

***DEVELOPMENT AND VALIDATION OF
AN IMPROVED LC-MS/MS METHOD
FOR THE ANALYSIS OF 1-
HYDROXYPYRENE IN HUMAN URINE***

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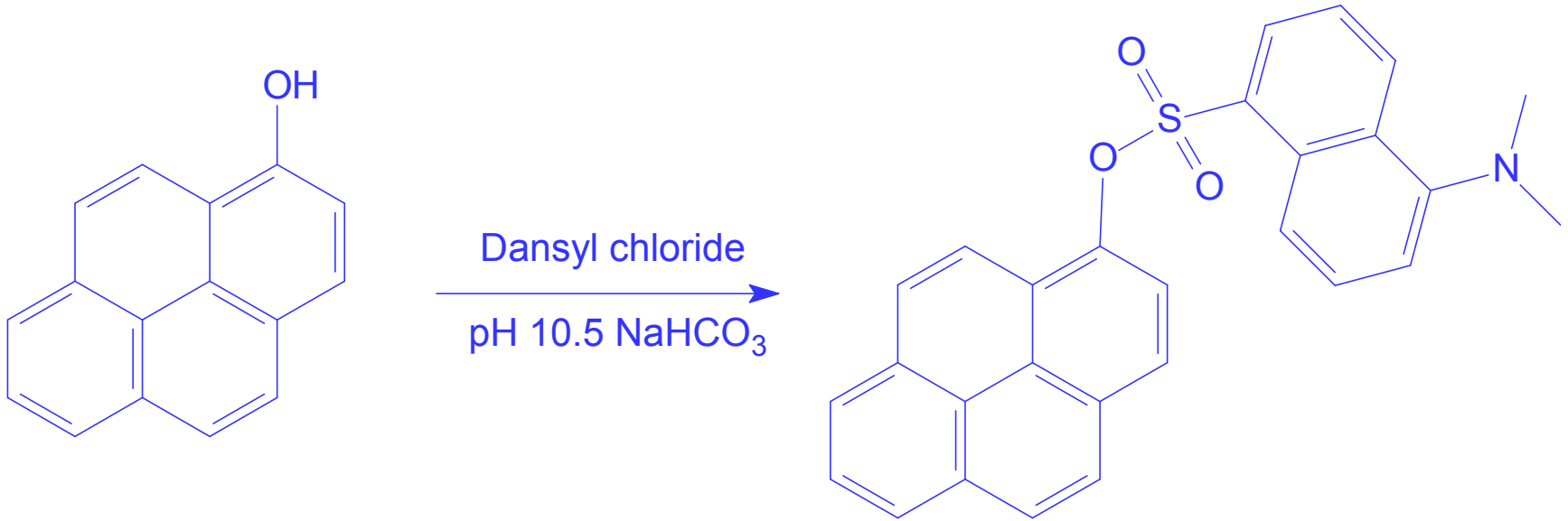
Background

- 1-Hydroxypyrene (1-OHP) is a biomarker for pyrene and is present (mostly in its glucuronide form) in the urine at pg/mL range
- A fast, reliable LC-MS/MS method for measuring 1-OHP has not been reported
- Due to lack of favorable MS fragmentation, 1-OHP itself can only be measured at ng/mL range

Analytical strategy

- Use well-established derivatization strategy to enhance the method sensitivity
- Dansyl chloride has been used to enhance the sensitivity by introducing a moiety with favorable ionization and fragmentation
- Deuterated 1-OHP (1-hydroxypyrene- d_9) was synthesized and was used as the internal standard (ISTD) to improve the method ruggedness

Derivatization of 1-OHP with dansyl chloride



Sample preparation (1)

- A properly agitated sample of urine (0.1 mL) was treated with β -glucuronidase with sulfatase activity (20.0 μ L of 50,000 units/mL, 2 hr at 37°C) to deconjugate the 1-OHP glucuronide
- The free 1-OHP and 1-OHP- d_9 , were extracted with 3 mL of methyl tert-butyl ether
- After freezing the aqueous layer, the organic phase was decanted into a clean test tube and evaporated to dryness

