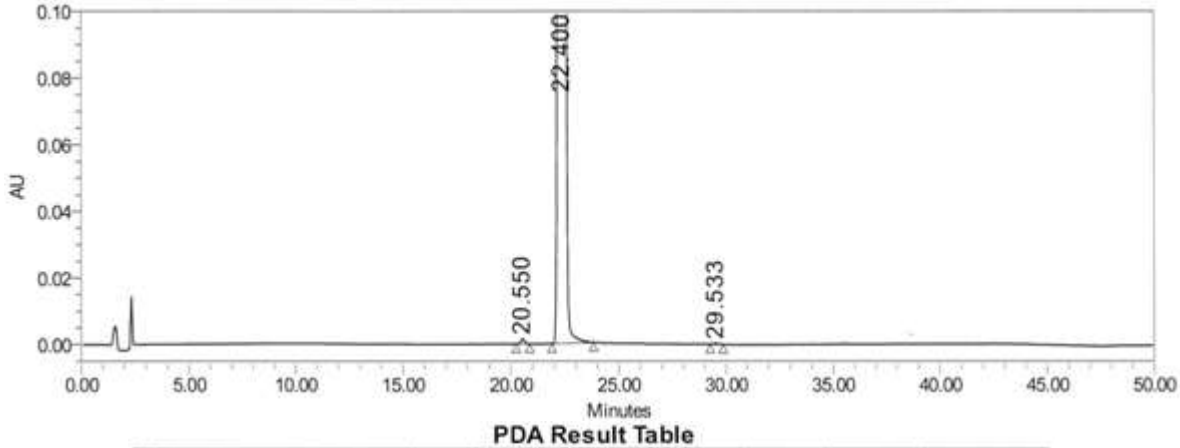


Figure 17: HPLC Chromatogram of acid treated Sample



PDA Result Table

	Peak Name	RT	RT Ratio	Area (µV*sec)	% Area	Purity1 Angle	Purity1 Threshold	PurityCriteria
1	N-Deshydroxyethyl Dasatinib	20.550	0.92	16901	0.05	0.70	0.93	Pass
2	Dasatinib	22.400	1.00	36543079	99.94	2.88	3.49	Pass
3	DAS-01	29.533	1.32	4670	0.01	1.96	3.13	Pass

Figure 18: HPLC Chromatogram of Dasatinib drug substance

3. Results and Discussion

Detection of Impurities. Laboratory batches of Dasatinib drug substance were analyzed for their related substances identification using the developed RP-LC method. Two related substances were detected in the Dasatinib drug substance batch sample (Figure-18).The developed method is capable to identify six impurities [a-f]. The impurities [a-f] were synthesized and co-injected with Dasatinib drug substance to confirm the retention times. All the related substances were well resolved from each other and the representative chromatogram of spiked test preparation is shown in Figure 4. Among the identified impurities, KSM-01is the key starting material and DAS-01is intermediate in the process; others are process related impurity.

Spectroscopic data All impurities were characterized using MS, FT-IR, and NMR spectroscopic techniques. The mass, FT-IR spectral data, and 1H NMR chemical shift values of these impurities are presented in Table 4.

