ORGANIC PROCESS RESEARCH & DEVELOPMENT
OPR&D

Communication

A Single Quadrupole Compact Mass Spectrometer Enabling Early Stage Synthetic Optimization of Verubecestat (MK-8931)

Daniel Zewge, Xiaodong Bu, Li Zhang, David A. Thaisrivongs, Yanke Xu, and Xiaoyi Gong

Org. Process Res. Dev., **Just Accepted Manuscript** • DOI: 10.1021/acs.oprd.9b00370 • Publication Date (Web): 19 Nov 2019

Just Accepted

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

A Single Quadrupole Compact Mass Spectrometer Enabling Early Stage Synthetic Optimization of Verubecestat (MK-8931)

Daniel Zewge,*[a] Xiaodong Bu,*[a] Li Zhang,[a] David Thaisrivongs,[b] Yanke Xu,[c] and Xiaoyi Gong[a]

[a]*Small Molecule Analytical Research and Development;* [b]*Small Molecule Process Research and Development;* [c]*Chemical Engineering Research and Development. Merck & Co., Inc., P.O. Box 2000, Rahway, NJ 07065, USA*

**Corresponding author, E-mail address: daniel_zewge@merck.com; xiaodong_bu@merck.com*

ABSTRACT:

A low-cost compact mass spectrometer was effectively utilized for identifying and tracking process impurities in the early stage development of a multi-step synthetic protocol used to prepare the verubecestat drug substance. Process optimizations were influenced by the type, quantity, and final fate of impurities generated during synthesis. Employing a compact mass spectrometer for impurity identification and tracking served as a guide to redesign synthetic strategies, which enabled the minimization or avoidance of undesired side products.

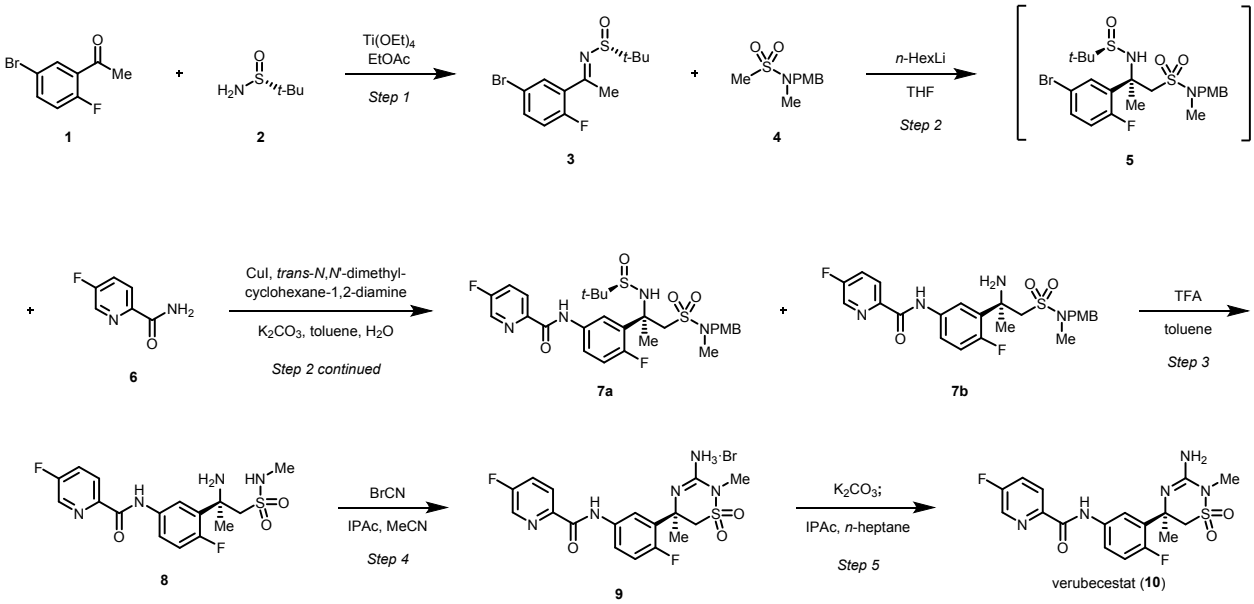
Keywords: Verubecestat; Compact mass spectrometer; Impurity identification; Process chemistry development

Process chemists' efforts at designing and executing efficient and robust synthetic routes to produce multi kilogram batches of active pharmaceutical ingredients (APIs) rely on accurate, precise, and reliable analytical techniques.[1,2] Process related impurities are tracked throughout synthetic protocols and their fate is determined to avoid compromising drug safety and efficacy. As the production of high-quality pharmaceuticals requires continued optimizations of reaction conditions geared towards developing efficient synthetic protocols, these efforts must be coupled with continuous generation of actionable analytical data to guide the optimization processes. While traditional analytical support is focused around developing chromatographic methodologies that identify products from starting material and intermediates, the characterization and identification of side products and impurities via mass spectrometry (MS) is becoming more prevalent. As process chemists face the difficult challenge of developing processes for the commercial manufacturing of an API within a short period of time, rapid understanding of the nature of undesired side products and low-level impurities during synthetic protocols is critical towards understanding molecular interactions that guide the development of the commercial synthetic route.