Sample preparation (2)

- The residue was derivatized with dansyl chloride (150 μL of 100 *mM* sodium bicarbonate, pH 11.0, and 150 μL of 0.250 mg/mL dansyl chloride in acetone, 45°C for 30 min) and back-extracted with 1.5 mL of hexane
- After evaporation of the organic phase under nitrogen, the residue was reconstituted in 150 μL of 50% acetonitrile in water, and was analyzed using LC/MS/MS

LC conditions

- Column: Betasil C8, 50 x 3 mm, 5 μm
- Column oven: 30°C
- Mobile phase: A: Water with 0.1% formic acid
B: Acetonitrile with 0.1% formic acid

- Gradient Program:

Time (minute)	% Mobile Phase B	Flow Rate (mL/min)
0.00	70	0.80
2.10	90	0.80
2.11	70	0.80

- Injection volume: 10 μL
- Run time: 3.6 min

MS/MS conditions (1)

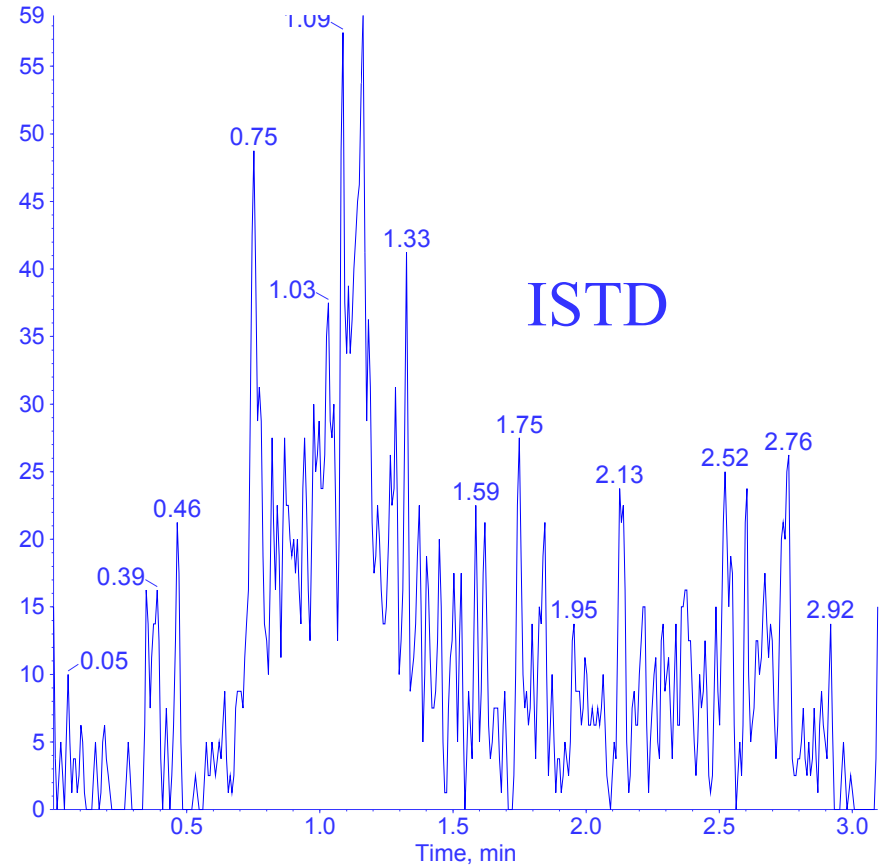
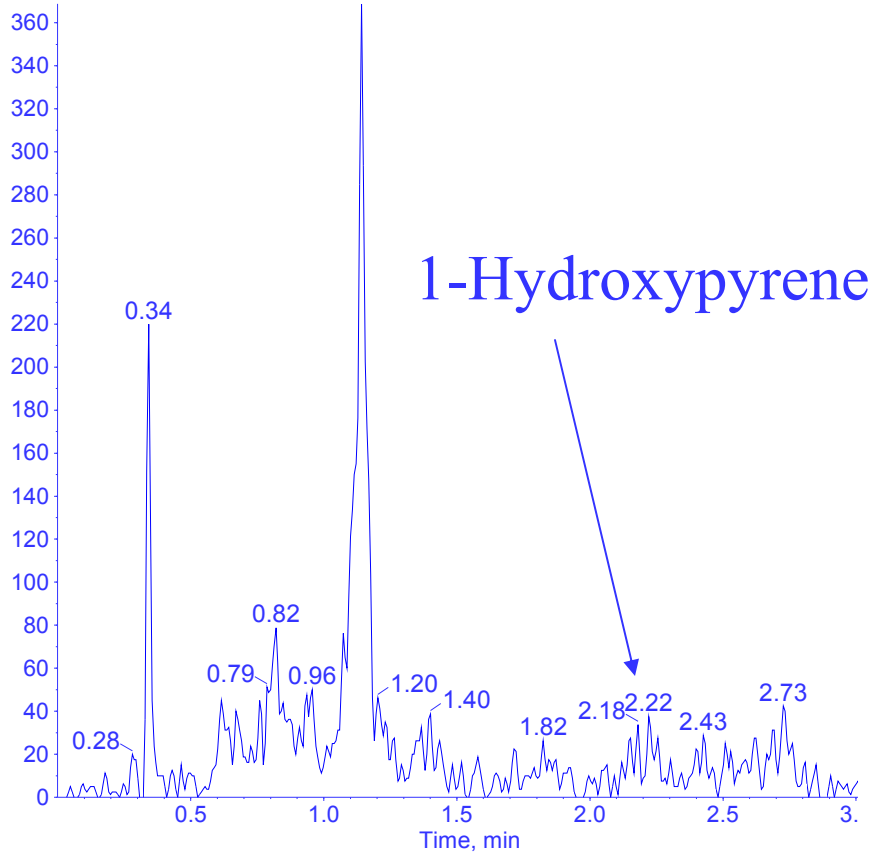
- Mass spectrometer: Sciex API 3000
- Ionization: ESI+
- Mode: MRM
- Ion spray voltage: 4000 V
- Ion spray temperature: 450°C
- Curtain gas: Nitrogen Setting: 8
- CAD gas: Nitrogen Setting: 7
- Nebulizing gas: Nitrogen Setting: 10
- Auxiliary gas flow: 8 L/minute

