



Pictured above: HIV absorption

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# Introduction

- Validated LC-MS/MS method for Ibrutinib and its metabolite
- Protein precipitation with acetonitrile, using STIL internal standards
- LC-MS/MS-conditions

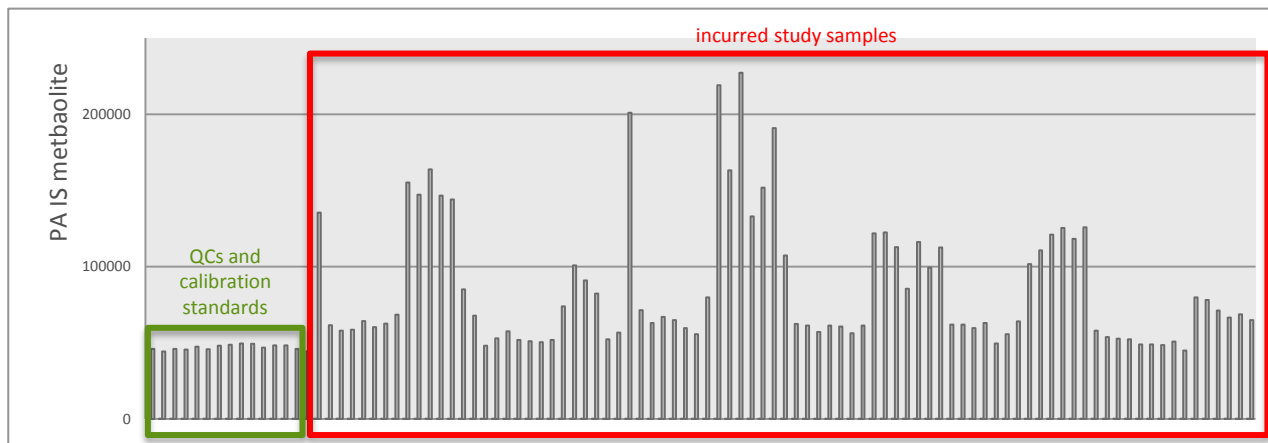
LC-parameters		
Column	Acquity BEH C18 50 X 2.1mm , 3.5 $\mu$	
Mobile Phase A	10 mM $(\text{NH}_4)_2\text{CO}_3$	
Mobile Phase B	$\text{CH}_3\text{OH}/\text{isopropanol}$ (80/20)	
Flow rate	0.6 ml/min	
Column temperature	50 $^\circ\text{C}$	
Injection volume	40 $\mu\text{L}$	
LC-MS/MS	Shimadzu LC-20 + API-4000	
Gradient time (min)	% A	% B
0.00	70	30
2.50	25	75
2.51	2	98
3.50	2	98
3.51	70	30
5.00	70	30
Analyte	Q1 (m/z)	Q3 (m/z)
UD	441.2	138.1
IS UD	446.2	138.1
metabolite	475.2	304.1
IS metabolite	480.2	309.1



- Used successfully in 22 clinical studies (more than 23,000 samples)

# Observed issue

- a study in non-cancer hepatic impaired patients was conducted
  - Unusual behaviour of the internal standard of the metabolite



→ Detailed overview of the impacted samples

	healthy	mild	moderate	severe
# impacted subjects	0 (5)	1 (6)	5 (9)	9 (9)

# Investigation of the selectivity problem

Samples extracted with and without internal standard:

	<b>PA + IS</b>	<b>PA – IS</b>	<b>difference</b>	<b>degree of hepatic impairment</b>
subject 1	94901	74269	20632	moderate
subject 2	136533	114744	21789	moderate
subject 3	57954	31644	26310	moderate
subject 4	81197	53566	27631	severe
subject 5	117669	91708	25961	severe
subject 6	206310	179650	26660	severe
subject 7	31732	655	31077	not impaired

# Investigation of the selectivity problem

Results selectivity experiment for the metabolite during the method validation in human plasma:

