Synopsis

Of

The Thesis Entitled

Impurity profiling and method validation of drug substances

To be submitted to The Maharaja Sayajirao University of Baroda

For the Degree

of

DOCTOR OF PHILOSOPHY

In Chemistry

By

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Under guidance of

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August-2019

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Name of the student: Chandrakant M Sojitra

Faculty: Science

Subject: Chemistry

Name of Guide: Dr. Sameer Agarwal/ Prof. Padmaja Sudhakar

Title of the Thesis: Impurity profiling and method validation of drug substances

Registration Number: F0S/2063

Date of Registration: 03-08-2017

Place of Work **:** Department of Chemistry, Faculty of Science,
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Chapter-1:

Liquid chromatographic method development for determination of process and degradation related Impurities in Solid Dispersion of Dasatinib

1. Introduction:

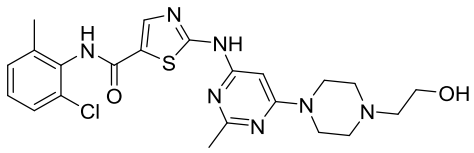
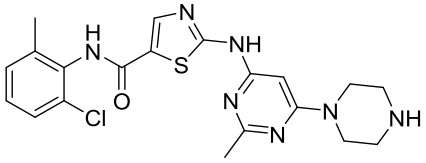
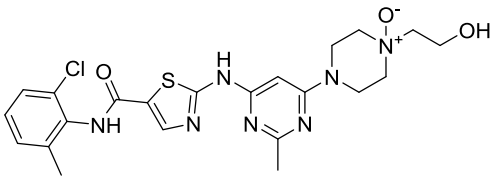
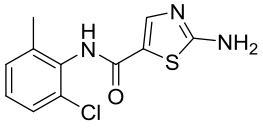
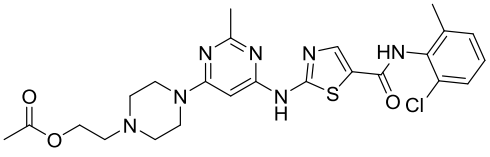
Dasatinib is an inhibitor of multiple tyrosine kinases, inhibiting the growth of chronic myeloid leukemia and acute lymphoblastic leukemia cell lines overexpressing BCR-ABL. Dasatinib is an approved drug, sold under the brand name Sprycel, and is a chemotherapy medication used for the treatment of chronic myelogenous leukemia and acute lymphoblastic leukemia. Stability testing is an integral part of the new drug development process because it provides quality of drug substances in different storage condition having variable temperature and humidity.

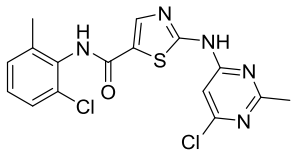
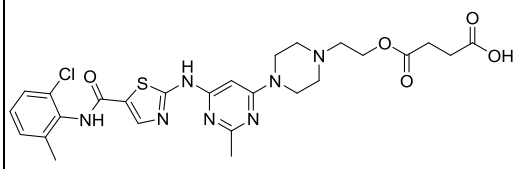
As per the International Conference on Harmonization (ICH) Guideline Q1A (R2), the shelf life of any drug substances is determined by stability studies. The quantification of impurities and dasatinib API is required to be determined using stability indicating chromatographic method, as suggested by the previously mentioned ICH guideline and United State Pharmacopoeia (USP). However, a few methods have been used for quantification of major tyrosine kinase inhibitors i.e. imatinib, dasatinib and nilotinib in human plasma.

In the present work we herein report the development and validation of a ‘stability indicating chromatographic method’ for determination of process and degradation related impurities in solid dispersion of Dasatinib drug substance by evaluating Response Factor (RF) values of each impurity as per validation guideline of ICH.

The developed method will be of high importance for the commercial production of Dasatinib, an efficient oncology drug, with over $ 2000 million market size.

2. Chemical structure of Dasatinib and its impurities:

Sr. No	Impurity particulars	Chemical structure	IUPAC name	Source
1	Dasatinib		N-(2-chloro-6-methylphenyl)-2-((6-(4-(2-hydroxyethyl) piperazin-1-yl)-2-methylpyrimidin-4-yl) amino) thiazole-5-carboxamide.	Target API
2	Imp-1		N-(2-chloro-6-methylphenyl)-2-((2-methyl-6-(piperazin-1-yl) pyrimidin-4-yl) amino) thiazole-5-carboxamide.	Degradation impurity
3	Imp-2		4-(6-((5-((2-chloro-6-methylphenyl) carbamoyl) thiazol-2-yl) amino)-2-methylpyrimidin-4-yl)-1-(2-hydroxyethyl) piperazine 1-oxide.	Degradation impurity
4	Imp-3		2-amino-N-(2-chloro-6-methylphenyl) thiazole-5-carboxamide	Key Starting Material
5	Imp-4		4-(2-(4-(6-((5-((2-chloro-6-methylphenyl)carbamoyl)thiazol-2-yl)amino)-2-methylpyrimidin-4-yl)piperazin-1-yl)ethoxy)-4-oxobutanoic acid	Degradation impurity due to HPMC

6	Imp-5		2-((6-chloro-2-methylpyrimidin-4-yl)amino)-N-(2-chloro-6-methylphenyl) thiazole-5-carboxamide	Intermediate
7	Imp-6		2-(4-(6-((5-((2-chloro-6-methylphenyl) carbamoyl)thiazol-2-yl)amino)-2-methylpyrimidin-4-yl)piperazin-1-yl)ethyl acetate	Degradation impurity due to HPMC

3. Optimized method parameter:

The main criteria for developing chromatographic method was that it must be stability indicating and easy to perform routine analysis in quality control laboratory. The first step for method development was the selection of wavelength. Analysis was performed by using diode array detector for selection of wavelength and to check homogeneity of peaks. The wavelength for analysis and quantification was selected based on UV spectrum of each impurity and analyte peak. Each peak is showing two UV maxima at about 220 nm and 320 nm. 310 nm was selected as cross section wavelength of Impurity-3 and all other peaks. Thus detection wavelength was selected as 310 nm for related substance analysis. For a method development, spiked solution of dasatinib was used.

