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MARTIN, Scott, LENZ, Eva M, SMITH, Robin, TEMESI, David G, ORTON, Alexandra L and CLENCH, Malcolm <http://orcid.org/0000-0002-0798-831X>

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Methanol adducts leading to the identification of a reactive aldehyde metabolite of AZX in human liver microsomes

Scott Martin[1†], Eva M. Lenz[1], Robin Smith[2], David G. Temesi, Alexandra Orton[1], Malcolm R.Clench[3]

[1†] Oncology iMED, Hodgkin building, Chesterford Science Park, AstraZeneca UK Ltd., Saffron Walden, Essex, CB10 1XL United Kingdom.

[2] Oncology iMED, Alderley Park, AstraZeneca UK Ltd., Macclesfield, Cheshire SK10 4TG, United Kingdom.

[3] Biomedical Research Centre, Sheffield Hallam University, Howard Street, Sheffield S1 1WB, United Kingdom.

[†]**Corresponding Author**

Scott Martin

Oncology iMED,

AstraZeneca UK Ltd.,

B900

Chesterford Science Park,

Saffron Walden,

Essex CB10 1XL

United Kingdom

Tel. +447780493703

e-mail: Scott.Martin2@astrazeneca.com

Abbreviations (non standard)

AZX (1-[(2R)-2-[[4-[3-chloro-4-(2-pyridyloxy)anilino]quinazolin-5-yl]oxymethyl]-1-piperidyl]-2-hydroxy)

HLM human liver microsomes

NADPH Nicotinamide adenine dinucleotide phosphate

GSH glutathione

nm nanometer

HCD higher energy collisional dissociation

CID collision induced dissociation

UHPLC ultra high performance liquid chromatography

Abstract

The incubation of compound AZX (1-[(2R)-2-[[4-[3-chloro-4-(2-pyridyloxy)anilino] quinazolin-5-yl]oxymethyl]-1-piperi-dyl]-2-hydroxy) with human liver microsomes generated several metabolites that highlighted the hydroxyacetamide side chain was a major site of metabolism for the molecule. The metabolites were derived predominantly from oxidative biotransformations, however two unexpected products were detected by LC-UV-MS and identified as methanol adducts. It became apparent that a metabolite formed in the microsomal incubation reacted with methanol in the mobile phase when no methanol adducts were detected in the analysis where acetonitrile was used. This observation prompted further investigations into the metabolic modification of the parent. Although this reactive metabolite could not be isolated or structurally characterised by LC-MS, several metabolic indications enabled the proposal of a reactive aldehyde. Experiments using methoxyamine post-incubation showed the disappearance of the two methanol adducts and appearance of a methoxyamine adduct, confirming the presence of an aldehyde group. The proposed structure of the reactive aldehyde derived from oxidation of the terminal hydroxyl group on the hydroxyacetamide side chain, leading to the formation of the diastereoisomeric methanol adducts detectable by LC-UV-MS.

Keywords: 6 keywords

Reactive aldehyde, methanol adducts, Mass spectrometry

Introduction

Metabolite identification studies within drug discovery are generally used to identify metabolically labile sites on chemical structures, human metabolites which may not be represented in the toxicological species, active metabolites or potential reactive/toxic metabolites, which are of concern for the pharmaceutical industry [1 2 3]. These discovery studies are conducted *in vitro* and generally involve incubation of a test compound in hepatocytes or microsomes, which mimic the most prevalent metabolic processes occurring in the liver. Typically, samples are taken from the test compound incubates, then added to acetonitrile to quench the reaction at t=0 min (control) and at a terminal time point (usually 30–60 min). Subsequently, samples are analysed on high resolution accurate mass UHPLC-MS/MS systems and comparison of the t=0 min *vs* the t=60 min samples enables easier identification of metabolites from endogenous components. The analysis can be both challenging and time consuming even when identifying only a small number of metabolites. Often the data has to be generated and reported quickly to impact the chemistry design and unusual unexpected metabolites/products are not fully investigated due to time constraints. Such products can occasionally be formed through reactive or toxic metabolites.

Here we present an unusual finding from an *in vitro* metabolite identification study of compound AZX, a representative compound from a structurally related chemical series, in human liver microsomes (HLM). AZX (Figure 1) generated several metabolites in HLM that were predominantly oxidative biotransformation products, which highlighted the hydroxyacetamide side chain as a major site of metabolism for the molecule. Additionally, two unexpected methanol adduct products were detected with different retention times yet identical mass. Methanol adducts are fairly common in mass spectrometry and are normally identified as analytical artefacts of the parent drug, generated during the positive ion electrospray ionisation process. Pozo et al. (2007)[4] reported the analysis of steroids using

electrospray generated adducts such as [M + MeOH] or [M+ Na +MeOH]. However, in this case AZX reacted with methanol in the mobile phase prior to the mass spectrometry ionisation process. The methanol adducts not only eluted at a different retention time to the parent drug, but two chromatographically separate methanol adduct peaks were observed, indicating the presence of isomers. These adducts were not detected in either the t=0 control samples or when acquired with acetonitrile as the mobile phase. This inferred a metabolic transformation to a reactive metabolite formed in the t=60 sample, which subsequently generated the methanol adducts on injection onto the UHPLC column. The formation of these methanol adducts is subject to further investigation in this paper.