Mass Spectrometry, therefore, is becoming a much more common instrument in the pharmaceutical industry, playing a critical role in the structural elucidation of small and large molecules.[3] While its potential in assisting analytical, process, and medicinal chemists is undeniable, it remains less abundant as a laboratory instrument than high-performance liquid chromatography (HPLC) and gas chromatography (GC) due mainly to cost and space constraints. In that regard, the availability of an affordable compact mass spectrometer (less than $50,000)[4] as a chromatographic detector to analyze and track small quantity side products while also analyzing the yield and purity of crude reaction mixtures would be appealing to the synthetic chemist. The recently introduced Waters ACQUITY QDa mass spectrometer, a quadrupole-based instrument, can reach up to 1250 ± 0.1 m/z in both negative and positive modes, and can operate at flow rates of up to of 1 mL/min, with full scan and SIM (single ion monitoring) modes at a scan rate of 10,000 m/z units*sec-1. Although the mass range and resolution (0.7 Da) are somewhat reduced relative to conventional MS instruments, the compact mass spectrometer is sufficient for most of the early stage analyses to support synthetic chemistry, where the sample is generally abundant and unit mass resolution is often acceptable.

Herein, we demonstrate the use of QDa compact mass spectrometer in the identification and tracking of impurities during early stage process optimizations of the synthesis of a beta secretase inhibitor, verubecestat (MK-8931). Three case studies were selected to illustrate how rapid data generation assisted in the immediate redesign and optimization of reaction protocols.

The presence of a quaternary chiral center in **10** makes synthesis of structurally distinct novel thiadiazine core in verubecestat challenging.[5] Depicted in Scheme 1, a five-step protocol was developed by the process chemistry team which involves installation of a chiral auxiliary on 5-bromo-2-fluoroacetophenone **1** to generate intermediate **3**. Early stage synthesis of ketimine **3** indicated that **1** is not inert to basic conditions and could generate polymeric impurities.[6] Synthesis of *para*-methoxybenzyl protected *N*-methylmethanesulfonamide **4** is performed via reductive amination of 4-methoxybenzaldehyde followed by sulfonylation of the resulting secondary amine with methanesulfonyl chloride. Lithiation of **4** followed by nucleophilic addition to **3** resulted in the formation of **5** (not isolated), which was subsequently subjected to a copper catalyzed C–N coupling with 5-fluoropicolinamide **6** generating key intermediates **7a** and **7b** (scheme 1).



Scheme 1: Early stage five step synthetic protocol developed to prepare verubecestat (**10**)

Case study 1: Copper catalyzed coupling of **5** and **6** was investigated using various ligands including dimethylethane-1,2-diamine (**L1**) and cyclohexane-1,2-dimethylamine (**L2**). During process development, we were puzzled by the observation of a 10 to 15% drop in the yield when using **L1** compared to **L2**, because UPLC-UV analysis showed a similar profile for crude samples

from both ligands with no new impurities observed. Subsequently, UPLC with compact MS detector was utilized to investigate the challenging issue. Further examination of the MS data revealed that there was a suspicious MS peak co-eluting with the toluene solvent peak (Figure 1) with molecular mass corresponding to a derivative of the new ligand, compound **26**. The sample was then subjected to overnight freeze drying to remove the solvent and re-injected to confirm the generation of up to ~3% of ligand coupled impurity **26** compared to ≤ 0.02% of **27** (impurity generated from coupling of **L2** to **7a**). This resulted in quick selection of **L2** as the best option. Use of compact MS detector to discover the new impurity co-eluting with the toluene solvent peak was the key to understanding the yield issue and the subsequent identification and determination of the impurity generation pathway. Identification of **26** provided an insight into reactivity of the ligand towards the fluoropyridyl side chain and assisted in the final decision to further develop the process with the less reactive and optimal ligand, **L2**.[7]