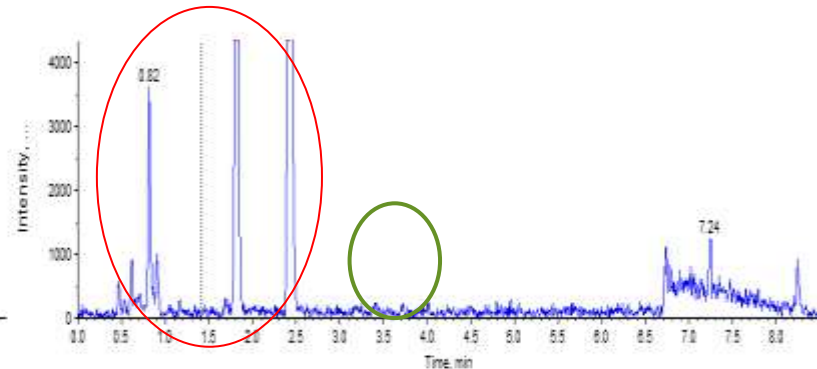
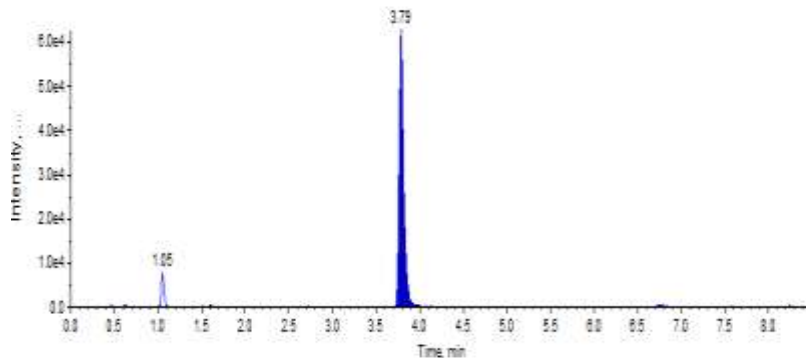
	metabolite			internal standard		
	6 Blanks PA	6 LLOQ's PA	% contribution in blank	6 Blanks PA	6 LLOQ's PA	% contribution in blank*
source 1	0	1378	0	630	73319	0.91
source 2	0	1665	0	756	79631	1.09
source 3	0	1598	0	791	71669	1.14
source 4	0	1354	0	1052	67798	1.52
source 5	0	1277	0	141	50094	0.20
source 6	0	1558	0	1265	74025	1.82
	mean	1472		mean	69423	

\* % contribution in blank for the internal standard < 5.0%

# Investigation of the selectivity problem

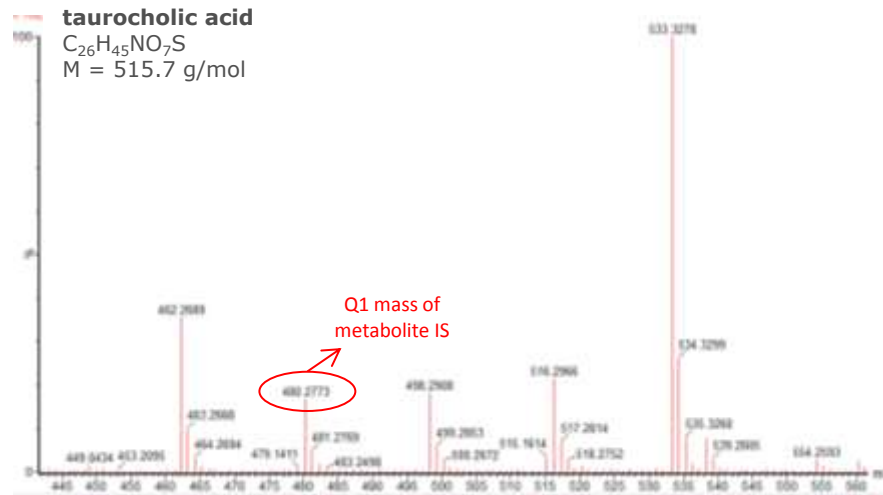
Results longer, slower chromatography:

LC-parameters		
Column	Acquity BEH C18 100 X 2.1mm , 1.7 $\mu$	
Mobile Phase A	10 mM $(\text{NH}_4)_2\text{CO}_3$	
Mobile Phase B	$\text{CH}_3\text{OH}/\text{isopropanol}$ (80/20)	
Flow rate	0.6 ml/min	
Column temperature	50 $^\circ\text{C}$	
Injection volume	5 $\mu\text{L}$	
LC-MS/MS	Nexera + API-6500	
Gradient time (min)	% A	% B
0.00	70	30
6.00	25	75
6.01	2	98
7.50	2	98
7.51	70	30
8.50	70	30



# Identification of the interference

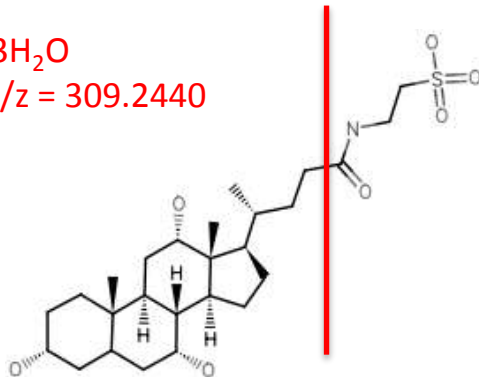
- Injection on the Synapt G2S:



Theoretical m/z  
taurocholic acid -  $2H_2O$  = 480.2784

- Formation of the product ion of the internal standard:

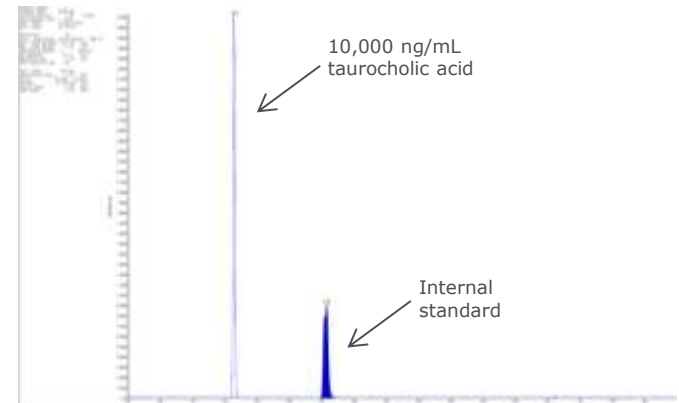
-  $3H_2O$   
m/z = 309.2440



# Confirmation of the observed issue

## Reanalysis of the samples

LC-parameters		
Column	Acquity BEH C18 100 X 2.1mm , 1.7 $\mu$	
Mobile Phase A	10 mM $(\text{NH}_4)_2\text{CO}_3$	
Mobile Phase B	$\text{CH}_3\text{OH}$ /isopropanol (80/20)	
Flow rate	0.6 ml/min	
Column temperature	50 $^\circ\text{C}$	
Injection volume	5 $\mu\text{L}$	
LC-MS/MS	Nexera + API-6500	
Gradient time (min)	% A	% B
0.00	70	30
6.00	25	75
6.01	2	98
7.50	2	98
7.51	70	30
8.50	70	30



Standard curve of taurocholic acid was extracted and injected together with the reanalysis:

	healthy	mild	moderate	severe
# impacted subjects	0 (5)	1 (6)	5 (9)	9 (9)
conc. taurocholic acid (ng/mL)	BQL < 250	600 (3 subj. BQL)	4200	7000



# Conclusions

- Unusual behavior of the internal standard area revealed selectivity issue with the internal standard of the metabolite;
- Interference in the MRM transition of the internal standard of the metabolite due to presence of taurocholic acid;
- Mitigation through chromatographic separation;
- Assay revalidated;
- Selectivity samples with taurocholic acid will be included as part of system suitability test in every run;
- Impact on the development program was evaluated:
  - Preclinical species did not show the interference
  - In clinical studies hepatic impairment was an exclusion criterion.

# Acknowledgement

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*de Vries R, Huang M, Bode N, Jejurkar P, de Jong J, Sukbuntherng J, Sips L., Weng N, Timmerman P, Verhaeghe T.*  
Bioanalysis of ibrutinib and its active metabolite in human plasma; selectivity issue, impact assessment and resolution. *Bioanalysis*, Vol. 7, No. 20, Pages 2713-2724 , DOI 10.4155/bio.15.159

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