Materials and Methods

Chemicals

AZX 1-[(2R)-2-[[4-[3-chloro-4-(2-pyridyloxy)anilino]quinazolin-5-yl]oxymethyl]-1-piperidyl]-2-hydroxy was synthesized at AstraZeneca R&D, Alderley Park, Macclesfield, UK. Methanol, acetonitrile, formic acid were all of analytical grade and supplied by Fisher Scientific (Loughborough, UK). Methoxyamine hydrochloride was sourced from Sigma-Aldrich (Poole, UK). All other chemicals or solvents were purchased from commercial suppliers and were of analytical grade. No specific safety considerations apply to any of these agents, although the agents should be handled with care in a fumehood to avoid inhalation or ingestion.

AZX stock solution

Solid AZX was dissolved in dimethyl sulfoxide to a concentration of 10 mM

In vitro incubation spiking solution

AZX dimethyl sulfoxide stock was diluted with 0.1 M potassium phosphate buffer (pH 7.4) to a concentration of 2 mM. This solution was then spiked into the *in vitro* incubation at 1:100 (v/v) to give a final concentration in the incubation of 20 uM.

In vitro incubation experimental methods

Three separate incubations were performed with parent compound (AZX):

A) AZX was incubated at 20 μM with HLM (2 mg protein/mL, BD-Gentest 150 donor ultrapool) in 0.1 M potassium phosphate buffer at pH 7.4 and 2.5 mM Nicotinamide adenine dinucleotide phosphate (NADPH) at 37 °C. An aliquot was removed after 0 min (control) and 60 min and the reaction stopped by the addition of ice cold MeCN (1:1, v/v).

B) AZX was incubated as described in A, but with 10 mM methoxyamine trapping agent added. An aliquot was removed after 60 minutes and the reaction stopped by the addition of ice cold MeCN (1:1, v/v).

C) AZX was incubated as described in A. An aliquot was removed after 60 min and the reaction stopped by the addition of ice cold MeCN (1:1, v/v) containing 10 mM methoxyamine. The extract was then incubated for a further 10 min at 37°C.

D) AZX was incubated as described in A, but with 10 mM GSH trapping agent added. An aliquot was removed after 60 minutes and the reaction stopped by the addition of ice cold MeCN (1:1, v/v).

Sample preparation – analytical incubation

Quenched incubates were centrifuged at ca. 3000*g* for 15 min, and the supernatant retained. An aliquot of the the supernatant was transferred to a Waters HPLC 2 mL vial and diluted 1:3 (v/v) with ultra purified distilled water prior to the LC-MS analyses.

Profiling and Structural characterisation of metabolites by UPLC-LTQ-Orbitrap Mass spectrometry

Accurate mass structural characterisation work was acquired on a LTQ Orbitrap XL (Thermo Fisher Scientific, Bremen, Germany) connected to a Waters Acquity UPLCTM system. The Waters Acquity system (Waters, Milford, MA, USA) consisted of a binary UPLC Pump, column oven, a sample manager and a photodiode array detector. Separation was carried out on a Waters BEH C18 (100 x 2.1 mm, 1.7 µM) (Waters, Milford, MA, USA), preceded by a guard filter in a column oven at 50 ºC.

