Waters™

Nota de aplicación

Modernized Impurity Analysis of the Kinase Inhibitor Imatinib by High-Resolution LC With MS-Compatible Mobile Phases

Peng Chen, Bonnie A. Alden, Matthew A. Lauber

Waters Corporation

Abstract

A UPLC method has been developed with a charged surface phenyl stationary phase for the separation of imatinib and its related impurities. A high efficiency, highly selective separation was achieved with an MS-compatible mobile phase based on 10 mM ammonium formate and 0.1% formic acid modifier. Imatinib and its nine related impurities were separated within six-minutes, and parent and fragmentation MS spectra of the impurities were readily obtained for peak confirmation.

Benefits

- Six-minute separation of imatinib and its nine related impurities
- The use of UPLC and a CSH Phenyl-Hexyl Column provides efficient separations with baseline component resolution
- MS-compatible mobile phases for sensitive MS and MS/MS analysis

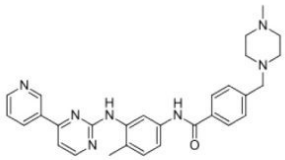
Introduction

Within the last couple of decades, small molecule pharmacology has expanded into multiple exciting areas. In particular, a new pipeline of cancer-fighting drug candidates has emerged, many of which aim at kinase inhibitors as their druggable target. These molecules are built upon peptide mimicking backbones and are thereby constructed with heteroatoms and conjugated substituents that look similar to amino acid residues that kinase inhibitors would naturally act upon. Drug candidates within this pharmaceutical space are chemically unique, and there is an ever-present need for high-resolution impurity analyses. Imatinib was the first kinase inhibitor to be approved by the FDA in 2001.[1–3] The European Pharmacopeia (EP) has issued a monograph HPLC method for its analysis as well as a set of its related impurities. This EP method uses three different HPLC conditions and two different HPLC columns to separate imatinib from seven of its impurities – A, B, C, D, F, H, and J. Additionally, the EP method calls for a non-volatile ion-pairing agent (sodium octanesulfonate) to facilitate the separation, which precludes the use of mass spectrometry if ever there was a need for a quality investigation upon the observation of a spurious peak.

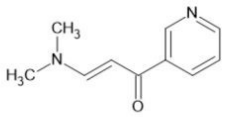
In this application note, we have taken advantage of a charged surface hybrid phenyl column and MS-compatible mobile phases to achieve significantly higher separation efficiency and selectivity for Imatinib and nine of its related impurities, seven of which are documented in the EP monograph. Moreover, because the method is readily hyphenated with mass spectrometry, it was possible to apply MS and MS/MS analysis to support component identification and structural analysis. In all, it is believed that the approaches outlined here will be adaptable to other kinase inhibitors, many of which feature similar N-containing heterocyclic moieties.

Experimental

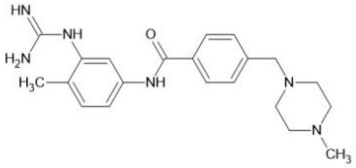
Chemicals and Materials



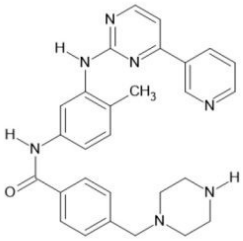
Imatinib (CAS 152459-95-5)
Free base $C_{29}H_{31}N_7O$
Exact mass 493.25901
$(M+H)^+$ 494.26629



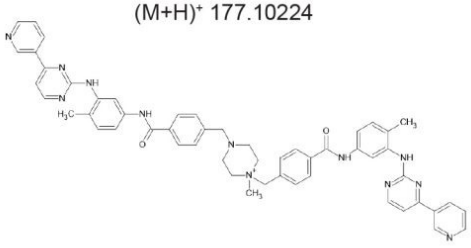
Impurity A (CAS 123367-26-0)
$C_{10}H_{12}N_2O$
Exact mass 176.09496
$(M+H)^+$ 177.10224



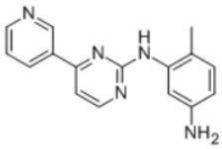
Impurity B (CAS 581076-67-7)
$C_{21}H_{28}N_6O$
Exact mass 380.23245
$(M+H)^+$ 381.23974



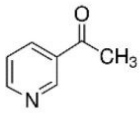
Impurity C (CAS 404844-02-6)
$C_{28}H_{29}N_7O$
Exact mass 479.24336
$(M+H)^+$ 480.25064