MS/MS conditions (2)

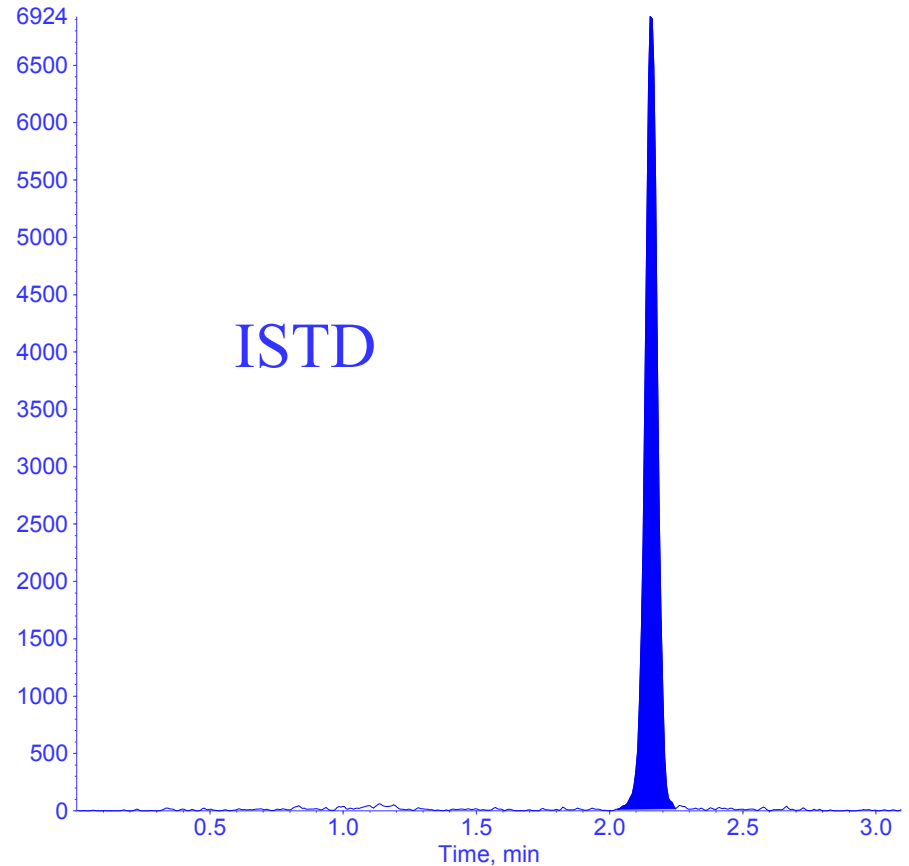
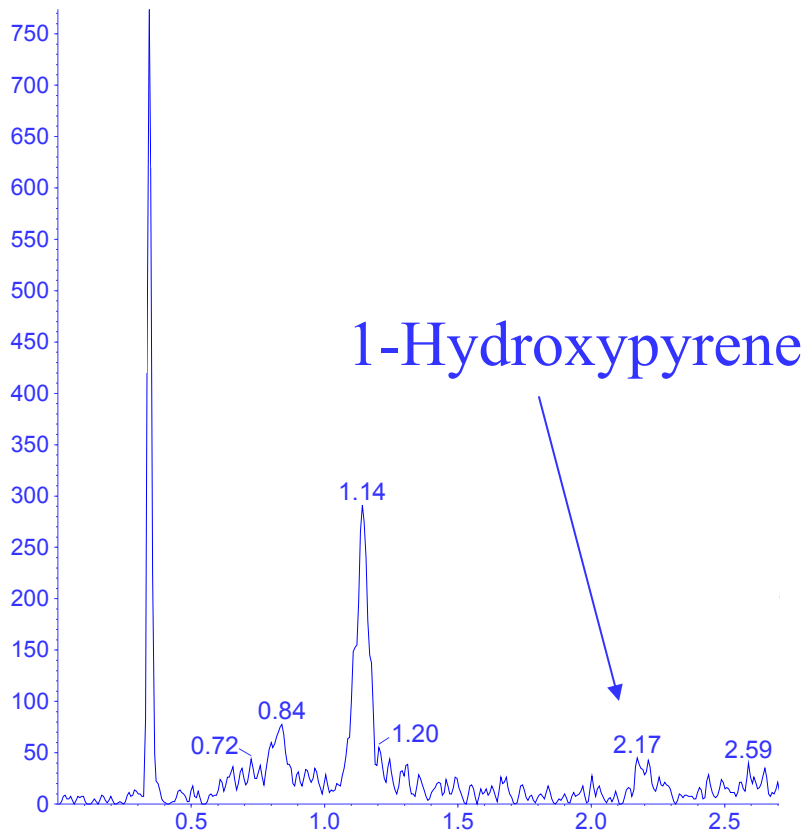
Compound Name	Transition Monitored	Dwell Time (msec)	Collision Energy (eV)	Approximate Retention Time (min)
1-hydroxypyrene	452.1→217.1	200	26	2.2
ISTD	461.1→226.1	200	26	2.2

- Quantitation
 - Regression type: Linear
 - Weighting: $1/(\text{concentration})^2$