Method parameter	**Optimized value**
Column	Sunniest C18 or Equivalent (250mm X 4.6mm i.d., 5µm particle size)
Mobile Phase-A	20mM ammonium acetate (pH 5.0) with acetic acid
Mobile Phase-B	Methanol, buffer and acetonitrile in a ratio of 90:5:5 (%, v/v/v)
Flow rate	1.2 ml/min
Retention time	Dasatinib 18.5min
Detection Wavelength	UV Detector – 310 nm
Temperature	35°C

	Time	%A	%B
Gradient Programming	0.01	50	50
	23	45	55
	42	33	67
	50	10	90
	65	10	90
	68	50	50
	75	50	50

Using these chromatographic conditions, significant separation (>2.0) for all the six impurities and dasatinib was achieved. The retention time of dasatinib was 20 min. It was confirmed that no blank interference observed at the retention time of any of the impurities and dasatinib.

4. Forced degradation study summary:

Specificity can be performed by analysis of the sample spiked with process and degradation related impurities; no interference was observed at the retention time of interest analytes. Stress studies were performed at concentration of 1000 µg mL-1. Degradation was performed under stress condition of UV light (254 nm), heat (105 °C), acid (1.0 N HCl at 60 °C), base (1.0 N NaOH at 60 °C) and oxidation (3% H2 O2 at 25 °C) to evaluate the capability of the proposed method to separate dasatinib and all impurities including process and degradation products. For thermal and photo stress studies, the study period was 24 h, whereas for acid hydrolysis approximately 1 h; alkali hydrolysis 1 h and oxidation 1.5 h. The purity of each peak was checked using PDA detector and the purity angle was found to be less than the purity threshold, directly demonstrated that peak is pure. Mass balance of each condition stressed samples was calculated by addition of %content of dasatinib + %known impurities + %unknown impurities in %, w/w.

Degradation condition	Time	Temp	Assay (%, w/w)	RS by HPLC % degradation	Mass balance (% assay + % deg. products)	Remarks/observation
A control sample (untreated)	-	-	100.6	0.27	100.8	NA
HCl, 1.0 N (acid degradation)	1 h	60°C	100.8	0.22	101.0	No significant degradation observed
NaOH, 1.0 N (base degradation)	1 h	60°C	100.9	0.25	101.1	No significant degradation observed
Oxidation by 3.0% H2O2	1.5 h	25°C	84.7	12.26	96.9	Imp-2 was formed
Thermally treated	24 h	105°C	98.1	0.68	98.79	Imp-4 and Imp-6 impurities were formed
UV treated (254nm)	24 h	25°C	99.7	0.64	100.4	Imp-1 and Imp-6 impurities were formed

5. Conclusion:

An accurate, selective and sensitive gradient RP-HPLC method has been developed and validated as per regulatory guideline for the determination of process and degradation related impurities for the oncology drug, Dasatinib. In addition, this method is cost effective as there is no need to inject expensive impurities standard solution during method validation. Taken together, developed RP-HPLC method demonstrated precise, economical and commercially viable quantitative determination of Dasatinib impurities which will also be useful for industrial scale manufacturing.

References:

1. Ali, M. Mol. Diagn. Ther., 2016, 20, pp 315-333. doi: 10.1007/s40291-016-0208-1
2. Miura, M. Bio. Pharm. Bull., 2015, 38, pp 645-654. doi: 10.1248/bpb.b15-00103
3. Drug Bank: Dasatinib (DB01254).
4. International Conference on Harmonization. ICH Q1A(R2). Stability Testing of New Drug Substances and Products, 2003.
5. The United States Pharmacopoeia 39th ed., US Pharmacopoeia Convention, MD. 2017.
6. Zeng, J.; Cai, H. L.; Jiang, Z. P.; Wang, Q.; Zhu, Y.; Xu, P.; Zhao, X. L. J. Pharm. Anal., 2017, 7, pp 374-380. doi: 10.1016/j.jpha.2017.07.009
7. Pirro, E.; De Francia, S.; De Martino, F.; Fava, C.; Ulisciani, S.; Cambrin, G.; Racca, S.; Saglio, G.; Di Carlo, F. J. Chromatogr. Sci., 2011, 49 (10), pp 753-757. doi: 10.1093/chrsci/49.10.753
8. Gonzalez, A. G.; Taraba, L.; Hranicek, J.; Kozlik, P.; Coufal, P. J. Sep. Sci., 2017, 40, pp 400-406. doi: 10.1002/jssc.201600950

9. Prinesh, P.; Gananadhamu, S.; Veeraraghavan, S.; Rambabu, A.; Kanthi Kiran, V. S.; Swaroop Kumar, V. V. S. Anal. Methods., 2014, 6, pp 433-439. doi: 10.1039/C3AY41287C
10. D'Avolio, A.; Simiele, M.; De Francia, S.; Ariaudo, A.; Baietto, L.; Cusato, J.; Fava, C.; Saglio, G.; Di Carlo, F.; Di Perri, G. J. Pharm. Biomed. Anal., 2012, 59, pp 109-116. doi: 10.1016/j.jpba.2011.10.003
11. Lankheet, N.; Hillebrand, M.; Rosing, H.; Schellens, J.; Beijnen, J.; Huitema, A. Biomed. Chromatogr., 2013, 27 (4), pp 466-476. doi: 10.1002/bmc.2814
12. Hesham, K.; Motiur, R. A. F. M.; Mohammed, K. Dasatinib. In: Brittain, H. G. (Ed.). Profiles of Drug Substances, Excipients and Related Methodology. Elsevier, San Diego, CA, 2014, 39, Chapter 4, pp 205-237.
13. De Francia, S.; D'Avolio, A.; De Martino, F.; Pirro, E.; Baietto, L.; Siccardi, M.; Simiele, M.; Racca, S.; Saglio, G.; Di Carlo, F.; Di Perri, G. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci., 2009, 877, pp 1721-1726. doi: 10.1016/j.jchromb.2009.04.028
14. Andriamanana, I.; Gana, I.; Duretz, B.; Hulin, A. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci., 2013, 926, pp 83-91. doi: 10.1016/j.jchromb.2013.01.037