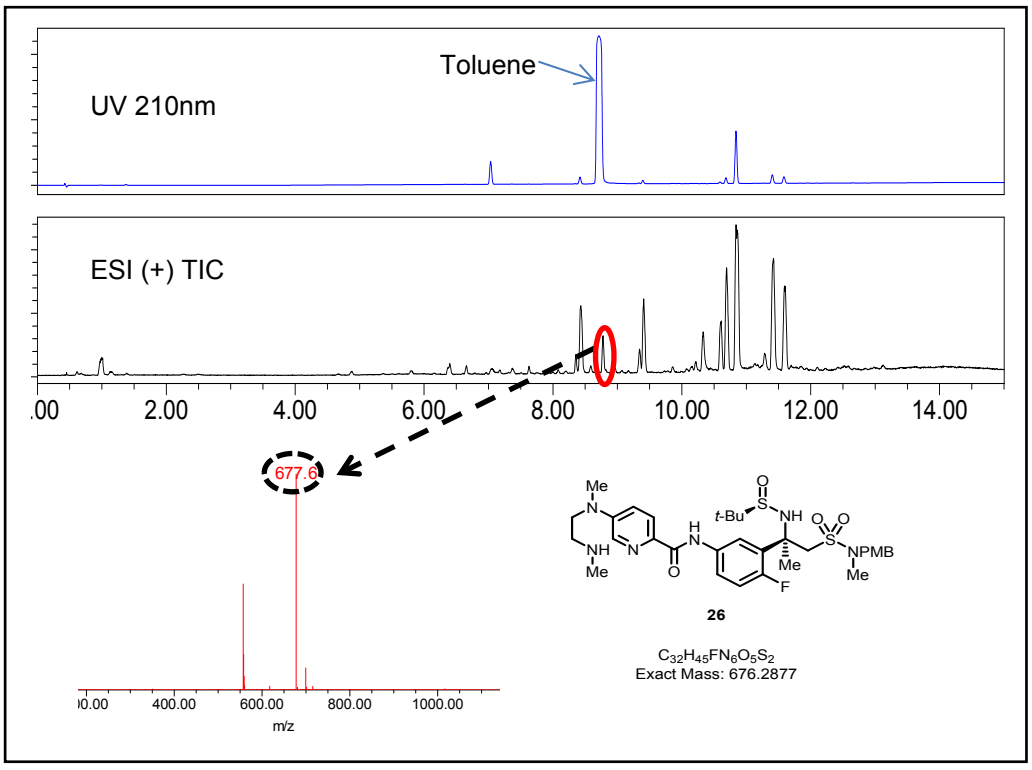
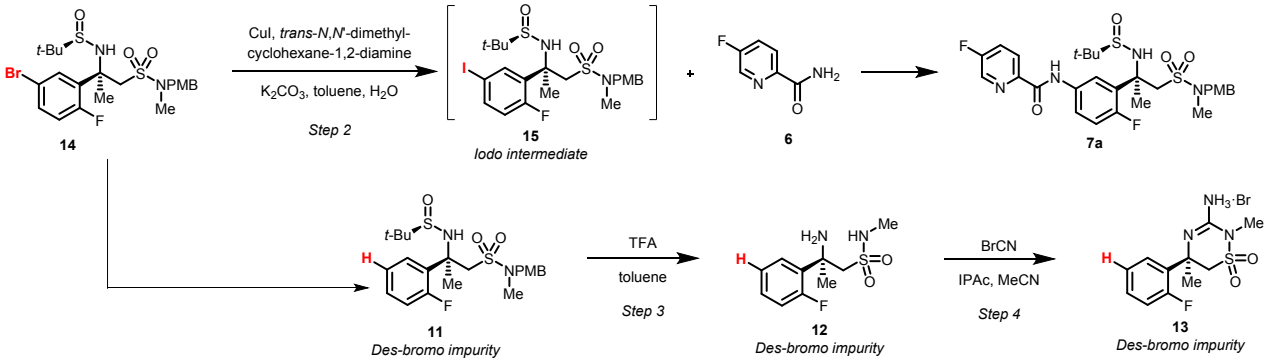


Figure 1: Crude reaction mixture injected on UPLC/QDa (method A) to evaluate the unintended side reaction generating **26**.

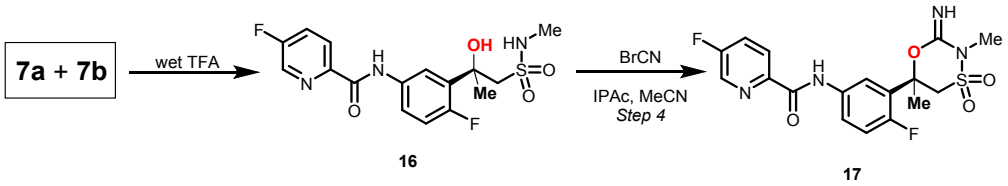
The coupling reaction with optimized conditions was efficient, affording intermediate **7a** in 85% yield. While the process resulted in approximately 12% of the partially de-protected impurity **7b**, once identified via MS analysis the partially de-protected impurity was considered as part of the product, as de-protection will eventually remove the sulfinamide chiral auxiliary exposing the free amine (Scheme 1). The rapid identification of this impurity enabled the process chemists to redirect their optimization efforts.

The use of copper (I) iodide in the process (coupling of **5** and **6**) had resulted in an iodo derivative **15** (due to incomplete conversion of a presumed reactive intermediate to coupling products **7a**) and des-bromo impurity **11**. However, the use of catalytic amounts of copper (I) iodide eliminated the iodo derivative and minimized the des-bromo impurity. Subjecting **11** to subsequent de-protection and cyanation resulted in the ring closed product **13**. Identification of the ring closed impurity (Scheme 2) was very useful for impurity tracking purposes. Other side products **28** and **29** (Scheme 5) resulting from coupling of **1** with **6** and nucleophilic addition of **4** to **1** were also detected and tracked in early optimizations.