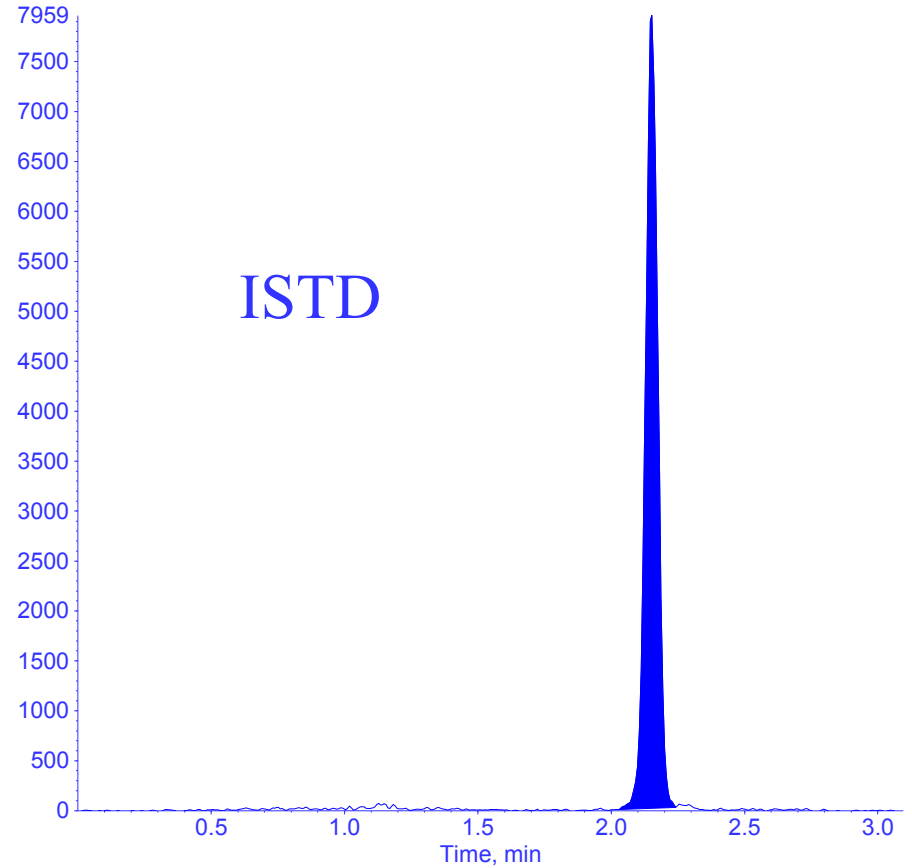
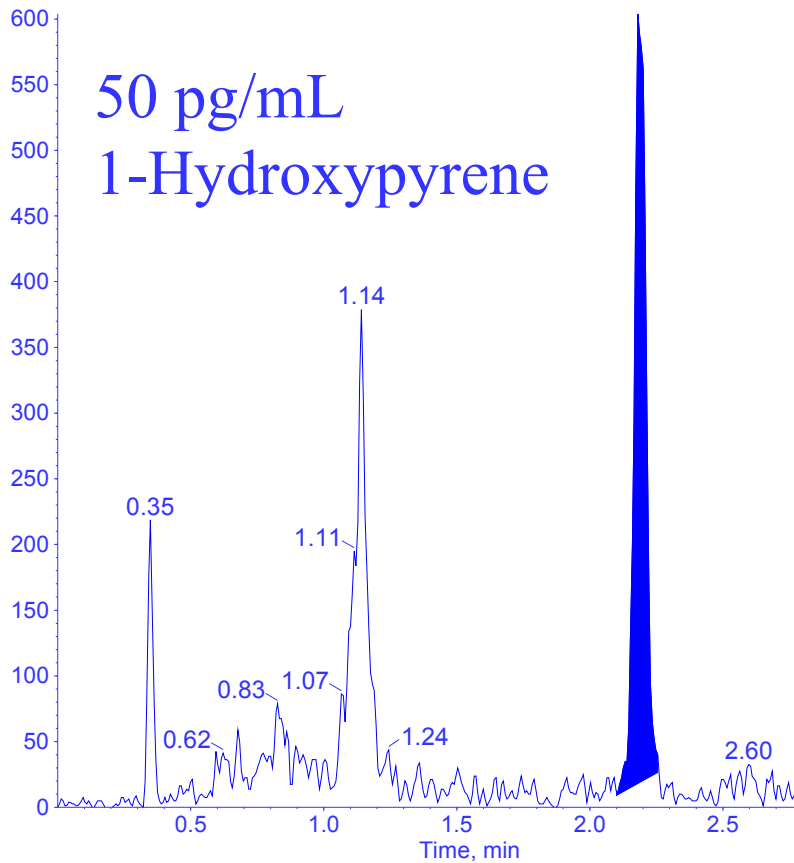
Chromatograms of blank urine