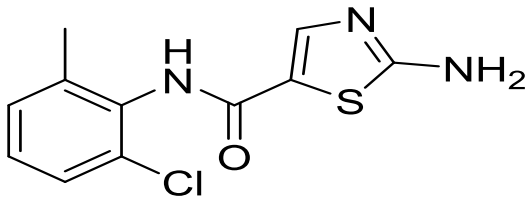
Chapter-2:

Characterization of related impurities present in dasatinib

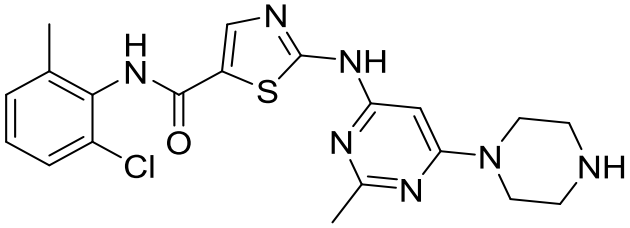
List of Impurities present in Dasatinib:

1.	2-amino-n-(2-chloro-6-methylphenyl)thiazole-5-carboxamide
2.	N-(2-chloro-6-methylphenyl)-2-((2-methyl-6-(piperazin-1-yl) pyrimidin-4-yl) amino) thiazole-5-carboxamide.
3.	4-(6-((5-((2-chloro-6-methylphenyl) carbamoyl) thiazol-2-yl) amino)-2-methylpyrimidin-4-yl)-1-(2-hydroxyethyl) piperazine 1-oxide.
4.	4-(2-(4-(6-((5-((2-chloro-6-methylphenyl)carbamoyl)thiazol-2-yl)amino)-2-methylpyrimidin-4-yl)piperazin-1-yl)ethoxy)-4-oxobutanoic acid
5.	2-(4-(6-((5-((2-chloro-6-methylphenyl)carbamoyl)thiazol-2-yl)amino)-2-methylpyrimidin-4-yl)piperazin-1-yl)ethyl acetate
6.	N-(2-chloro-6-methylphenyl)-2-[(6-chloro-2-methyl-4-pyrimidinyl)amino]-5-thiazole carboxamide

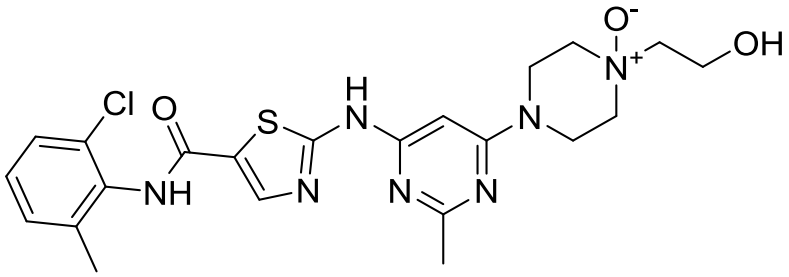
1. 2-amino-n-(2-chloro-6-methylphenyl)thiazole-5-carboxamide



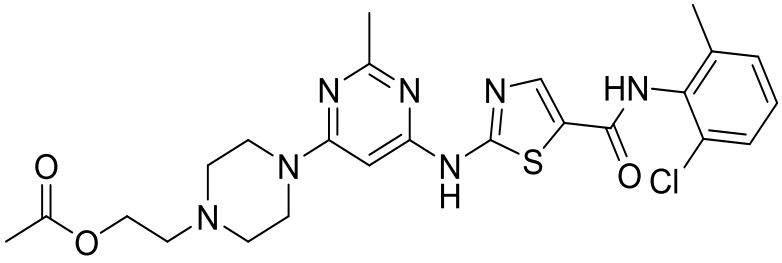
2. N-(2-chloro-6-methylphenyl)-2-((2-methyl-6-(piperazin-1-yl) pyrimidin-4-yl) amino) thiazole-5-carboxamide



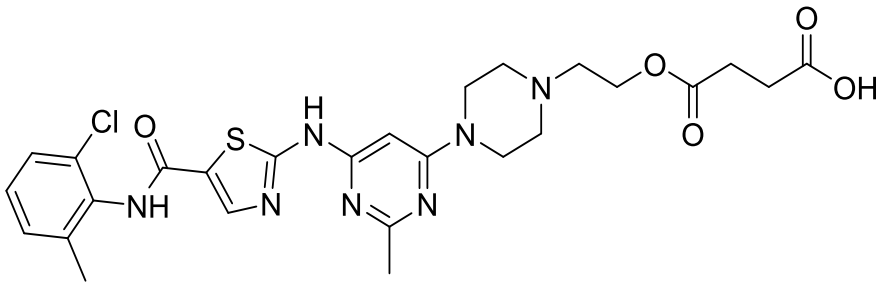
3. **4-(6-((5-((2-chloro-6-methylphenyl) carbamoyl) thiazol-2-yl) amino)-2-methylpyrimidin-4-yl)-1-(2-hydroxyethyl) piperazine 1-oxide**



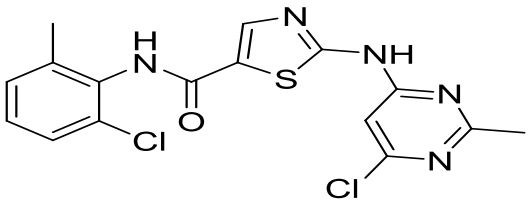
4. **4-(2-(4-(6-((5-((2-chloro-6-methylphenyl)carbamoyl)thiazol-2-yl) amino)-2-methylpyrimidin-4-yl)piperazin-1-yl)ethoxy)-4-oxobutanoic acid**



5. **2-(4-(6-((5-((2-chloro-6-methylphenyl)carbamoyl)thiazol-2-yl) amino)-2-methylpyrimidin-4-yl)piperazin-1-yl)ethyl acetate**



6. N-(2-chloro-6-methylphenyl)-2-[(6-chloro-2-methyl-4-pyrimidinyl) amino]-5-thiazole carboxamide



- **Conclusion:**

All the six impurities present in Dasatinib are identified and characterized by Mass Spectroscopy, ^{1}H-NMR spectroscopy, ^{13}C-NMR Spectroscopy and IR Spectroscopy.