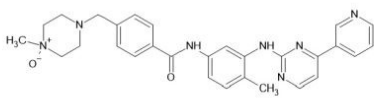
Impurity D (CAS 1821122-73-9)
Cation $C_{53}H_{51}N_{12}O_2^+$
Exact mass of M^+ 887.42525



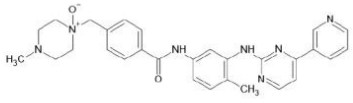
Impurity F (CAS 152460-10-1)
$C_{16}H_{15}N_5$
Exact mass 277.13275
$(M+H)^+$ 278.14002



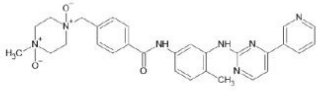
Impurity H (CAS 350-03-8)
C_7H_7NO
Exact mass 121.05276
$(M+H)^+$ 122.06004



Impurity J (CAS 571186-91-9)
$C_{29}H_{31}N_7O_2$
Exact mass 509.25392
$(M+H)^+$ 510.26120



Imatinib (Piperidine)-1-oxide
(CAS 938082-57-6)
$C_{29}H_{31}N_7O_2$
Exact mass 509.25392
$(M+H)^+$ 510.26120



Imatinib (Piperidine)-N,N-dioxide
(CAS 571186-93-1)
$C_{29}H_{31}N_7O_3$
Exact mass 525.24884
$(M+H)^+$ 526.25611

Imatinib and impurity F were acquired from Sigma. Imatinib impurities A, B, D, and H were obtained from Toronto

Research Chemicals. Imatinib impurities C and J as well as imatinib (Piperidine)-1-oxide (Oxide in short) and imatinib (Piperidine)-N,N-dioxide (Di-oxide in short) were purchased from BocSciences.

Sample Preparation

Each standard chemical listed above was used to prepare a stock standard solution of 1.5 mg/mL in a mixed solvent of methanol and acetonitrile (1:1). 100 µL of each stock standard solution was mixed together, and the mixture was further diluted with methanol (1:5) for UPLC analysis. Each stock standard solution was diluted with methanol (1:50) for UPLC analysis and retention time confirmation.

UPLC Method Conditions

UPLC system:	ACQUITY UPLC I-Class
Detection:	UV detection at 227, 254, and 267 nm
Vials:	Total Recovery 12 x 32 mm glass screw neck vials (p/n: 186000384C)
Column 1:	Figures 1 and 2: ACQUITY UPLC HSS C_{18} 1.8 µm Column, 100 Å, 2.1 mm x 100 mm (p/n: 186003533)
Column 2:	Figure 3: ACQUITY Premier UPLC CSH Phenyl-Hexyl 1.7 µm Column, 130 Å, 2.1 mm x 100 mm (p/n: 186009475)
Column temp.:	35 °C for Figure 1, and 40 °C for Figures 2 and 3
Sample temp.:	10 °C
Injection volume:	0.5 µL (sample)
Flow rate:	0.50 mL/min for Figure 1, and 0.40 mL/min for Figures 2 and 3
Mobile phase A:	Figure 1: 2.3 g of sodium octanesulfonate

	monohydrate and 1.2 mL of phosphoric acid in 700 mL of water and 300 mL of acetonitrile Figures 2 and 3: 0.1% (v/v) formic acid and 10 mM ammonium formate in water
Mobile phase B:	Figure 1: 2.3 g of sodium octanesulfonate monohydrate and 1.2 mL of phosphoric acid in 100 mL of water and 900 mL of acetonitrile Figures 2 and 3: 0.1% (v/v) formic acid in acetonitrile

MS Conditions

MS system:	Vion IMS QTof
Ionization mode:	ESI positive, resolution
Acquisition range:	50–1000 *m/z*
Capillary voltage:	2.0 kV
Sampling cone:	80 V
HD-MS^E collision energy:	6 eV (low energy), and 10–40 eV ramp (high energy)
HS-MSMS collision energy:	10–40 eV ramp @ 50 *m/z* to 20–50 @1000 *m/z*
Source temperature:	110 °C
Desolvation temperature:	400 °C
Desolvation gas:	800 L/h