Scheme 2: De-halogenated/halogen exchanged impurities detected in early process development

Case study 2: Dual de-protection of sulfinamide chiral auxiliary and *para*-methoxybenzyl groups in compounds **7a** and **7b** was done using trifluoroacetic acid (TFA). While the de-protection step is very efficient, giving a 96% isolated yield of de-protected **8** (Scheme 1), the presence of small amounts of water (0.5 wt.%) in TFA resulted in generation of up to 1.12% of hydrolyzed product **16** (Figure 2) which eventually converts to the API analog **17** (Scheme 3). Based on these observations, a specification was set to control the water in TFA to <0.1 wt%.



Scheme 3: Tracking fate of hydroxylated impurity **16**.

Despite these corrective measures, a more effective de-protection reagent, methanesulfonic acid (MSA), was later selected to remove the protecting groups and gain better control of the impurity. De-protection reaction using MSA in acetic acid reduced the hydroxy impurity **16** to ND levels (<0.03%). The valuable information obtained from UPLC/compact MS detector analysis was useful in helping to optimize and implement a synthetic step thus avoiding formation of **16** as well as its derivative **17**.

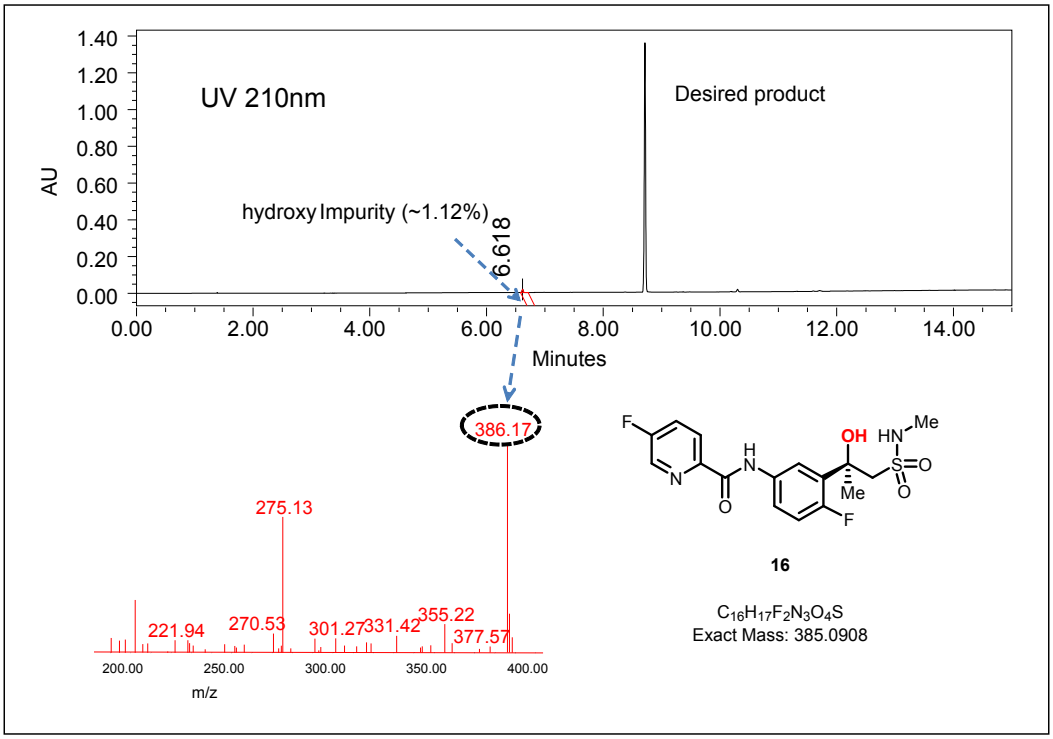


Figure 2: Crude reaction mixture injected on UPLC/QDa (method A) to track hydrolysis impurity **16**.

Case study #3: In the late stage cyclization of the penultimate intermediate **8** with cyanogen bromide (CNBr), a new impurity **19**, with (MW = M-14) was observed (Figure 3). Subsequent

pseudo-MS/MS experiments (in-source ion fragmentation) confirmed that an *N*-demethylation had occurred. As *N*-demethylation commonly occurs under oxidative conditions, this impurity was initially considered unlikely (no oxidation conditions involved in the protocol). However, the experimental data was further supported by available literature which indicated the possibility of *N*-demethylation (Von Braun reaction) in the presence of cyanogen bromide. As excess cyanogen bromide (1.2 eq) was found to be the culprit the use of 0.98 to 1.0 equivalents of CNBr did eventually minimize/eliminate the generation of the Von Braun product **19**.