Chapter-3:

Headspace gas chromatographic method development and validation for Organic Volatile impurities in Imatinib Mesylate

1. Introduction:

Imatinib Mesylate is a well-known tyrosine kinase inhibitor used to treat hematological malignancies in patient or malignant sarcomas such as gastrointestinal stromal tumors, chronic myeloid leukemia, acute lymphoblastic leukemia, gastrointestinal stromal tumors. Imatinib mesylate is a specific kind of tyrosine kinase inhibitor in Bcr-Abl+ cell 4-[(4-methylpiperazin-1-yl)methyl]-N-(4-methyl-3-(11) phenyl) benzamide methane sulfonate. Imatinib mesylate is approved for gastrointestinal stroma tumor treatment since 2002. Imatinib mesylate is also used for chronic myelogenous leukamia disease since 2011 and approved by USFDA.

Residual solvents in pharmaceuticals are defined here as organic volatile chemicals that are used or produced in the manufacture of drug substances or excipients, or in the preparation of drug products. The solvents are not completely removed by practical manufacturing techniques. Appropriate selection of the solvent for the synthesis of drug substance may enhance the yield, or determine characteristics such as crystal form, purity, and solubility. Therefore, the solvent may sometimes be a critical parameter in the synthetic process. This guideline does not address solvents deliberately used as excipients nor does it address solvates. However, the content of solvents in such products should be evaluated and justified.

This method describes the separation of six residual solvents, among which two pair of solvents was critical i.e. n-hexane and ethyl acetate, DCM and acetone using headspace gas chromatography (HSGC) with Flame Ionization Detector (FID) in Imatinib Mesylate API. Moreover, method was developed considering sample matrix and accuracy, precision and linearity has been established.

2. Classification of Residual solvents as per ICH

- **Class I solvents** (which covers 5 residual solvents) are known or suspected human carcinogens and environmental hazards, the use of these solvents should be avoided. It should be identified and quantified
- **Class II solvents** (which covers 29 residual solvents) are non-genotoxic animal carcinogens or possible causative agents of other irreversible toxicity such as neurotoxicity or teratogenicity. Use of these solvents should be limited
- **Class III solvents** (which covers 26 residual solvents) having low toxic potential to man; no health-based exposure limit is needed. Class 3 solvents have PDEs of 50 mg or more per day
- **Class IV** solvents are those for which no adequate toxicological data have been found.

Therefore determination of residual solvents becomes a necessary procedure for quality control of drug substances and drug product to meet regulatory guideline and ensure patient safety. Six solvents are required for the synthesis of Imatinib Mesylate i.e. methanol, acetone, dichloromethane, n-hexane, ethyl acetate and pyridine and these should be controlled in final API.

S. No	Name of residual solvent in Imatinib Mesylate	Class of solvent	Permissible daily exposure (PDE) (mg/day)	ICH Limit (ppm)	Density (kg/m3)
1	Methanol	II	30.0	3000	791.80
2	Acetone	III	50.0	5000	791.00
3	Dichloromethane	II	6.0	600	1326.00
4	n-Hexane	II	2.9	290	659.1
5	Ethyl acetate	III	50	5000	897.00
6	Pyridine	II	2.0	200	981.90

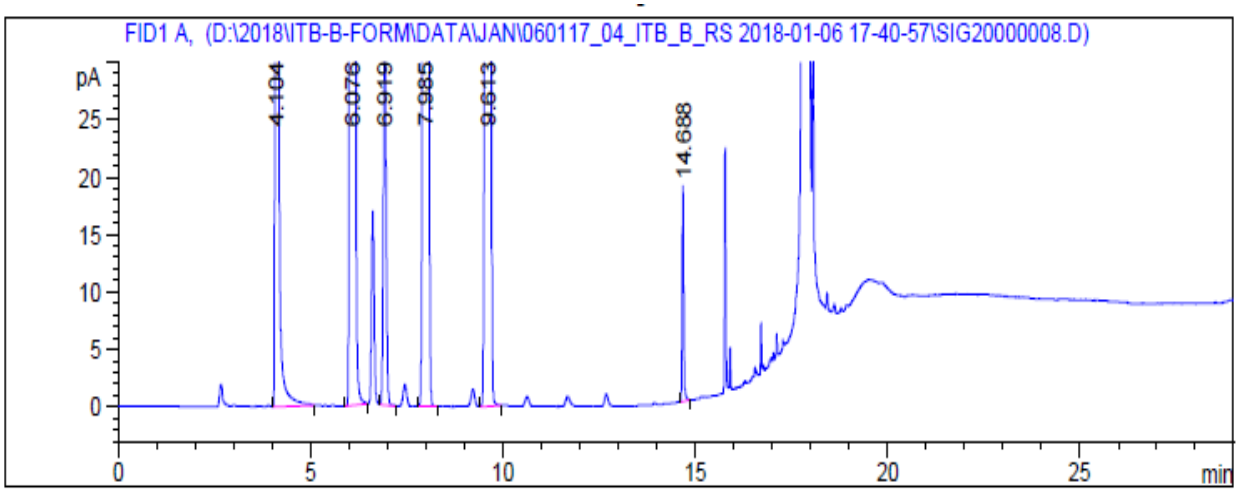
3. Optimized HSGC experimental method:

Components	Parameters	Requirements
Headspace condition	Injection volume	1ml
	G.C cycle time	40 min
	Oven temperature	80°C
	Loop temperature	90°C
	Transfer line temperature	110°C
	Sample equilibration time	30 min
	Loop equilibration time	0.20 min
	Loop fill time	0.10 min
	Inject time	1 min
Injector	Carrier gas	Nitrogen
	Injector Temperature	200°C
	Gas flow (Constant Pressure)	2.3 psi
	Injection mode	Split 1:2
	Liner	Glass liner
Column	DB-624 (30m long x 0.53mm I.D x 3µm film thickness) 6%Cyanopropylphenyl and 94% Dimethylpolysiloxane	
Detector Parameter	Total program time	29.0 min
	Type	FID
	Temperature	260°C
	Hydrogen flow	40 ml/min
	Airflow	400 ml/min
	Make up flow (N2)	25 ml/min

	Increment rate (°C/min)	Temperature (°C)	Hold time (min)
Oven temperature program	--	35	2
	4	80	0
	40	230	12

4. System precision and System suitability parameter:

Solvent name	RT (min)	USP resolution	USP tailing factor	USP theoretical plate	%RSD (n=6) of peak area
Methanol	4.10	--	1.292	20863	4.6
Acetone	6.07	16.0	1.028	33847	1.9
Dichloromethane	6.92	6.3	1.065	42971	2.5
n-Hexane	7.98	7.3	1.003	39627	2.1
Ethyl acetate	9.61	10.4	0.996	62912	2.3
Pyridine	14.69	45.0	1.162	652171	4.3



Typical chromatogram of the six composite standard solution

5. Conclusion:

A selective and sensitive fast static HSGC method has been successfully developed for the determination of methanol, acetone, dichloromethane, n-hexane, ethyl acetate and pyridine in Imatinib Mesylate API through consideration of route of synthesis and solvents nature. The developed method was successfully validated as per regulatory guideline and found to be precise, accurate, linear, robust and specific. Additionally, our method is suitable for analysis of pyridine and other solvents in one single method, which is accurate, precise and linear in presence of sample matrix. However only a limited number of solvents are used in Imatinib Mesylate API, this method may be used to separate the residual solvents present in other drug substances and can be used for routine analysis to monitor in-process drying and in quality control for bulk drug manufacturing. Taken together, our developed HSGC method demonstrated precise, economical and commercially viable quantitative technique for residual solvents determination in Imatinib Mesylate API which will also be advantageous for industrial scale manufacturing.

6. References:

1. Otero R, Carrera G, Dulsat JF, Fábregas JL, Claramunt J. Static headspace gas chromatographic method for quantitative determination of residual solvents in pharmaceutical drug

substances according to European Pharmacopoeia requirements. Journal of Chromatography A. 2004 Nov 19;1057(1-2):193-201.

2. Harmonized Tripartite Guideline on Maintenance of Note for Guidance on Impurities: Residual Solvents (Q3C (M)) (2016) International Conference on Harmonization of Technical Requirements for Registrations of Pharmaceuticals for Human Use (ICH), Geneva.
3. Residual Solvents <467>, the United States Pharmacopoeia, USP38/NF33 (2012) the United States Pharmacopeial Convention, Rockville, MD, USA.
4. Identification and control of residual solvents (2.4.24), in: European Pharmacopoeia (2013) 8th ed., European Directorate for the Quality of Medicines &HealthCare, Strasbourg, France.
5. Witschi C, Doelker E (1997) Residual solvents in pharmaceutical products: acceptable limits, influences on physicochemical properties, analytical methods and documented values. Eur J Pharm Biopharm 43:215-242.
6. Proceedings of International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH-2016), Tripartite harmonized guideline (Q3C) Impurities: Residual Solvents.
7. Kolb B, Ettre LS (1991) Theory and practice of multiple headspace extraction. Chromatographia 32:505-513.
8. Zhu JY, Chai XS (2005) some recent developments in headspace gas chromatography. Curr Anal chem 1:79-83.
9. B'Hymer C (2003) Residual solvent testing: a review of gas-chromatographic and alternative techniques. Pharm Res 20:337-344.
10. Camarasu CC, Mezei-Szuts M, Varga GB (1998) Residual solvents determination in pharmaceutical products by GC-HD and GC–MS-SPME. J Pharm Biomed Anal 18:623-638.
11. Otero R, Carrera G, Dulsat JF, Fabregas JL, Claramunt J (2004) Static headspace gas chromatographic method for quantitative determination of residual solvents in pharmaceutical drug substances according to European Pharmacopoeia requirements. J Chromatogr A 1057:193-201.

Chapter-4:

Quantification of Hydrazine Hydrate in Imatinib Mesylate at TTC (Threshold of Toxicological Concern) level by High pressure liquid chromatography

1. Introduction:

Increased concern for impurities present in drug substance and involvement of regulatory body provides threshold to the research of impurity profiling study. Even regulatory body becomes more stringent for impurities in generic products which increase recalls of product from markets. Definition of impurities as per ICH Q3A guidelines “impurity in a drug substance is any component of the drug substance that is not the chemical entity defined as the drug substance” and as per ICH Q3B guidelines “impurity in any component of the drug product that is not the chemical entity defined as the drug substance or an excipient in the drug product”

Imatinib Mesylate is a well-known tyrosine kinase inhibitor used to treat hematological malignancies in patient or malignant sarcomas such as gastrointestinal stromal tumors, chronic myeloid leukemia, acute lymphoblastic leukemia, gastrointestinal stromal tumors. Imatinib mesylate is a specific kind of tyrosine kinase inhibitor in Bcr-Abl+ cell 4-[(4-methylpiperazin-1-yl)methyl]-N-(4-methyl-3-(11) phenyl) benzamide methane sulfonate. Imatinib mesylate is approved for gastrointestinal stroma tumor treatment since 2002. Imatinib mesylate is also used for chronic myelogenous leukamia disease since 2011 and approved by USFDA.