Data Management

UPLC and MS software:	UNIFI v1.8 for Data Acquisition and Analysis

Results and Discussion

The EP monograph method for imatinib served as a starting point for our LC method development. For optical detection of impurities, the EP monograph calls for the use of a long 4.6 x 250 mm column packed with 5 µm silica C_{18} and the use of an ion pair containing mobile phase. A relatively high flow rate of 2.3 mL/min is also specified. This method was scaled to a UPLC condition before starting experiments. An ACQUITY UPLC HSS $C_{18,}$ 1.8 µm, 2.1 x 100 mm Column was selected to match the L/dp, and a flow rate of 0.5 mL/min was applied to provide an optimal pairing with MS detection (even though it corresponds to a lower linear velocity than that cited in the EP monograph). Under these conditions, imatinib impurities A and H co-eluted around 0.64 min, so did impurity J and Oxide at 5.87 min, as shown in Figure 1.

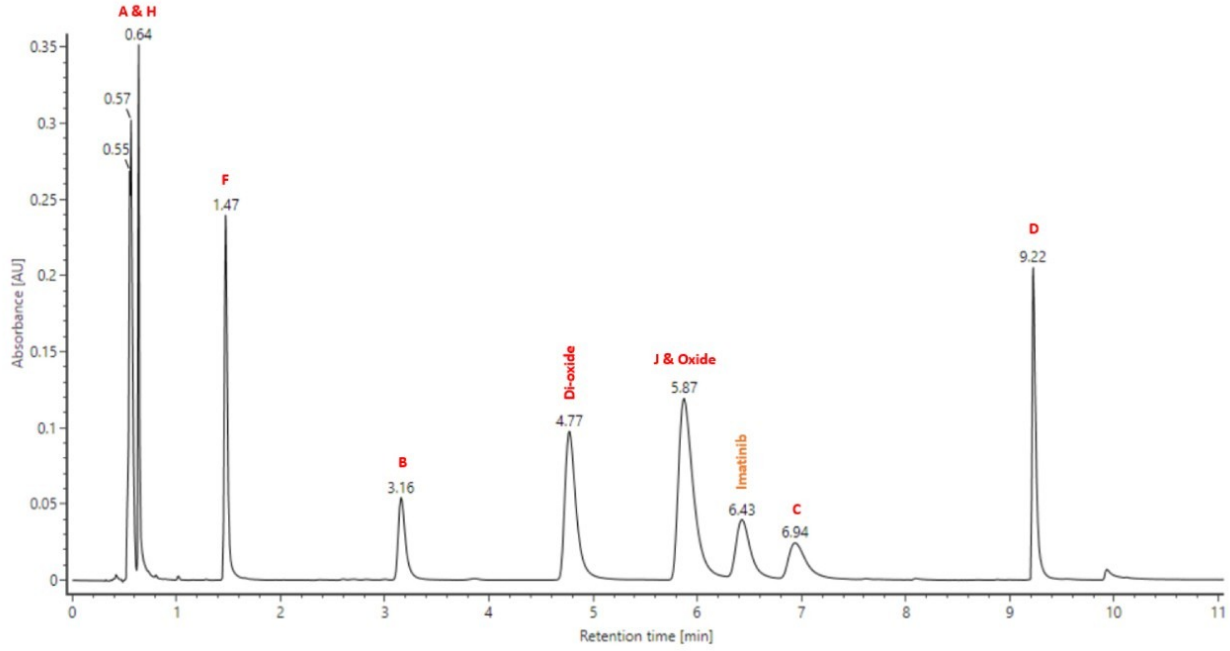


Figure 1. Separation of imatinib and nine related impurities under UPLC-modified EP method conditions with an ACQUITY HSS C_{18} Column (2.1 mm x 100 mm C_{18} 1.8 µm 100 Å). Detection: 267 nm. Flow rate: 0.5 mL/min. Gradient: 2% B isocratic for 6.14 min, and 2% B to 50% B linear gradient in 5.36 min.

With the same C_{18} Column, a new UPLC gradient with MS-compatible mobile phases was chosen to shorten the analysis time and to begin obtaining precursor and fragment ion MS spectra for component identification and structural analysis. However, the peak pair of Oxide/Di-oxide could not be separated, and peaks A/B/H and C/imatinib/J were found to partially co-elute, as shown in Figure 2.