Two chromatographic methods were used. Method 1: The mobile phase consisted of formic acid (0.1% in water, eluent A) and methanolic formic acid (0.1%, eluent B). Method 2: The mobile phase consisted of formic acid (0.1% in water, eluent A) and acetonitrile containing formic acid (0.1%, eluent B). The elution profile for both methods was: Initial conditions 95% A, then a linear gradient to 20% A from 1.01 min to 9.00 min, isocratic hold at 2% A from 9.01 to 11.00 min and re-equilibration 95% A from 11.00 min to 14.00 min. The flow rate was 0.45 mL/min and the eluent was introduced into the mass spectrometer via the LTQ divert valve at 1 min. The injection volume was 20 µL and UV spectra were acquired over 200-350 nm. The LTQ-Orbitrap XL was equipped with an electrospray ionisation (ESI) source which was operated in positive ion mode. Positive ion source settings were: capillary temperature 300 ºC, sheath gas flow 25, auxiliary gas flow 17, sweep gas flow 5, source voltage 3.5 kV, source current 100.0 µA, capillary voltage 18 V, tube lens 75.0 V. Full scan MS data were obtained over the mass range of 100 to 1200 Da. Full scan MS data were obtained over the mass range of 100 to 1200 Da. Targeted MS/MS experiments were acquired in the Orbitrap using Higher Energy Collisional Dissociation (HCD) fragmentation, isolation width 3 Da, normalised collision energy 60 eV, and activation time 30 ms. All Ion trap MSn experiments were acquired using collision induced dissociation (CID), isolation width 3 Da, normalised collision energy 35 eV and activation time 30 ms. All ions acquired in the Orbitrap were monitored at 7500 resolution FWHM (full width at half maximum). LTQ and Orbitrap mass detectors were calibrated within one day of commencing the work using Proteomass LTQ/FT-Hybrid ESI positive mode calibration mix (Supelco, Bellefonte, USA).

Data Analysis

Mass spectrometric data were collected using Xcalibur version 2.1 (Thermo Fisher Scientific, Bremen, Germany). Components were identified as being derived from AZX by common fragments, isotopic pattern (Chlorine), UV absorbance and accurate mass. Comparisons with T=0 incubations were conducted to minimise the potential for false positives from system impurities and endogenous components.

Results

The initial full characterization of test compound with accurate mass MS/MS fragmentation often allows structural motifs and characteristic fragment ions to be identified, which assist the elucidation of the metabolite structures. MS/MS fragmentation experiments were undertaken in positive ion for the structural characterisation of metabolites or chemical addition products. All accurate mass measurements including the MS/MS fragmentation were within ±3ppm of the theoretical accurate mass.

Structural characterization of AZX by LC-MS/MS

In positive ion mode AZX yielded a protonated molecular ion at $[M+H]^+$=520.1749 Da (+0.56 ppm mass error) and showed a characteristic chlorine pattern. The proposed dissociation pattern and LTQ Orbitrap HCD MS/MS spectrum (Figure 1) revealed 3 key product ions at m/z 365.0797 (corresponding to loss of the methylpiperidylethanone), m/z 156.1018 (methylpiperidylethanone) and m/z 98.0964 (methylpiperazine).

Structural characterization of the acid metabolite (+O -H2) by LC-MS/MS

In positive ion mode the acid metabolite yielded a protonated molecular ion displaying the characteristic chlorine pattern with an accurate mass of $[M+H]^+$ =534.1539Da (0.0ppm mass error), which is consistent with addition of one oxygen and loss of two hydrogen atoms. The proposed fragmentation pattern and LTQ Orbitrap HCD MS/MS spectrum (Figure 2) revealed diagnostic product ions; m/z 365.0789, 170.0808, 126.0911 and 98.0963. Product ion m/z 170.0808 corresponded to the addition of 14 Da to m/z 156.1018 observed in AZX. By accurate mass it was confirmed this addition was one oxygen atom and loss of two hydrogens to the methylpiperidylethanone group. Detection of the methylpiperazine fragment (m/z 98.0963, also observed in AZX) confirmed the piperazine ring was unchanged and the

biotransformation had occurred on the hydroxyacetamide side chain. The product ion m/z 126.0911 represents loss of CO_2 from the hydroxyacetyl side chain, which is consistent with an acid on the terminal side chain carbon.

Structural characterization of the hydrate product (+O) by LC-MS/MS

The hydrate metabolite was only detected at trace levels when methanol was used as a mobile phase eluent, but it was the predominant species when acetonitrile.was used Therefore the MS/MS spectra shown in Figure 3 for the hydrate are from the LC-MS analysis where acetonitrile mobile phase eluent was employed. In positive ion mode the hydrate product yielded a protonated molecular ion displaying a characteristic chlorine pattern with an accurate mass of $[M+H]^+$ =536.1691 Da, (+0.79ppm mass error), which is consistent with addition of one oxygen atom. The proposed dissociation pattern and LTQ Orbitrap HCD product ion MS/MS spectrum (Figure 3) revealed diagnostic product ions; m/z 172.0964, 126.0911 and 98.0963. The m/z 172.0964 product ion corresponded to the addition of 16 Da to the m/z 156.1018 product ion observed in AZX and the accurate mass confirmed addition of one oxygen atom to the methylpiperidylethanone group. Detection of the methylpiperazine product ion (m/z 98.0963, also observed in AZX) confirmed the piperazine ring was unchanged and the oxygen atom addition had occurred on the hydroxyacetamide side chain. The m/z 126.0911 product ion represents loss of CH_2O_2 from this side chain, which is consistent for a hydrate on the terminal carbon.

