DOING MORE WITH LESS: ADDRESSING THE MICROSAMPLING SENSITIVITY CHALLENGE IN DMPK STUDIES USING VACUUM JACKETED COLUMN & UHPLC-CYCLIC ION MOBILITY MS

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PHARMACOKINETICS

UHPLC/MS/MS QUANTIFICATION

Sample Preparation

- Study plasma samples (10µL extracted using 4:1 MeCN protein ppt, supernatant diluted 1 / 100 in 25% Water

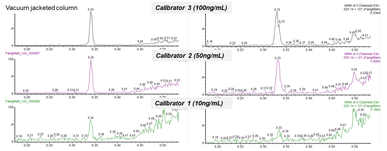
UHPLC/MS/MS Methodology

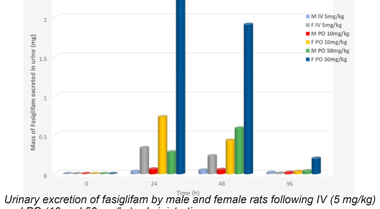
- ACQUITY™ Premier UPLC™, Xevo™ TQ-S micro MS
- Reversed—phase chromatography, aqueous formic scid (0.1%) vs acetonitrile, 50 – 60% B over 0.9 mins @ 0.5ml/min. BEH™ C18 2.1 x 50mm, 1.7 µm, in *Vacuum Jacketed Column Format, temperature* 60 °C
- Positive Ion Esi, MRM 525.14 —> 121
- MassLynx™ software, 4.2, TargetLynx™ software

VACUUM JACKETED COLUMN

Improved LC/MS/MS Performance Using Vacuum Jacketed Column

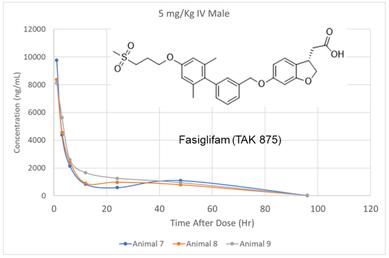
- Improved Sensitivity
- Faster Analysis
- Improved resolution from endogenous material





Urinary excretion of fasiglifam by male and female rats following IV (5 mg/kg) and PO (10 and 50 mg/kg) administration.

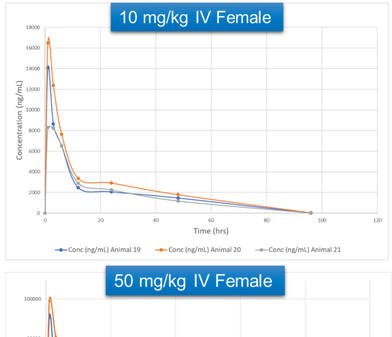
INTRAVENOUS DATA

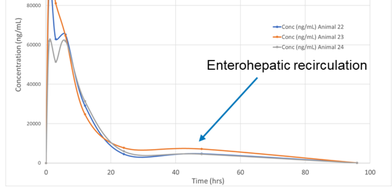


	Units	5mg/kg IV Female	5mg/kg IV Male	10mg/kg Oral Male	10mg/kg Oral Female	50mg/kg Oral Male	50mg/kg Oral Female
T max_obs	h	1	1	1	1	1	1
C max	(µg/mL)	8.8	9.2	12.4	12.9	76.2	83.7
Vd	L	0.56	0.47	0.61	0.67	0.56	0.53
t 1/2	Hr	12.4	11.2	11.5	11.6	10.3	9.8
Cl	L/hr	1.70	1.40	1.71	1.51	1.14	0.83
AUC 0-96 h	mg/ml*h	0.10	0.11	0.17	0.20	1199	1189
Vd (derived)	mL/kg	542.4	528.3	326.8	359.4	68.5	88.9
Oral bioavailability (%)	F	---	---	85.0	90.9	119.9	108.1

Pharmacokinetics following IV administration at 5mg/kg, and oral administration at 10mg/kg & 50mg/kg PO to male and female rats

ORAL DATA





CHALLENGE

- **Understanding metabolic fate of a molecule using early in vivo studies**
- **Maximizing information from small sample volumes**
- **Sensitivity for PK elimination phase and metabolite identification**
- **Rapid "data turn around"**
- **Simplifying data analysis and structural elucidation in complex matrices**

STUDY

- Fasiglifam (TAK-875), GPR40 agonist developed for the treatment of Type II diabetes [1]
- Terminated in Phase III clinical trails due to concerns regarding liver toxicity [2]
- However, no toxicity observed in preclinical studies
- Aim understand the DMPK of TAK 875 and identify possible toxic biotransformations
- Blood samples (50µL) taken using tail bleed capillary sampling

4 Dose Groups (males & Females)

Vehicle | *Oral Dose 10mg/Kg* | *Oral Dose 50mg/Kg* | *IV Dose 5mg/Kg*

Plasma collection

Pre dose, 1hr, 3hr, 6hr, 12hr, 24hr, 48hr, & 96hrs

Urine collection

Pre dose, 0-24hr, 24-48hr, 48-96hrs

Tissue collection

24, 48, and 96hrs

CONCLUSION

- **A rapid, specific, method for the quantification of fasiglifam in rat plasma using VJC UHPLC-MS-MS was developed [3]**
- **Microsampling facilitated full PK profile and metabolite identification, using just 3 animal per dose group.**
- **IV (5 mg/kg) peak plasma concentrations = 8.8/9.2mg/ml for male / female rats respectively [4].**
- **PO peak plasma concentrations = 12.4/12.9mg/mL (10 mg/kg) & 76.2/83.7mg/mL (50mg/kg) doses for male / female rats respectively.**
- **$T_{1/2}$ = 12.4 (male) and 11.2 h (female).Oral bioavailability was estimated to be 85-120% in males and females.**
- **UHPLC/clM/MS analysis of plasma identified 15 biotransformation and 3 novel metabolites of the drug including acyl glucuronide.**
- **Novel a ide-chain shortened metabolite via elimination of CH_2 from the acetyl side chain identified**