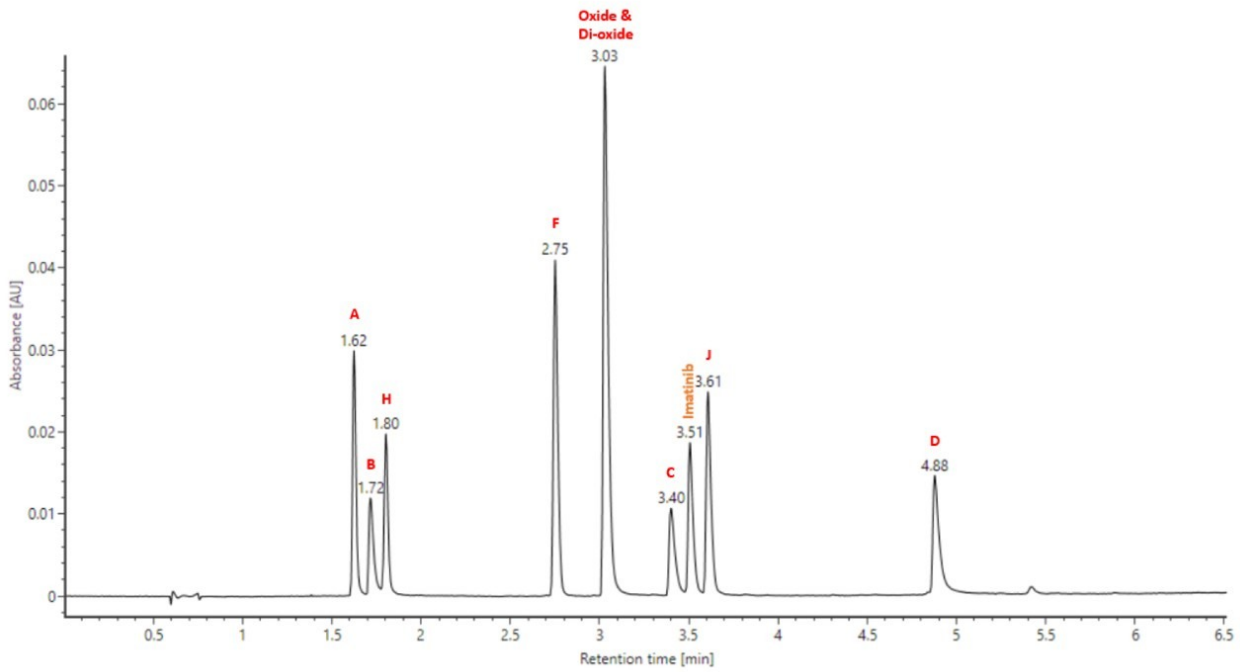


Figure 2. Separation of imatinib and nine related impurities with MS-compatible mobile phases and an ACQUITY HSS C_{18} Column (2.1 mm x 100 mm, $C_{18,}$ 1.8 µm, 100 Å). Detection: 267 nm. Flow rate: 0.4 mL/min. Gradient: 5% B to 100% B linear gradient in 10-min.

To enhance selectivity towards the N-containing heterocyclic structures of the imatinib impurity molecules, an ACQUITY Premier CSH 130 Å Phenyl-Hexyl Column was next applied. The CSH Phenyl-Hexyl Particle is a unique stationary phase that is modified to bear a phenyl hexyl ligand along with a positive surface potential under acidic pH conditions. Accordingly, this stationary phase exhibits unique selectivity versus a more traditional C_{18} RP-LC phase. With the CSH Phenyl-Hexyl Column, imatinib impurities A/H and J/C were well separated, and a change in peak elution order was observed due to the unique properties of the sorbent, as shown in Figure 3.

Mass spectra were acquired during this separation to verify peak identifications and to corroborate peak assignments based on individual injections. Extracted ion chromatograms (XICs) for the protonated molecular/precursor ions $(M+H)^+$ or native cation M^+ of each analyte have been prepared and are overlaid in the lower panel of Figure 3.

In addition to its retentivity and selectivity, the CSH Phenyl-Hexyl Packing Material is relatively resistant to dewetting. As a result, it was possible to optimize the initial conditions of the method for even greater retention of the analytes. An initial condition of 0.5% B was therefore employed as a way to more effectively retain Impurity B. To further optimize the retention of this molecule, we incorporated 10 mM ammonium formate into mobile phase A to attenuate some of the charge repulsion imparted by the CSH particle's positive surface potential.