Structural characterization of the methanol addition product (a hemiacetal) by LC-MS/MS

Two distinct LC-UV/MS peaks were detected in the chromatogram for this product. In positive ion mode the methanol addition products yielded protonated molecular ions

displaying characteristic chlorine patterns with an accurate mass of $[M+H]^+$ =550.1854 Da (+0.41ppm mass error) and $[M+H]^+$ =550.1852 Da (+0.12ppm), which is consistent with addition of one carbon, two hydrogens and an oxygen atom. The proposed dissociation pattern and LTQ Orbitrap HCD product ion MS/MS spectrum for both adducts (Figure 4) revealed diagnostic product ions m/z 186.1121, m/z 126.0911 and m/z 98.0963. The product ion m/z 186.1121 corresponded to the addition of 30 Da to m/z 156.1018 observed in the MS/MS spectrum of AZX, and accurate mass confirmed addition of one carbon, two hydrogens and an oxygen to the methylpiperidylethanone group. Detection of the methylpiperazine product ion (m/z 98.0963, also observed in AZX) confirmed the piperazine ring was unchanged and addition of CH_2O occurred on the hydroxyacetamide side chain. The m/z 126.0911 product ion represents loss of $C_2H_4O_2$ from this side chain, confirming it as the site of methanol addition.

The observation of two distinct LC-UV-MS peaks both with accurate masses within 2ppm of $[M+H]^+$= 550.1854 (theroetical mass of AZX+CH_2O) and identical MS/MS fragmentation (Figure 4), suggested formation of isomers. The addition of methanol to the terminal carbon on the hydroxyacetamide side chain creates a second chiral centre and therefore diastereoisomers, which could be separated chromatographically (Figure 5a).

These data (the observation of the hydrate and the hemi-acetal) suggested that the potential reactive intermediate had undergone structural changes on the hydroxyacetyl side chain, producing a reactive aldehyde, whilst the formation of the acid indicated further oxidation of this moiety.

Glutathione and methoxyamine trapping experiments

To confirm the reactive intermediate, the proposed reactive aldehyde, two trapping experiments in HLM were undertaken. The first involved using glutathione (GSH), the routine industry standard for trapping electrophilic reactive metabolites and methoxyamine which forms adducts with reactive aldehydes [5 6 7 8]. The incubation of AZX with GSH did not produce an adduct (data not shown), instead, only the methanol adducts were detected, confirming that the reactive intermediate was not a soft electrophilic species**.** In contrast, the incubation of AZX with methoxyamine generated the corresponding methoxyamine adduct, whilst the methanol adducts were not detected. Incubation of AZX in HLM showed almost complete turnover of the AZX at t=60 min (Figure 5a), however when co-incubating with methoxyamine the overall metabolic turnover was reduced, resulting in a significant amount of AZX-parent remaining unmetabolised (Figure 5b). Additionally hydroxyl metabolites of the methoxyamine adduct were detected at significantly greater concentration than the methoxyamine adduct itself (Figure 5b). It was therefore decided to repeat the methoxyamine trapping experiment, this time spiking the methoxyamine post-incubation into the HLM immediately after quenching (at t=60 min). This resulted in increased metabolic turnover of AZX and the formation of only one methoxyamine adduct with significantly increased yield, simplifying the data interpretation (Figure 5c).

Structural characterization of the methoxyamine adduct by LC-MS/MS

In positive ion mode the methoxyamine adduct yielded a protonated molecular ion displaying a characteristic chlorine pattern with an accurate mass of $[M+H]^+$ =547.1855 Da (+0.35ppm mass error), which is consistent with addition of one carbon, one hydrogen and one nitrogen (CHN). The addition of CHN was an expected modification upon reaction of an aldehyde with methoxyamine. The proposed dissociation pattern and the LTQ Orbitrap HCD MS/MS spectrum (Figure 6) revealed diagnostic product ions m/z 183.1124, m/z 152.0942, m/z

123.0552 and m/z 96.0808. The product ion m/z 186.1121 corresponded to the addition of 27 Da to m/z 156.1018 observed in the MS/MS spectrum of AZX and accurate mass confirmed addition of CHN to the methylpiperidylethanone group. The m/z 152.0942 ion corresponded to the loss of a methanol radical from m/z 183.1124 ion.

The adduct formation with methoxyamine provided evidence to the presence of an aldehydic functional group, and therefore confirmed the formation of a reactive aldehyde intermediate following oxidation of the terminal hydroxy.