Table 4: Spectroscopic data

Name of Impurities	FT-IR bands (cm−1)	1H NMR, δ in ppm,	m/z(ES+)
KSM-01 (Fig.-1)	771,1219,1577,1477, 1384,1539, 1608, 3113, 3375	9.61 (s,1H),7.85(s,1H), 7.59(s,1H), 7.35-7.36(d, 1H), 7.22-7.25(m,2H), 2.20(s, 3H)	268.03
DAS-01(Fig.-2)	767, 1122, 1573,1504,1454, 1384, 1635, 2789, 3236	12.24 (br s,1H) 10.02(s,1H), 7.41(dd, 1H), 8.31(s,1H), 7.23-7.31 (m,2H), 6.94(t,1H)	394.03
Dimer of DAS-01(Fig.-3)	725, 1122, 1558,1527,1415, 1381, 1627,1651, 2854,2924	12.44(s,1H), 7.24 (s,1H),8.78 (s,1H) 8.33-7.92 (m,4H), 2.94(s,1H), 3.16(s,3H), 3.40(s,3H),	519.8
N-Oxide Dasatinib(Fig.-4)	775, 1192, 1319, 1573,1496,1454, 1388, 1608, 3012, 3197, 956	9.92(s,1H), 4.13(bs,1H), 7.39(dd,1H), 8.22(s,1H), 7.28-7.21(dd,2H), 6.14(s,1H), 4.10(bd,2H), 3.92(m,2H), 3.62(t,2H), 3.40(m,6H), 2.42(s,3H), 2.22(s,3H)	504.16
N-Deshydroxyethyl Dasatinib (Fig.-5)	767, 1570, 1504, 1408, 1392,1620,2831, 3194	9.91 (s,1H), 7.37(s,1H), 8.23(s,1H), 7.26-7.24(m,1H), 6.03(s,1H), 2.47(t,4H), 3.45(m,1H), 2.76(br s,1H), 2.38(s,3H), 2.22(s,3H)	444.13
Dimer of Dasatinib (Fig.-6)	771, 1404, 1612, 1573, 1176, 1195, 1323,1261, 2850,2924, 3255	11.76(br,1H), 7.82-7.42(m,4H), 7.36(s,1H), 6.32(s,1H), 4.81(br,2H), 3.90-3.84(m,12H), 3.68(s,12H), 2.84(s,6H), 2.68(s,3H)	708.10

Development of Chromatographic Conditions

Preliminary experiments for separation of Dasatinib and all the related substances using RP-LC method under isocratic (Buffer: Solvent Mixture: 50:50(v/v)) conditionsusing Symmetry C-18, 150 x 4.6, 5µ and employing different eluents were unsuccessful. Further method development trials were performed on gradient mode of separation. The parameters assessed include the type and quantity of organic modifier, the column, thesalt concentration, the pH of mobile phase (3 to 7), andcolumn temperature. Dasatinib drug substance showed UV-absorption maxima at about 323nm, but based on study of other process impurities and Dasatinib responses, was found equal response on 315nm, which was selected for further method development. Based on the pK*a* value of Dasatinib method development was carried out at mobile phase with pH 6.0.The selection of proper stationary phase plays a major role in method development to achieve reproducible results. Initial method development trials conducted using Sunfire C-18250mm x 4.6mm, 5µm, and X-Select C-18 250mm x 4.6mm, 5µ but run time was more. Further trials were performed using short length nonpolar column, such as, Symmetry C-18, 150mm x 4.6mm, 5µ and Inertsil ODS 3V,150mm x4.6mm, 5µ. All process impurities and Dasatinib well separated and aim of method development achieved by using Inertsil ODS 3V, 150mm x4.6mm, 5µ which was successful as it provides exceptional stability and maximize lifetime and reproducibility.The different gradient RP-LC trials were performed on Inertsil ODS 3V,150mm x4.6mm, 5µ using phosphate buffer with ion pair and without ion pair reagent and organic modifier (acetonirile and methanol) to achieve optimum separation. The good separationbetween all process impurities is achieved only when Inertsil ODS 3V,150mm x4.6mm, 5µ is used, with potassium dihydrogen phosphate buffer and sodium-1-octane sulphonic acid in mixture at pH 6.0. Finally, satisfactory peak shape and resolution was achieved on Inertsil ODS 3V,150mm x4.6mm, 5µ mobile phase-A (buffer) and mobile phase-B (acetonitrile). The flow rate of the mobile phase was 1.0mLmin−1. The HPLC gradient program was optimized as shown in table-1. The column oven temperature was maintained at 50◦C with PDA detector set at 315nm. It was observed thatDasatinib and its process impurities [a-f] were well separated under the optimized conditions with a resolution greater than 2.0 (Figure-8) and they indicated the specificity of the developed HPLC method for Dasatinib drug substance and its impurities (process and degradation-related impurities) as shown in Figure-10. Thus, the proposed method is simple and could be suitable for the determination of process related impurities of Dasatinib drug substance.