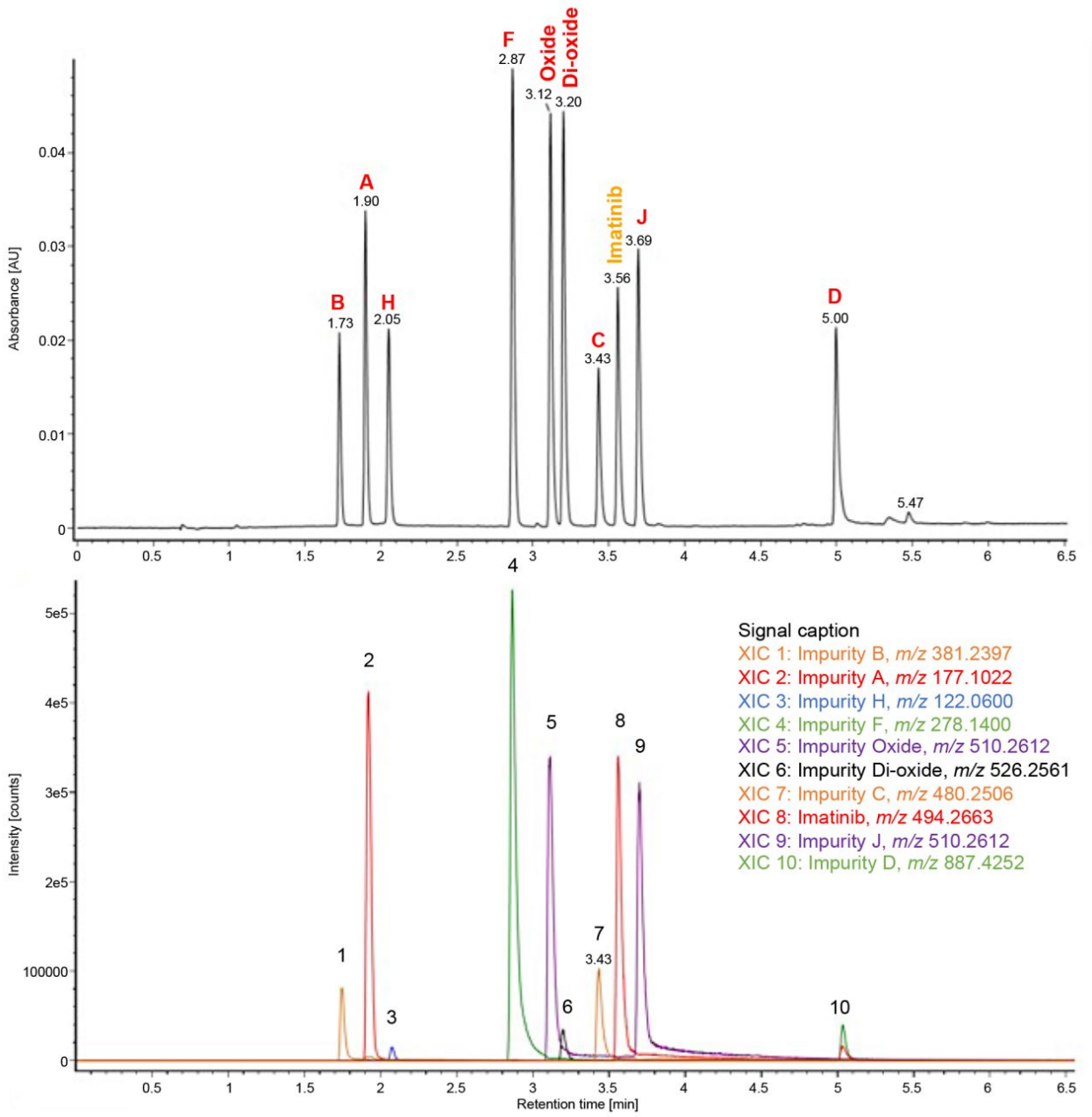


Figure 3. Separation and MS XICs of imatinib and nine related impurities with MS-compatible mobile phases and an ACQUITY Premier CSH Phenyl-Hexyl Column (2.1 mm x 100 mm, $C_{18,}$ 1.7 µm, 130 Å). Detection: 267 nm. Flow rate: 0.4 mL/min. Gradient: 0.5% B to 100% B in 10 min with a linear gradient.