The metabolic fate of AZX, the formation of the methanol adduct and the methoxyamine adduct are summarised in Figure 7 and TOC**.**

Discussion

This study demonstrated that compound AZX on incubation with HLM generated a reactive aldehyde metabolite that subsequently formed two methanol adduct isomers (Figure 7). Whilst the reactive aldehyde metabolite was not directly detected and therefore not structurally verified by UHPLC-MS, the methoxyamine trapping experiment proved the presence of an aldehydic functional group. MS^n experiments carried out on the methoxyamine adduct confirmed that addition occurred on the hydroxyacetamide side chain. Detection of the hydrate product, gave further support for metabolism of the hydroxyacetamide side chain to the corresponding oxoacetamide (reactive aldehyde). The identification of two chromatographically distinct methanol adduct peaks with identical accurate masses (within ± 1ppm) and dissociation patterns is indicative of the formation of diastereoisomerisms. Chemical addition of methanol to the reactive aldehyde metabolite created an additional chiral centre and resulted in the formation of diastereoisomers, which were easily separated by UHPLC (Figure 5a). Overall, the metabolic profile of AZX incubated in HLM showed that AZX was readily metabolised, with little evidence of AZX

parent in the LC-UV chromatograms (Figure 5). The aldehyde, hydrate and methanol adduct were likely to be in equilibrium. In acidified methanol/water mobile phase the methanol adducts appeared to be the predominant species, with the hydrate detected only at trace levels and the reactive aldehyde not detected. However in acidified acetonitrile/water mobile phase only a single hydrate product was detected. Chemical reactions of methanol with aldehydes forming hemiacetals have been reported previously by Bateman et al. (2008)[9], who observed methanol addition to numerous components of secondary organic aerosol when methanol was used for extraction and/or storage. They also observed an increased rate of methanol adduct formation in acidified solutions. A series of publications have reported the formation of artefacts when methanol was used as a diluent to spike test compounds into microsomal incubations. Yin et al (2001) [10] and Li et al (2006)[11]described the formation of +12 Da artefacts (having gained 1 carbon atom over incubated parent compound) from microsomal incubations, where methanol was present in the spiking diluent at 1% (*v/v*). Similarly Cunningham et al (1990)[12] reported the formation of an unusual -CH_2 bridged dimer of 2,4-diaminotoluene again through the presence of methanol in the diluent. In these examples, methanol in the spiking solution was metabolised in the microsomal incubation to formaldehyde, which subsequently reacted with the test compounds containing 1,2-diamino, 1,2 amino hydroxy or 2,4 diaminotoluene. This is in contrast to this study, where methanol had not been added to the incubation or the sample extract prior to injection onto the LC-UV-MS system, confirming the addition occurred post-injection on the LC-column, with the mobile phase the source of the methanol

The initial methoxyamine HLM incubation with AZX resulted in a significant reduction of metabolic turnover of parent compared to the control (incubation without methoxyamine, as shown in Figure 5a) and the generation of additional products due to further metabolism of the methoxyamine adduct. The reduction in metabolic turnover was almost certainly due to

enzyme inhibition by addition of methoxyamine to the incubation. Zhang et al[13] reported on the inhibitor properties of methoxyamine against P450 enzymes CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5 at concentrations up to 10mM. To improve data quality and yield of the methoxyamine adduct the experiment was repeated, adding methoxyamine 'post-quench' then incubating for a further 10 min at 37°C. The result was a much cleaner metabolic profile; with no reduction in metabolic turnover and a significant increase in yield of the methoxyamine adduct (shown in Figure 5). Although the aldehyde metabolite is reactive, it was present at a high enough concentration immediately post-incubation to produce the methoxyamine adduct. This is in agreement with the facile reaction of the reactive aldehyde and methanolic mobile phase on column forming the isomeric methanol adducts. Aldehyde metabolites are capable of reacting with macromolecules such as proteins, forming covalent adducts potentially triggering direct cell toxicity or an immune response and have been implicated in adverse drug reactions observed in the clinic. Several drugs are suspected of generating reactive aldehyde metabolites implicated in adverse drug reactions, which include Acyclovir nephrotoxicity [14], Abacavir idiosyncratic hypersensitivity with increased risk of cardiac dysfunction [15] and Felbamate, aplastic anemia and hepatotoxicity [16]. It is therefore prudent to identify compounds or structural moieties that form reactive aldehyde metabolites early in drug discovery to modify or change the chemistry and remove the liability. It is also important to note that AZX did not form an adduct in the HLM GSH trapping experiment, thus escaping detection from the early routine in-house reactive metabolite screen. Without the formation of the methanol adducts, the aldehyde may not have been identified until much later in drug discovery.

In conclusion, analytical artefacts (such as methanol adducts) are not desired and can confuse the analysis or lead to misinterpretation. However, here we presented an example where the

methanol adducts were diagnostic and pivotal in identifying a reactive aldehyde metabolite, thus highlighting the importance of fully investigating any unusual adducts observed in metabolite identification studies.