Reduction of nitro group during the synthesis of Imatinib can be achieved by several reducing reagents such as Fe/HCl, SnCl2/HCl, hydrazine hydrate/Raney Ni, hydrazine hydrate/FeCl3/C. The use of Fe/HCl and SnCl2/HCl as reducing agent were not preferred as due to presence of metallic hydroxides emulsion formation occurs during isolation process of imatinib. SnCl2is an expensive reagent and also toxic. In comparison to other reduction process, the reduction with hydrazine hydrate produces harmless byproducts such as nitrogen gas and water.

2. Selection and optimization of derivatizing agent:

The derivatization agent should bump conversion of free impurity (hydrazine hydrate) to the derivatized product (1,2-dibenzylidenehydrazine). The absorption of derivatized product should be far away from the absorption of reagents and solvents so the interference should be minimum. For derivatized product 1,2-dibenzylidenehydrazine a strong UV absorption at 300nm is noted which is far away from any interference of solvents and reactive species. A derivatized product 1,2-dibenzylidenehydrazine seems to be highly suitable for the High pressure liquid chromatography due to its high lipophilicity which could help in good mark on retention in C18,C8 and even in C8 columns.

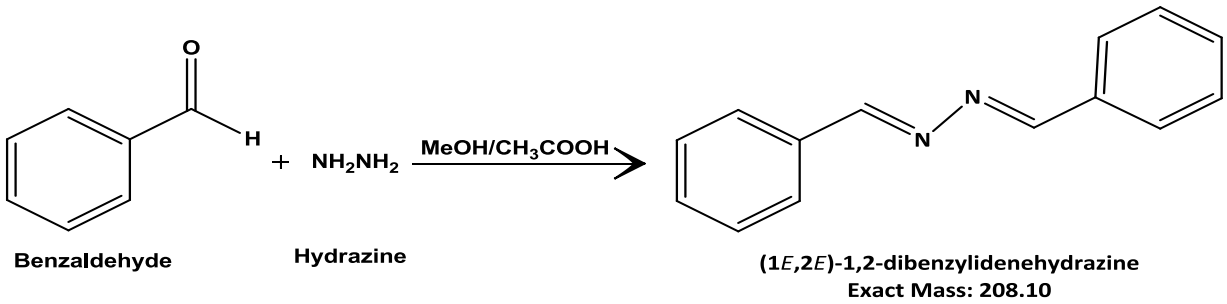
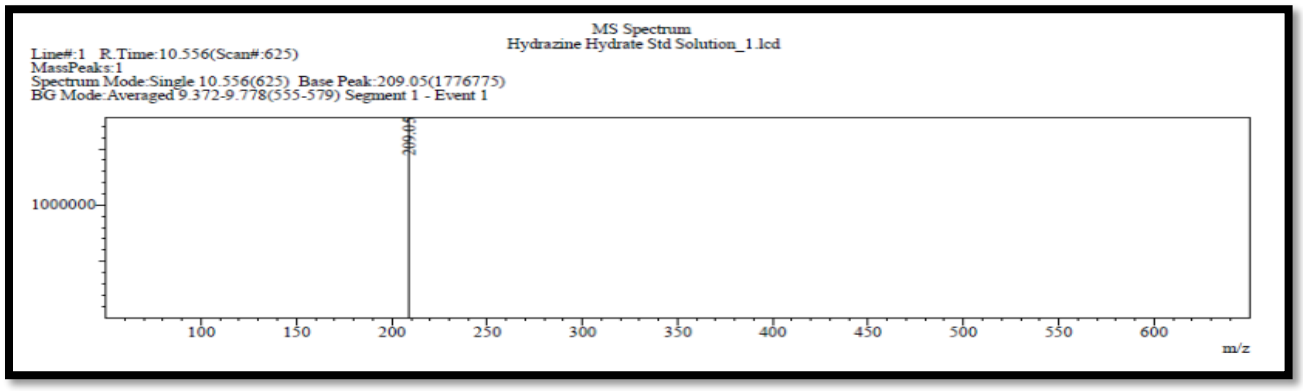


Fig.1. Reaction scheme of Benzaldehyde with Hydrazine in acidic condition for Derivatization

3. Detection of impurity by LC-MS:

LC-MS chromatogram shows molecular ions at m/z 209.05 (M^++H) confirmed that resultant product was 1,2-dibenzylidenehydrazine.



4. Optimized HPLC parameters:

Parameter	**Conditions**
HPLC Column	Inertsil ODS-3V, 5µm, 4.6 × 250 mm
Mobile Phase	A: water
	B: Acetonitrile:Water-90:10 %v/v
Injection Volume	50µl
Isocratic	Solvent A:Solvent B-30:70
Flow Rate	1.5ml/min
Column Oven Temp.	40°C
UV Wavelength	300nm
Run Time (min)	20min

5. System suitability criteria:

Parameters	**Observed Results (n=6)**	**Acceptance Criteria**	**Remarks**
Theoretical plates	11223	> 2000	Method passes the system suitability test
Tailing factor	1.03	T ≤ 1.5	
Repeatability (% RSD)	1.70	%RSD <5	
Resolution	23.6	Rs <2	

6. Application of method:

- The method presented above was very elective and even sensitive fir the determination of hydrazine hydrate present in pharmaceutical product or even in drinking water.
- Method validation is performed as per guidelines provided by ICH. Derivatization of hydrazine hydrate makes is detectable at UV range which is very convenient and easy to perform.

- As HPLC is involved in method of detection, method can be performed easily at academic and industrial level due to very cheap solvents and easily available agents are used during derivatization.
- Reproducibility of method is very high so it can be performed at any facility with basic instrumentation requirements.