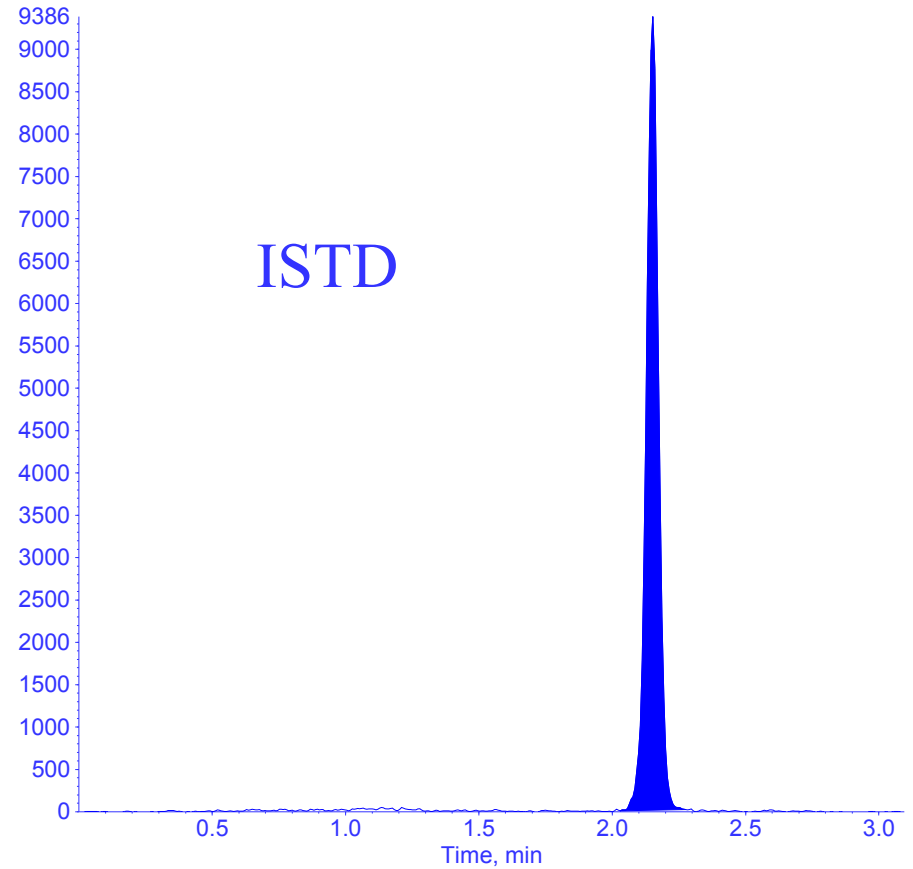
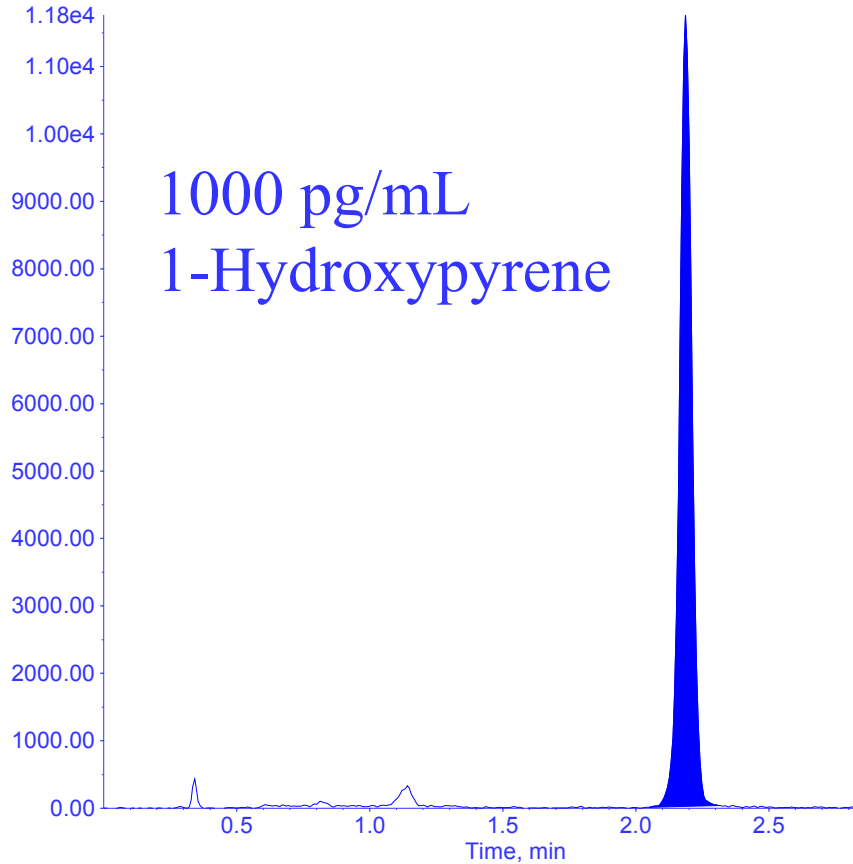
Chromatograms of blank urine with ISTD