With peaks baseline separated, additional MS analyses were performed. Figure 4 provides a fragmentation spectrum of imatinib, highlighting how this modernized UPLC method can be hyphenated with mass spectrometry to obtain information for further structural analysis of known and unknown peaks. The UNIFI elucidation tool kit can help confirm or propose molecular formulas of known or unknown MS signals based on accurate masses of molecular

ions and isotopic ions, as well as isotopic abundances and isotopic spacings. The tool kit can subsequently analyze the MS/MS fragmentation spectra and assign or propose structures for the fragments, as shown in Figure 4. Additional work is underway to investigate low-level impurities of imatinib.

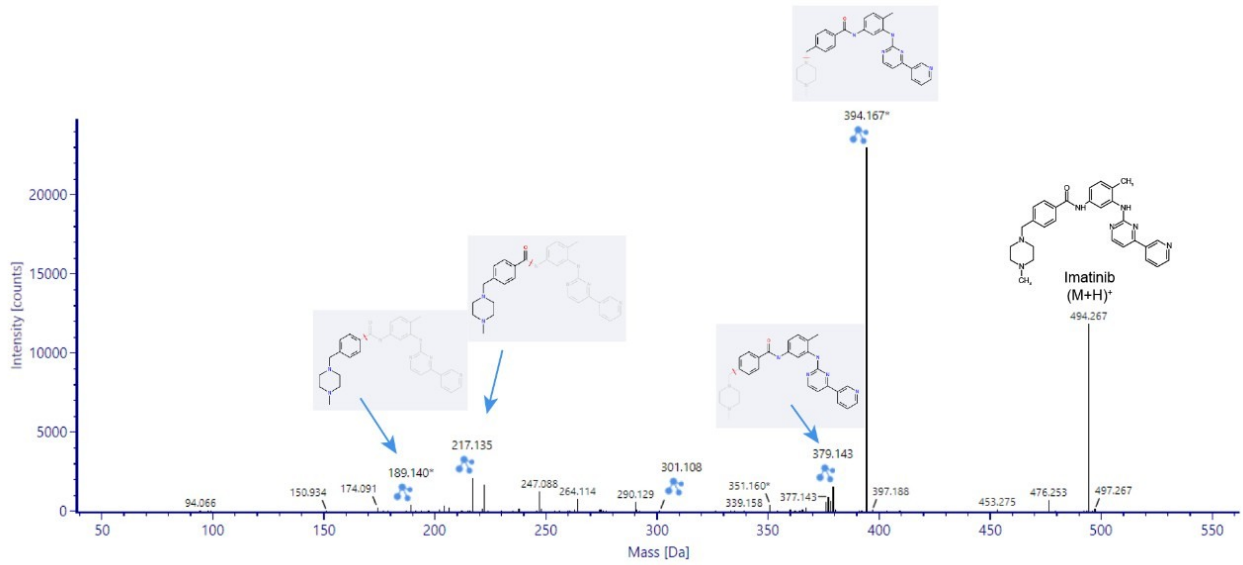


Figure 4. MS fragmentation spectrum of imatinib obtained through a High Definition MS^E mode analysis.

Conclusion

The use of UPLC with an ACQUITY Premier CSH Phenyl-Hexyl Column has facilitated the modernization of an EP method to analyze imatinib and its impurities. Considerations on L/dp and MS compatibility were applied to develop a separation that produces excellent peak shape and resolution for imatinib and nine of its impurities. Sensitive impurity analysis was made possible through both optical detection and accurate mass time-of-flight MS analysis. It is our hope that new analytical methods like this will help hasten the development and QC testing of kinase inhibitors for oncology indications and beyond.

References

1. Cohen, P., Cross, D. & Jänne, P.A. Kinase Drug Discovery 20 years after Imatinib: Progress and Future

Directions. *Nat Rev Drug Discov* 20, 551–569 (2021).

2. Bhullar, K. S. *et al.* Kinase-Targeted Cancer Therapies: Progress, Challenges and Future Directions. *Molecular Cancer* 17:48 (2018).

3. Roskoski, R. Jr. Properties of FDA-Approved Small Molecule Protein Kinase Inhibitors: A 2021 Update. *Pharmacological Research* 165,105463 (2021).

Featured Products

ACQUITY UPLC I-Class PLUS System <https://www.waters.com/134613317>

Vion IMS QTof Ion Mobility Quadrupole Time-of-flight Mass Spectrometry <https://www.waters.com/134845751>

UNIFI Scientific Information System <https://www.waters.com/134801648>

720007340, August 2021