7. Conclusion:

The presented method provides specific and meticulous quantization of hydrazine hydrate in a variety of pharmaceutical product and active ingredients using a derivatization technique which is very simple in nature and with help of HPLC. Derivatizing a hydrazine hydrate proved a key approach for the detection of impurity. A methanolic solution of benzaldehyde performed role of derivatizing agent and able to meet the requirements of all analytical tools. As hydrazine hydrate doesn't have chromophores in it derivatizations by benzaldehyde help to shift its wavelength to the detectable UV range. With addition derivatized product can be easily resolve by HPLC from rest of Active pharmaceutical ingredients peak. Moreover, current method were proved suitable on the basis of linearity and range, accuracy, specificity and precision. All the statistical results present i.e. R.S.D., % recovery and mean are seems to be in acceptable criteria. The usefulness of method is not only limited to the impurities in bulk drug but also it can be used for final formulation of active pharmaceutical ingredients.

8. References:

1. Guideline IHT, editor. Impurities in new drug substances Q3A (R2). Proceedings of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Geneva, Switzerland; 2006.
2. Guideline IHT. Impurities in new drug products. Q3B (R2), current step. 2006;4:1-5.
3. Görög S. Identification and determination of impurities in drugs: Elsevier; 2000.
4. Ahuja S, Alsante KM. Handbook of isolation and characterization of impurities in pharmaceuticals: Academic press; 2003.
5. Shukla PK, Verma A. Handbook of Inorganic Impurities in Pharmaceuticals.

6. Prabu SL, Suriyaprakash T. Impurities and its importance in pharmacy. Int J Pharm Sci Rev Res. 2010;3(2):66-71.
7. Guideline IHT. Impurities: Guideline for residual solvents Q3C (R5). Current Step. 2005;4:1-25.
8. Puranik S, Pai P, Rao G. Organic volatile impurities in pharmaceuticals. Indian Journal of Pharmaceutical Sciences. 2007;69(3):352.
9. Dwivedi SD, Singh KK, Charan GDS, Vasava CJ. Process for the preparation of amorphous imatinib mesylate. Google Patents; 2016.
10. Snodin DJ. Residues of genotoxic alkyl mesylates in mesylate salt drug substances: real or imaginary problems? Regulatory Toxicology and Pharmacology. 2006;45(1):79-90.
11. Amarnath V, Anthony DC, Amarnath K, Valentine WM, Wetterau LA, Graham DG. Intermediates in the Paal-Knorr synthesis of pyrroles. The Journal of Organic Chemistry. 1991;56(24):6924-31.
12. Guideline IHT, editor. Assessment and Control Of Dna Reactive (Mutagenic) Impurities In Pharmaceuticals To Limit Potential Carcinogenic RISK M7. International conference on harmonization of technical requirements for registration of pharmaceuticals for human use (ICH): Geneva; 2014.
13. Wahlich J, Carr G. Chromatographic system suitability tests—what should we be using? Journal of pharmaceutical and biomedical analysis. 1990;8(8-12):619-23.
14. Persson B-A, Vessman J, McDowall R. Is your method specific or just selective? LC GC. 1998;16(6):556-60.
15. Armbruster DA, Pry T. Limit of blank, limit of detection and limit of quantitation. The clinical biochemist reviews. 2008;29(Suppl 1):S49.

Chapter-5:

Impurity profiling of Novel ZY12201 (NCE)

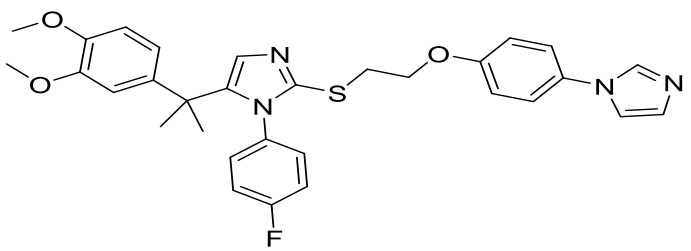
1. Introduction:

Diabetes is increasing globally at an alarming state. Type 2 diabetes is characterized by reduced insulin sensitivity combined with impaired insulin secretion resulting in higher blood glucose levels. Although a range of therapies are available such as sulfonylureas, metformin, and glinides, they are unable to achieve satisfactory glycemic control. Therefore, there is an urgent need for research exploring therapies with distinct unprecedented mechanisms of action.

Takeda G-protein-coupled receptor 5 (TGR5), also known as GPR 131, GPBAR1, or M-BAR is G protein-coupled receptor primarily expressed in monocytes and macrophages, lung, spleen, and the intestinal tract, and it is activated by bile acids. It has been suggested that bile acids induce glucagonlike peptide-1 (GLP-1) secretion from primary intestinal enteroendocrine cells by increasing intracellular cAMP levels via the TGR5 receptor.

Additionally, activation of TGR5 receptors in brown adipose tissue has been proposed to increase energy expenditure through the induction of type 2 iodothyronine deiodinase (D2). Thus, a small molecule TGR5 agonist may be beneficial for the treatment of type 2 diabetes with simultaneous management of glucose levels, body weight, and associated complications.

A wide range of structurally diverse modulators of TGR5 have been reported in the literature by various pharmaceutical companies. Most of these reported TGR5 agonists, however, possess insufficient potency and/or lack metabolic stability. This communication describes the discovery of 2-((2-(4-(1H-imidazol-1-yl)phenoxy)ethyl) thio)-5-(2-(3,4-dimethoxyphenyl) propan-2-yl)-1-(4-fluoro phenyl)-1H-imidazole (6g), a potent, selective, and orally efficacious TGR5 agonist.