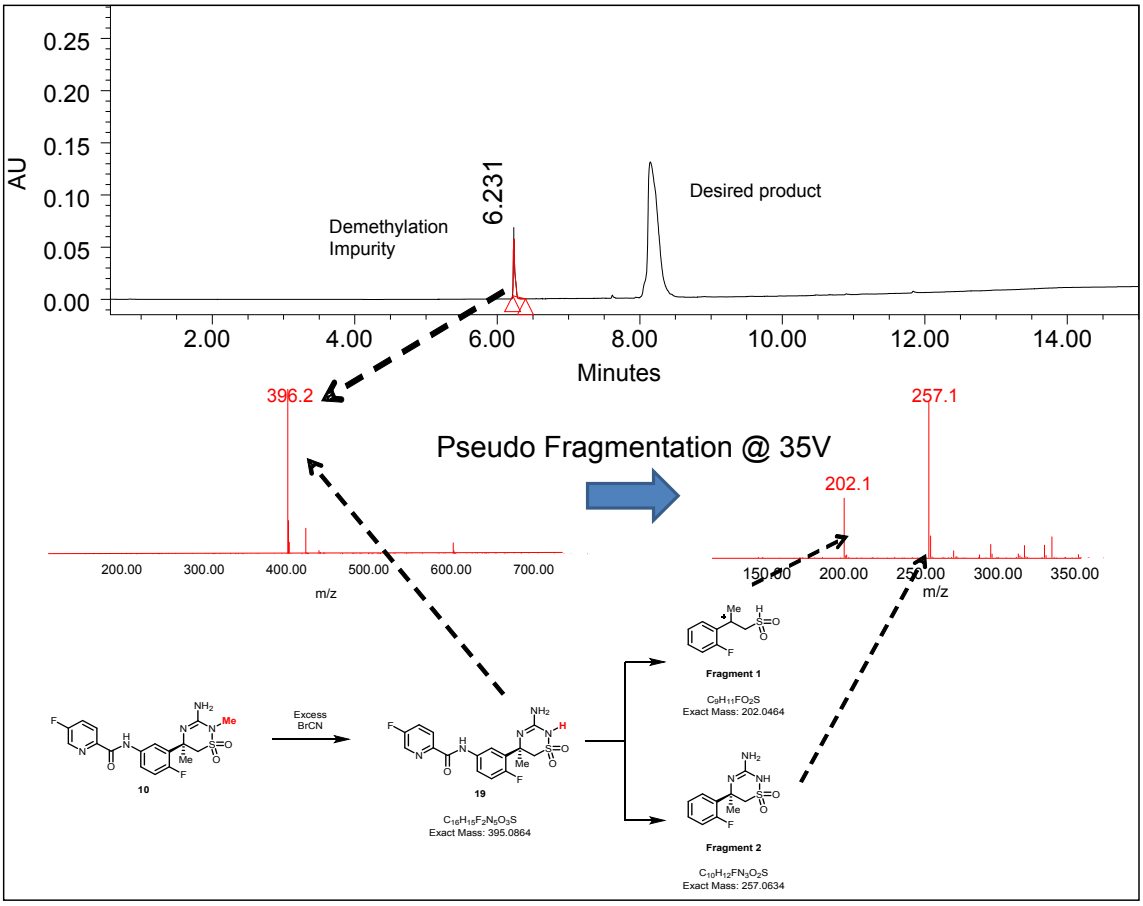
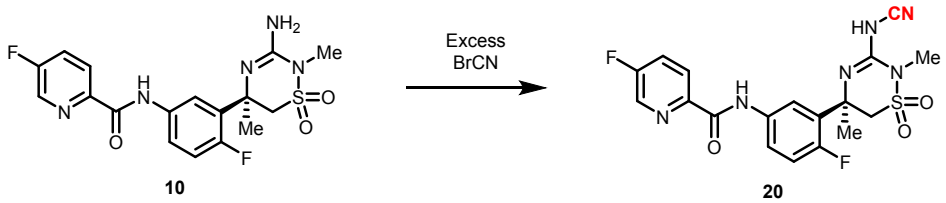


Figure 3: Crude reaction mixture with excess cyanogen bromide used for identification of demethylation impurity **19** via UPLC/QDa (method B). Pseudo MS/MS experiments at 35V show further fragmentation of demethylated ring.

Besides resulting in unintended demethylation, the use of excess CNBr also generated the over cyanation product **20** (Figure 4a). While the addition of a nitrile functional group on one of the active nitrogens was determined using UPLC/compact MS analysis data (pseudo MS/MS

experiments), an LR-HSQMBC NMR experiment involving long range C-H coupling was required to determine the exact position of cyanation.[8] Early LC/MS experiments have also identified over-cyanation on the pyridyl side chain **18**.[9]



Scheme 4: Excess cyanogen bromide intentionally charged to assess the effect of excess reagent on ring closed product

In addition to side products that may be expected during chemical reactions, the possibility of generating impurities via simple contamination of reaction vessels was examined. Indeed, contamination of the reaction vessel with MeOH was found to be responsible for generating a methoxy derivative (Figure 5B) via an S_NAr reaction. Rapid identification of the methoxy derivative **30** was critical in addressing and avoiding contamination issues, prompting elimination of methanol as cleaning solvent for reaction vessels.[10]