BIOTRANSFORMATIONS

UHPLC-cIM-MS METABOLITE ID

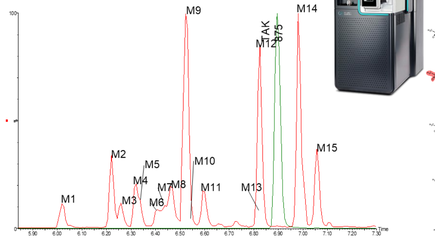
Sample Preparation

- Plasma samples extracted using 4:1 MeCN protein precipitation, diluted 1 / 100 in 25% Water prior to analysis (10µL injected)

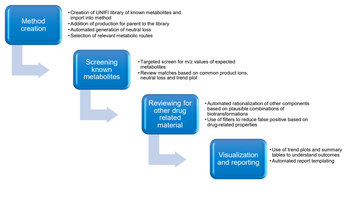
UHPLC/MS/MS Methodology

- ACQUITY Premier UPLC , Select Series™ Cyclic™ IMS Mass Spectrometer
- RPLC using ACQUITY Premier HSS T3 C18 2.1 x 100mm 1.7µm column (40 °C), eluted with , formic acid (aq) (0.1%) vs MeCN gradient at 0.5ml/min 40 – 70% B over 10 mins
- Positive & negative Ion Esi cIMS HDMSe 50 – 1200 m/z
- Nitrogen used for ion mobility gas

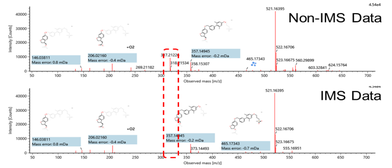
Plasma UHPLC-IM-MS Chromatogram



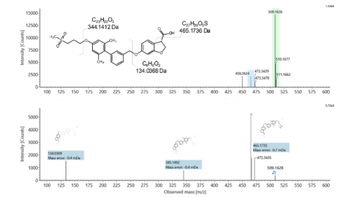
Data Analysis Workflow



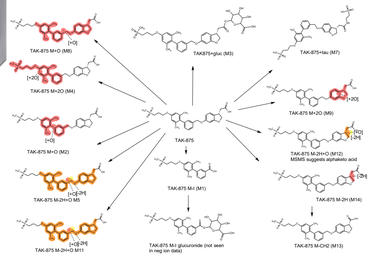
Improved MS Spectral Quality With Drift-Time Aligned Data



New, Possibly Toxic, Metabolite of Fasiglifam



Summary of Biotransformations



Label	Component Name	Formula	Observed m/z	Mass error (ppm)	Observed CCS (Å²)	Observed tR (min)	Common Neutral Losses	Common Fragment Ions	Total Fragments Found	Response
M1	Taki875 M-I	C19H22O5S	361.1111	−1.2	199.7	6.03	TRUE	FALSE	1	1908
M2	Taki875 + O	C29H32O8S	539.1739	−1.1	219.8	6.22	TRUE	TRUE	6	6020
M3	Taki875-G	C35H40O13S	699.2113	−0.5	246.3	6.26	FALSE	FALSE	1	2523
M4	Taki875 + O2	C29H32O9S	555.1687	−1.2	222.9	6.32	FALSE	TRUE	4	4281
M5	Taki875 + O-H2	C29H30O8S	537.1585	−0.7	220.2	6.34	TRUE	FALSE	3	1036
M6	Taki875 + O2	C29H32O9S	555.1688	−1.1	224.3	6.41	TRUE	FALSE	1	787
M7	Taki875-Mac	C31H37NO9S2	630.1836	−0.2	238.4	6.44	TRUE	FALSE	1	3834
M8	Taki875 + O	C29H32O8S	539.1743	−0.3	220.8	6.46	TRUE	FALSE	5	3812
M9	Taki875 + O2	C29H32O9S	555.1690	−0.7	221.1	6.53	FALSE	FALSE	12	19155
M10	Taki875 + O	C29H32O8S	539.1738	−1.2	220.3	6.54	FALSE	FALSE	7	1162
M11	Taki875 + O-H2	C29H30O8S	537.1586	−0.5	220.0	6.6	TRUE	FALSE	5	3173
M12	Taki875 + O-H2	C29H30O8S	537.1583	−1.1	219.0	6.83	TRUE	FALSE	5	12403
M13	Taki875-CH2 (from side chain)	C28H30O7S	509.1636	−0.8	214.8	6.83	TRUE	FALSE	4	2277
M14	Taki875-H2	C29H30O7S	521.1636	−0.6	217.8	6.98	TRUE	TRUE	8	19205
M15	Taki875-H2	C29H30O7S	521.1638	−0.3	218.1	7.06	TRUE	TRUE	7	6973

References

1. Kaku K, Enya K, Nakaya R, Ohira T, Matsuno R.2016. Long-term safetyand efficacy of fasiglifam (TAK-875), a G-protein-coupled receptor 40agonist, as monotherapy and combination therapy in Japanesepatients with type 2 diabetes: a 52-week open-label phase III study.Diabetes Obes Metab. 18 (9):925–929.
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4. Nikunj Tanna, Robert S. Plumb, Billy J. Molloy, Paul D. Rainville, Ian D. Wilson. Enhanced chromatographic efficiency obtained with vacuum jacketed columns facilitates the rapid UHPLC/MS/MS -based analysis of fasiglifam in rat plasma, Talanta, 2023. 254, 124089. https://doi.org/10.1016/

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