4. Conclusion

This paper presents a novel, simple, precise, robust, accurate and selective gradient RP-LC method that separates the related substances and degradation products of Dasatinib drug substancewith good resolution. The process and degradation related impurities present in Dasatinib drug substance sample were identified by LC-MS and characterized by using MS, FT-IR, and 1HNMR spectral data (table-4). Hence, this method can be used for routine testing and stability analysis of Dasatinib drug substance. This methodis suitable for evaluation of the quality of Dasatinib drug substance.

5. Acknowledgments

The authors wish to thank the management of Oncogen Pharma (Malaysia) Sdn Bhd for supporting this work. We would also like to thank colleagues in the division of Research and Development of Oncogen Pharma (Malaysia) Sdn Bhd for their cooperation in carryingout this work.

References

[1] Arun Kumar K, Ananta Rao B,Yaswanth A, Dayananda Chary P,Shanth Kumar S and Navya A. Development and validation of RPHPLC method for estimation of Dasatinib in bulk and its pharmaceutical formulation. Am JPharm Tech Res. 2012;2 (4):863-872.
[2] Elisa P, Silvia DF, Francesca DM, Carmen F, Stefano U, Giovanna Sand Francesco DC. A new HPLC-UV validated method for therapeutic drug monitoring of tyrosine kinase inhibitorsin leukemic patients. J ChromatogrSci. 2011; 49:753-757.
[3] Michael TF, Shruti A, Dara H, MichaelL, Steve U, Linda K and Bruce S. Avalidated LC-MS/MS assay for thesimultaneous determination of theanti-leukemic agent Dasatinib and two pharmacologically active metabolitesin human plasma: application to aclinical pharmacokinetic study. JPharm Biomed Anal. 2012; 58:130-135.
[4] Silvia DF, Antonio DA, Francesca DM,Elisa P, Lorena B, Marco S, Marco S,Silvia R, Giuseppe S, Francesco DCand Giovanni DP. New HPLC-MSmethod for the simultaneous quantification of the antileukemiadrugs Imatinib, Dasatinib, and Nilotinibin human plasma. J Chromatogr B.2009; 877(18-19):1721-1726.
[5] Eva K, Jurij T, Tadej P and Albin K.Simultaneous measurement of Imatinib, Nilotinib and Dasatinib in Dried blood spot by ultra-high performance liquid chromatography tandem mass spectrometry. JChromatogr B. 2012; 903:150-156.
[6] Zhao, Na; Yan, Zhengyu; Jiang, Xinmin. Determination of dasatinib and its related substances by HPLC. Zhongguo Yiyao Gongye Zazhi (2012), 43(2), 129-131. Language: Chinese, Database: CAPLUS.
[7] Xu, Jiagen; Zhao, Chuncai; Wu, Xueping; Xu, Kangkang Determination of dasatinib and related substances in dasatinib tablets by HPLC. Zhongguo Yaofang (2011), 22(1), 56-57. Language: Chinese, Database: CAPLUS.
[8] ICH Q3A (R2), Impurities in New Drug Substances, 2006. ICH Q3B (R2), Impurities in New Drug Substances, 2006.ICH Q3C (R5), Impurities: Guideline for Residual Solvents, 2011.
[9] Ramachandra B, Suguna B, Sivarami Reddy P, Naidu NVS. Development of a UV-visible spectrophotometric method for determination of dasatinib in pharmaceutical formulation and biological samples. Int J Pharm Sci Res 2015;6:293-303.
[10] Sreedevi A, Lakshmana Rao A. Development and validation of novel HPLC method for the estimation of dasatinib in bulk and pharmaceutical dosage forms. Int J Res Pharm Chem2013; 3:724-9.