Whilst the reactive aldehyde metabolite could be proposed from the detection of methanol adducts, the hydrate metabolite is simply addition of Oxygen (+16 amu) to parent compound, which is a very common biotransformation reaction. If the initial analysis had been undertaken in acetonitrile it is likely that the hydrate would have been reported as a simple hydroxylated metabolite and the reactive aldehyde liability for the chemical series missed.

In summary, the proposed bioactivation of AZX occurred *via* the reactive aldehyde intermediate, which It is important to note that readily reacted with methanol to form a pair of isomeric hemiacetal methanol adducts. The reactive aldehyde metabolite was not detected and could not be structurally characterized directly, in acidified methanol the equilibrium favoured the methanol adduct and in acidified acetonitrile it favoured the hydrate.

The aldehyde did not form an adduct with GSH, escaping detection in our conventional *in vitro* trapping screen, however, it was trapped with methoxyamine. A significant efficiency gain (>20 fold increase in yield of adduct) was observed when methoxyamine was added immediately post incubation over the traditional method, where methoxyamine is added pre incubation.

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Figure 1. Accurate mass HCD MS/MS positive product ion mass spectrum of AZX (bottom) and proposed product ions with theoretical accurate mass and mass error in ppm (top).

Figure 2. Accurate mass HCD MS/MS positive product ion mass spectrum of the acid metabolite (bottom) and proposed product ions with theoretical accurate mass and mass error in ppm (top).

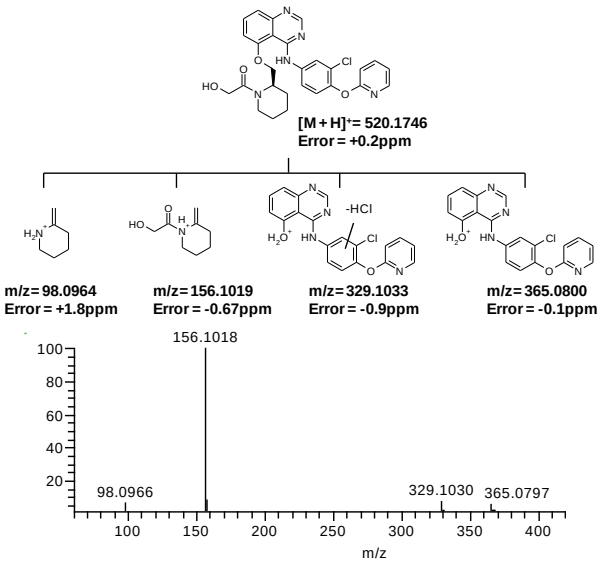
Figure 3. Accurate mass HCD MS/MS positive product ion mass spectrum of the hydrate (bottom) and proposed product ions with theoretical accurate mass and mass error in ppm (top).

Figure 4. Accurate mass HCD MS/MS positive product ion mass spectra of the methanol adducts the hemiacetal (bottom) and proposed product ions with theoretical accurate mass and mass error in ppm (top).

Figure 5. Extracted UV chromatograms (330 nm) of AZX incubated in (a) HLM (b) HLM + methoxyamine (c) HLM with addition of methoxyamine post-quench.

Figure 6. Accurate mass HCD MS/MS positive product ion mass spectrum of the methoxyamine adduct (bottom) and proposed product ions with theoretical accurate mass and mass error in ppm (top).