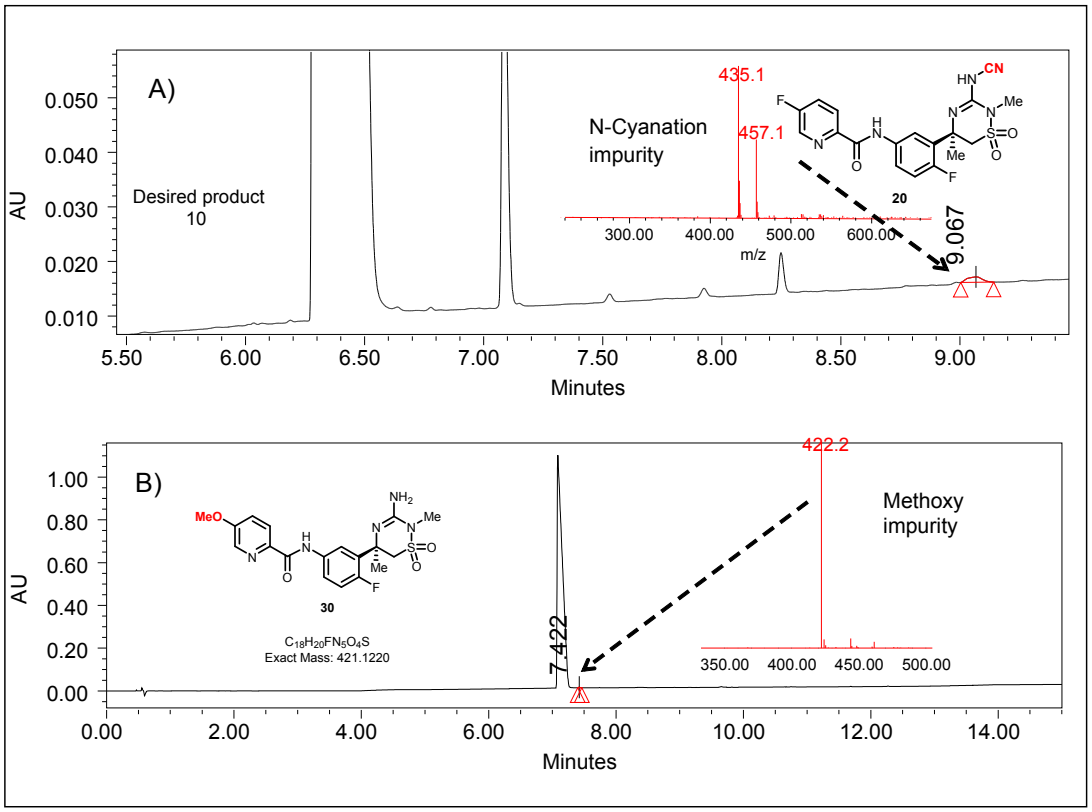
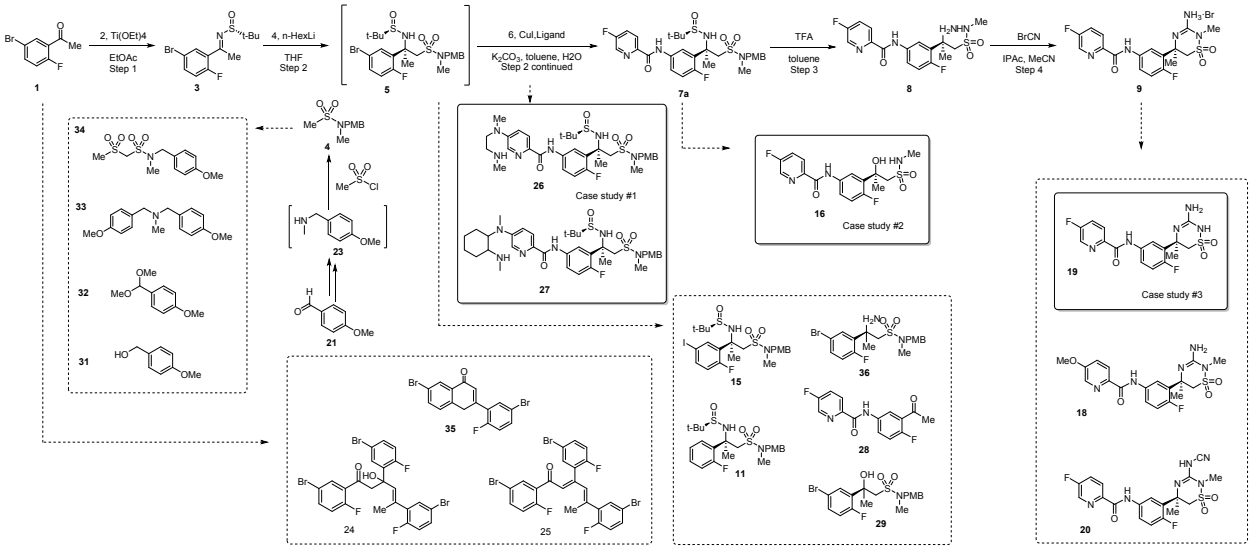


Figure 4: Crude reaction mixture injected on UPLC/QDa (method A) to identify/track (a) overcyanation side product **20** (b) Impurity **30** resulting from contamination with MeOH via SNAr reaction.