Chromatograms at LLOQ



Chromatograms at ULOQ



Precision and accuracy of QC samples (Intraday)

	QC 50.0 pg/mL (LLOQ)	QC 112 pg/mL (Low)	QC 321 pg/mL (Mid)	QC 522 pg/mL (High)	*QC 522 pg/mL (Dilution)
n	6	6	6	6	6
Mean (pg/mL)	46.5	108	295	502	473
Precision (%RSD)	7.8	5.2	2.4	4.2	1.6
Accuracy (%DMT)	-7.0	-3.6	-8.1	-3.8	-9.4

* Analyzed after 5-fold dilution with analyte-free matrix.

Precision and accuracy of QC samples (Interday)

	QC 112 pg/mL (Low)	QC 321 pg/mL (Mid)	QC 522 pg/mL (High)
n	18	18	17
Mean (pg/mL)	110	302	498
Precision (%RSD)	6.5	5.8	4.8
Accuracy (%DMT)	-1.8	-5.9	-4.6

Precision and accuracy of calibration standards

	Calibration Level (pg/mL)							
	50.0	80.0	100	200	400	600	800	1000
n	3	3	3	3	3	3	3	3
Mean (pg/mL)	51.0	77.5	98.1	208	395	582	821	1000
Precision (%RSD)	1.9	1.7	2.0	6.7	2.1	2.5	1.9	1.4
Accuracy (%DMT)	2.0	-3.1	-1.9	4.0	-1.3	-3.0	2.6	0.0

Method stability

Condition	Minimum stability
1-OHP standard solution	~ 25 hours at ambient
1-OHP- d_9 standard solution	~ 25 hours at ambient
Freeze-thaw in matrix	three cycles
Ambient (benchttop) in matrix	at least 23.5 hours
Processed samples stored at 10°C	~ 24 hours
Processed samples stored at 2-8°C	~ 24 hours
Total processed sample stability	~ 48 hours
Frozen matrix stability at -20°C	85 days

Extraction Recovery

- 1-Hydroxypyrene
 - 86.1% (RSD% = 3.7) at 100 pg/mL
 - 89.9% (RSD% = 4.1) at 400 pg/mL
 - 91.8% (RSD% = 2.6) at 800 pg/mL
 - Overall recovery: 89.3%
- 1-Hydroxypyrene-*d*₉ (ISTD)
 - Overall recovery: 91.6%

Conclusions

- A sensitive, robust and fast LC-MS/MS method was developed and validated to measure 1-hydroxypyrene in human urine.
- Derivatization strategy was used to significantly enhance the method sensitivity.
- The lower limit of quantitation has a signal to noise ratio >20 at 50 pg/mL using only 0.1 mL urine sample

Acknowledgement

- Dr. Heiko Junga from Covance Laboratories is acknowledged for synthesizing and characterizing 1-hydroxypyrene- d_9 .