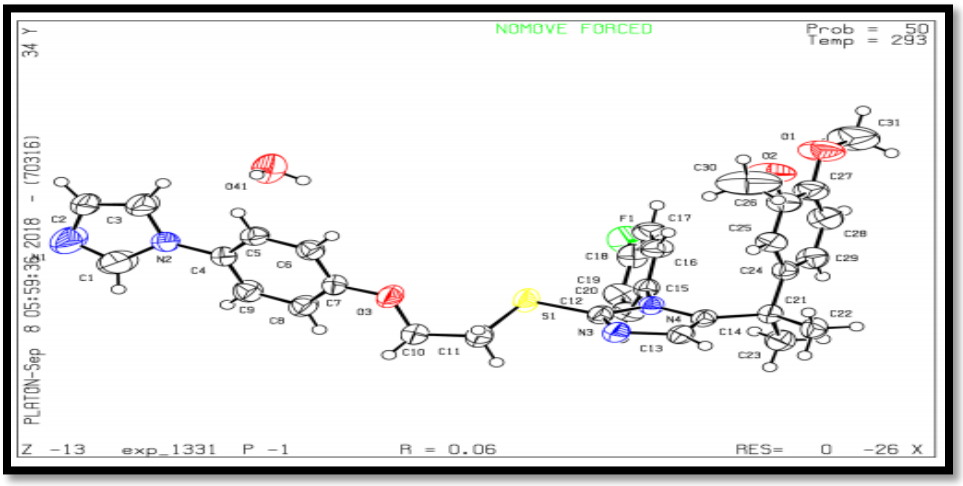
2. Force degradation study:

Degradation condition	Time	Temp	Assay (%, w/w)	Rs by HPLC % Degradation	Mass balance (% assay + % deg. Products)	Remarks/ Observation
A control sample (untreated)	-	-	100.6	0.27	100.8	NA
HCl, 1.0 N (acid degradation)	1 h	60°C	100.8	0.22	101.0	No significant degradation observed
NaOH, 1.0 N (base degradation)	1 h	60°C	100.9	0.25	101.1	No significant degradation observed
Oxidation by 3.0% H2O2	1.5 h	25°C	84.7	12.26	96.9	Imp-2 was formed
Thermally treated	24 h	105°C	98.1	0.68	98.79	Imp-4 and Imp-6 impurities were formed
UV treated (254nm)	24 h	25°C	99.7	0.64	100.4	Imp-1 and Imp-6 impurities were formed

3. Crystallography:

Table 1: crystal data and structure refinement for ZY12201.	
Empirical formula	$C_{31}H_{33}FN_4O_4S$
Formula weight	576.67
Temperature/K	293(2)
Crystal system	triclinic
Space group	P-1
a/Å	8.8548(6)
b/Å	10.3539(6)
c/Å	16.8284(10)
α/°	78.388(5)
β/°	80.158(5)
γ/°	83.078(5)
Volume/$Å^3$	1483.10(16)

Z	2
$\rho_{calc}g/cm^3$	1.291
μ/mm^{-1}	0.158
F(000)	608.0
Radiation	MoKα (λ = 0.71073)
2Θ range for data collection/°	6.32 to 58.16
Index ranges	-11 ≤ h ≤ 12, -14 ≤ k ≤ 14, -21 ≤ l ≤ 22
Reflections collected	32734
Independent reflections	7178 [R_{int} = 0.0347, R_{sigma} = 0.0324]
Data/restraints/parameters	7178/0/377
Goodness-of-fit on F^2	1.044
Final R indexes [I>=2σ (I)]	R_1 = 0.0593, wR_2 = 0.1501
Final R indexes [all data]	R_1 = 0.0892, wR_2 = 0.1714
Largest diff. peak/hole / e $Å^{-3}$	0.84/-0.53



4. Conclusion:

In conclusion we have developed a method to detect process and degradation related impurities in novel compound ZY12201.

5. References:

1. IBD Diabetes Atlas, 6th ed.; International Diabetes Federation: Brussells, Belgium, 2013;http://www.diabetesatlas.org.
2. De Fronzo, R. A. From the Triumvirate to the Ominous Octet: A New Paradigm for the Treatment of Type 2 Diabetes Mellitus. Diabetes2009, 58, 773−795.

3. Nazimek-Siewniak, B.; Moczulski, D.; Grzeszczak, W. Risk of macrovascular and microvascular complications in Type 2 diabetes Results of longitudinal study design.J. Diabetes Complications2002, 16, 271−276.
4. Kles, K. A.; Vinik, A. I. Pathophysiology and treatment of diabetic peripheral neuropathy: The case for diabetic neurovascular functions as an essential component. Curr. Diabetes Rev.2006, 2, 131−145.
5. Doyle, M. E.; Egan, J. M. Pharmacological agents that directly modulate insulin secretion. Pharmacol. Rev.2003, 55, 105−131.
6. Ashiya, M.; Smith, R. E. T. Non-insulin therapies for type 2 diabetes.Nat. Rev. Drug Discovery2007, 6, 777−778.
7. Maruyama, T.; Miyamoto, Y.; Nakamura, T.; Tamai, Y.; Okada, H.; Sugiyama, E.; Itadani, H.; Tanaka, K. Identification of membrane type receptor for bile acids (M-BAR). Biochem. Biophys. Res. Commun. 2002, 298, 714−719.
8. Tiwari, A.; Maiti, P. TGR5: an emerging bile acid G-protein coupled receptor target for the potential treatment of metabolic disorders. Drug Discovery Today2009, 14, 523−530.
9. Katsuma, S.; Hirasawa, A.; Tsujimoto, G. Bile acids promote glucagon-like peptide-1 secretion through TGR5 in a murine enteroendocrine cell line STC-1. Biochem. Biophys. Res. Commun. 2005, 329, 386−390.
10. Thomas, C.; Gioiello, A.; Noriega, L.; Strehle, A.; Oury, J.; Rizzo, G.; Macchiarulo, A.; Yamamoto, H.; Mataki, C.; Pruzanski, M.; Pellicciari, R.; Auwerx, J.; Schoonjans, K. TGR5-mediated bile acid sensing controls glucose homeostasis. Cell Metab.2009, 10, 167−177