Figure 7. The proposed metabolic profile of AZX generated in HLM, together with the analytical artifact, the methanol adduct, as well as the methoxyamine incubation product. The proposed reactive aldehyde intermediate is shown in the centre.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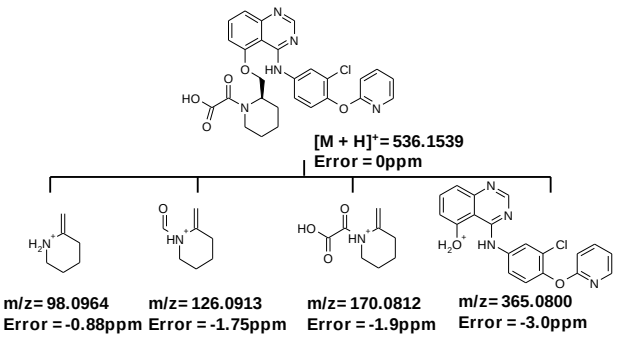
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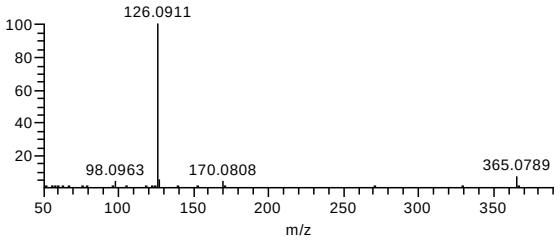
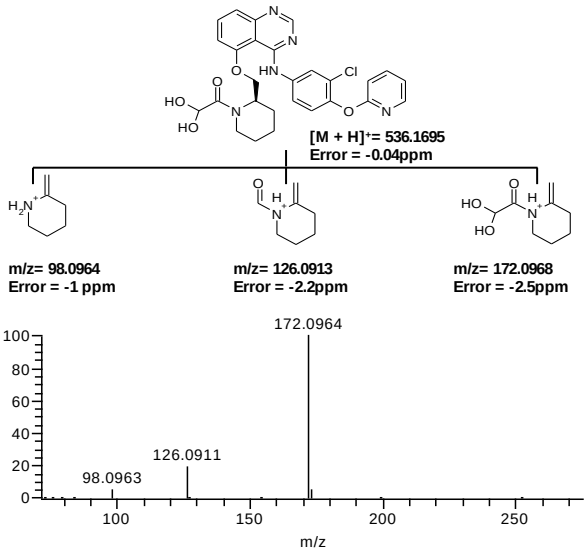
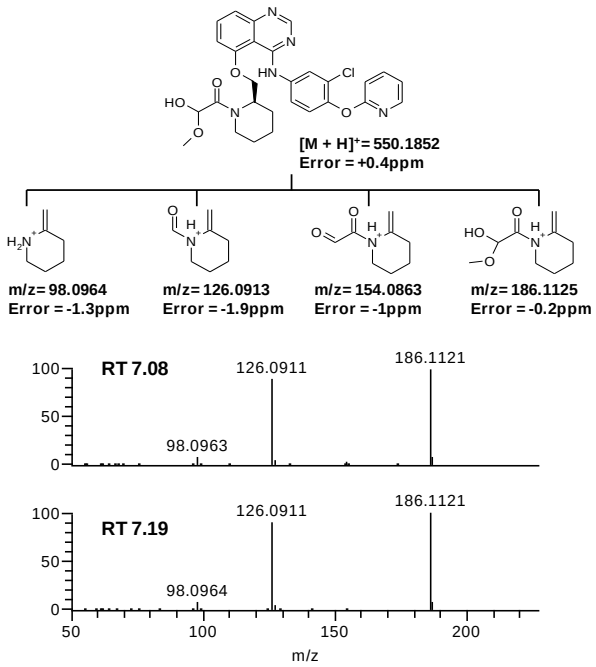
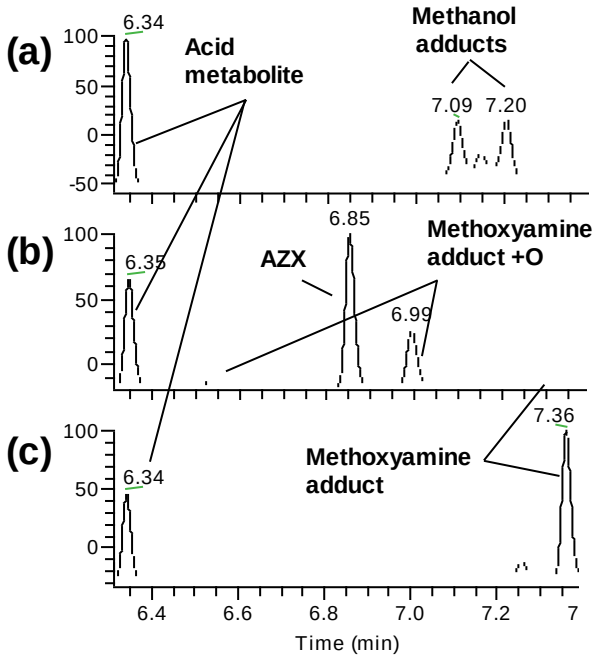
m/z = 365.0800
Error = -3.0ppm