Scheme 5: Impurity map for a five-step total synthesis of verubecestat. All impurities were identified and tracked using UPLC/QDa.[11]

In summary, this paper highlights impurity tracking case studies used in early process optimizations of verubecestat. The study demonstrates the value of a compact mass spectrometer to generate useful critical analytical data with significant contributions towards developing robust synthetic protocols to prepare pharmaceuticals. Diligent impurity tracking and mapping (Scheme 5) during reaction optimizations was a crucial part of successful process optimizations affording the API with improved overall yield (~50%) and high purity "≥ 99.8%". The efficiency and robustness of a compact mass spectrometer lend itself to the analysis and rapid generation of valuable information from crude reaction mixtures. Knowledge of where impurities are generated and determination of the fate of side products saves time and minimizes the risk of compromising the quality of the final API due to small amount of impurities that may not be identified via fit-for-purpose HPLC methodologies.

These studies have illustrated the benefit of a readily available LC-MS on every bench providing researchers in the chemical sciences with powerful, personalized analytical support tools. The revolution in low cost, miniaturized LC-MS promises to move beyond the traditional process research laboratory into new domains, while improving and accelerating the design and optimization of commercial routes for drug substances.

EXPERIMENTAL SECTION:

Chemicals and Reagents: All chemicals including ammonium formate (99%) and acetontrile were purchased from Sigma Aldrich. All other samples are from Merck & Co., Inc., Kenilworth, NJ, USA.

Preparation of samples and Buffers: All samples were dissolved in acetonitrile. A stock solution of ammonium formate (200 mM) was prepared by dissolving 12.61g of ammonium formate in water in 1L volumetric flask. Stock solution (10 mL) is diluted to 1L to prepare 2 mM ammonium formate solution. Required buffer pH conditions were attained by adjusting pH using (ammonium hydroxide, pH ~8.6) and (formic acid) for pH ~3.5.

Instrument: Waters ACQUITY QDa mass spectrometer is a compact quadrupole-based instrument. It can reach up to 1250 ± 0.1 m/z in both negative and positive modes and can operate at flow rates up to 1 mL/min, with full scan and SIM modes at a scan rate of 10,000 m/z units*sec^{-1}.

The instrument features include automated calibration, which virtually eliminates the need for the sample-specific or user based tuning typical of traditional mass spectrometers. and disposable sample cones and probes. The module is connected to standard Waters ACQUITY H-class UPLC chromatography system and is controlled via Empower software. The version of the system used in the studies also includes a floor pump with lower vacuum for improved sensitivity.

UPLC/MS method: Water acquity UPLC BEH C18 1.7 um, 100 x 2.1mm column, Temp = 40 ^{o}C, flow rate = 0.6 mL/min, UV = 210 nm, Time = 15 min, post time = 3 min. Mobile Phase: A = 2 mM Ammonium Formate (pH ~8.6), Mobile Phase B= ACN. **Method A:** Gradient Time = 0 min, %B = 5, Time = 3 min, %B = 5, Time = 13 min, %B = 95, Time = 15 min, %B = 95, Time = 15.1 min, %B = 5. Sample concentration = 0.5 mg/mL, injection volume = 2µL. **Method B:** Gradient Time = 0 min, %B = 3, Time = 3 min, %B = 3, Time = 13 min, %B = 95, Time = 15 min, %B = 95, Time = 15.1 min, %B = 5. Sample concentration = 0.5 mg/mL, injection volume = 2µL.

Acknowledgements

The authors acknowledge Dr. Steve Miller, Dr. Carmela Molinaro, Dr. Azzeddine Lekhal, Mrs. Sarah Pulicare and Mr. Ashish Shah for helpful discussions, Dr. Huaming Sheng for HRMS support and Mr. Ryan Cohen for NMR support.

References

[1] Thaisrivongs, D. A.; Miller, S. P.; Molinaro, C.; Chen, Q.; Song, Z. J.; Tan, L.; Chen, L.;Chen, W.; Lekhal, A.; Pilicare, S. K.; Xu, Y. *Org. Lett*., **2016**, *18*, 5780-5783.

[2] (a) Isolation and characterization of low-level impurities is a time-consuming process requiring resources from several parts of the organization. As a result, obtaining useful analytical data on crude reaction mixtures would be very useful in allowing process chemists to make rapid decisions towards optimization of reaction protocols. (b) Bari, S. B.; Kadam, B. R.; Jaiswal, Y. S.; Shirkhedkar, A. A. *Eurasian J. Anal.l Chem.*, **2007**, *2*, 32-53. (C) Grodowska, K.; Parczewski, K. *Acta Poloniae Pharmaceutica-Drug Research*. **2010**, *67*, 3-12.