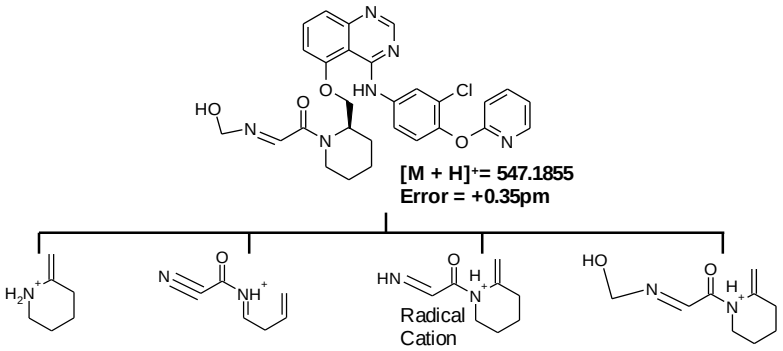

126.0911
98.0963
170.0808
365.0789
m/z

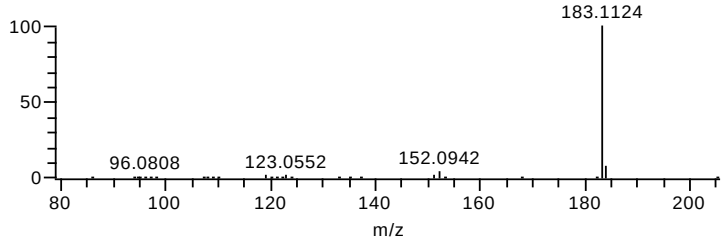

[M + H]+= 536.1695
Error = -0.04ppm
m/z= 98.0964
Error = -1 ppm
m/z= 126.0913
Error = -2.2ppm
m/z= 172.0968
Error = -2.5ppm

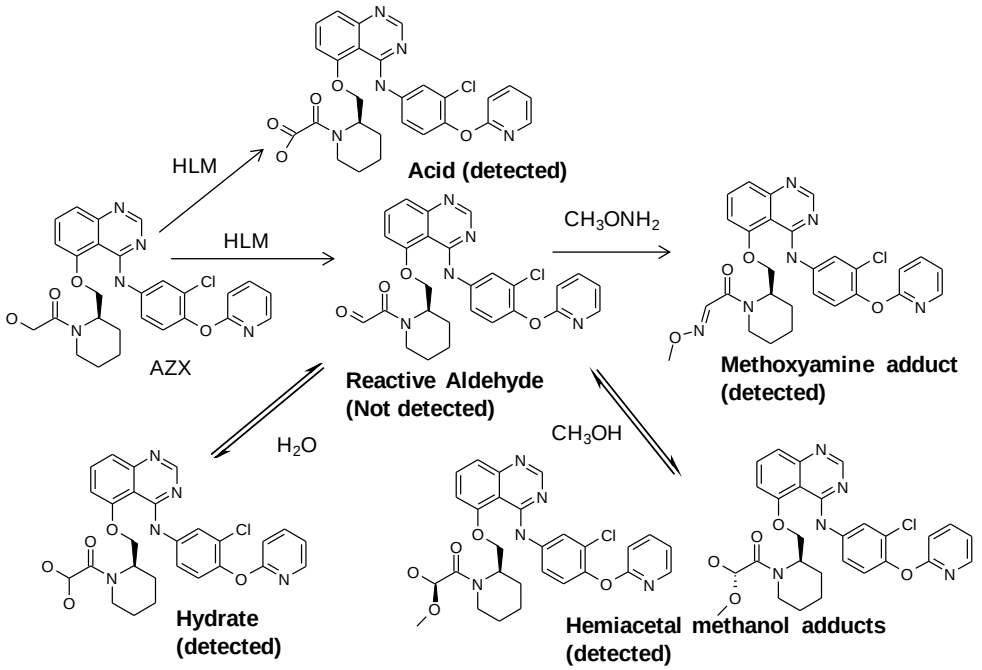
172.0964
126.0911
98.0963
100
80
60
40
20
0
100
150
200
250
m/z


[M + H]+= 550.1852
Error = +0.4ppm
m/z= 98.0964
Error = -1.3ppm
m/z= 126.0913
Error = -1.9ppm
m/z= 154.0863
Error = -1ppm
m/z= 186.1125
Error = -0.2ppm
RT 7.08
126.0911
186.1121
98.0963
RT 7.19
126.0911
186.1121
98.0964
m/z


(a)
(b)
(c)
6.34
Acid
metabolite
Methanol
adducts
7.09
7.20
100
50
0
-50
6.85
Methoxyamine
adduct +O
6.35
AZX
6.99
7.36
Methoxyamine
adduct
6.4
6.6
6.8
7.0
7.2
7
Time (min)


[M + H]+= 547.1855
Error = +0.35pm
Radical
Cation
m/z= 96.0808
Error = -0.27ppm
m/z= 123.0553
Error = -0.48ppm
m/z= 152.0944
Error = -1.3ppm
m/z= 183.1128
Error = -2.0pm


183.1124
96.0808
123.0552
152.0942
100
50
0
80
100
120
140
160
180
200
m/z


HLM
Acid (detected)
HLM
AZX
Reactive Aldehyde
(Not detected)
CH3ONH2
Methoxyamine adduct
(detected)
H2O
CH3OH
Hydrate
(detected)
Hemiacetal methanol adducts
(detected)