[3] (a) Chen, G.; Paramanik, B, N.; Liu, Y-H.; Mirza, U. A. *Journal of Mass Spectrometry*, **2007**, *42*, 279-287 (b) Hamilton, S. E.; Mattrey, F.; Bu, X.; Murray, D.; McCullough, B.; Welch, C. *J. Org. Process. Res. Dev*, **2014**, *18*, 103-108.

[4] (a) Bu, X.; Regalado, E. L.; Hamilton, S. E.; Welch, C.J. The emergence of low-cost compact mass spectrometry detectors for chromatographic analysis. *Trends in Anal Chem*. **2016**, *82*, 22-34. (b) Snyder, D. T.; Pulliam, C. J.; Ouyang, Z.; Cooks, R. G. Miniature and Fieldable Mass Spectrometers: *Recent Advances. Anal Chem*. **2016**, *88*, 2 – 29.

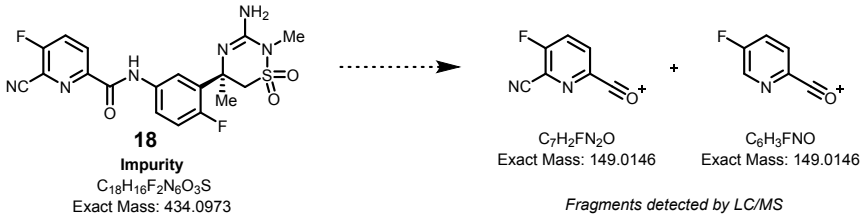
[5] Christoffers, J.; Baro, A. Stereoselective Construction of Quaternary Stereocenters. *Adv.Synth. Catal.* **2005**, *347*, 1473-1482.

[6] Minor polymerization of bromo fluoroacetophenone **1** (Scheme 1) was observed during early chiral auxiliary installation process development generating proposed impurity structures **24** and **25** (Scheme 5).

[7] Phillips, E. M.; Reibarkh, M.; Limanto, J.; Kieu, M.; Lekhal, A.; Zewge, D. Improved Process for a Copper-Catalyzed C−N Coupling in the Synthesis of Verubecestat. *J. Org. Process. Res. Dev*, **2019**, *23*, 1674 -1678.

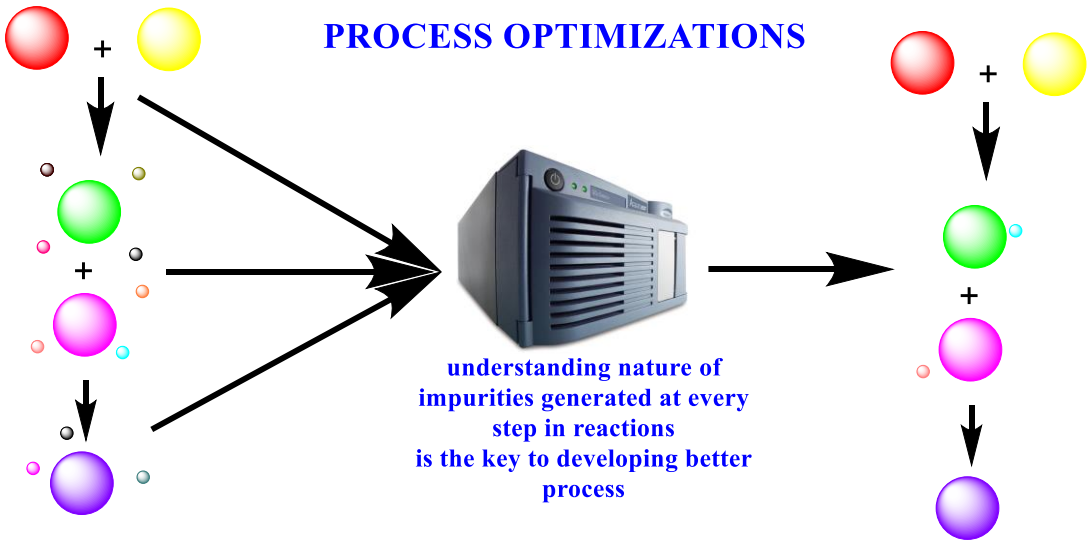
[8] Williamson, R. T.; Buevich, A. V.; Martin, G. E.; Parella, T. *J. Org. Chem*, **2014**, *79*, 3887–3894.

[9] Overcyanation on fluoropyridyl side chain in **10** was explained using pseudo MS/MS data using UPLC/QDa.



[10] Contamination of API with methanol in reaction vessels and generation of impurity **30** was avoided by replacing methanol with acetone.

[11] Additional impurities were identified using UPLC/QDa and will be discussed in future communications.


PROCESS OPTIMIZATIONS
understanding nature of
impurities generated at every
step in reactions
is the key to developing better
process