

1 **Supplemental**

2 **Table S1. Individual fish data for microsomal and liver slice preparations.**

<b>RBT Liver Slices</b>							
<b>Date</b>	<b>Chemical</b>	<b>fish</b>	<b>sex</b>	<b>maturity</b>	<b>body weight (g)</b>	<b>liver weight (g)</b>	<b>HSI (%)</b>
Sept 27 2017	AC	1	M	immature	369	3.31	0.90
Oct 16 2017	AC	1	M	immature	417	3.9	0.94
Jan 23 2018	IMI	1	F	1/3	541	6.7	1.24
Nov 30 2018	AC, IMI	1	M	immature	425	3.65	0.86
Jan 30 2019	AC, IMI	1	M	immature	435	5.02	1.15
Feb 7 2019	AC, IMI	1	M	immature	401	4.6	1.15
Feb 7 2019	AC, IMI	2	F	1/3	412	4.69	1.14
<b>RBT usomes</b>							
<b>Date</b>	<b>Chemical</b>	<b>fish</b>	<b>sex</b>	<b>maturity</b>	<b>body weight (g)</b>	<b>liver weight (g)</b>	<b>HSI (%)</b>
April 9 2018		2	F	1/3	512	5.5	1.07
		3	M	1/6	624	7.3	1.17
		5	F	1/4	524	6.1	1.16
		6	F	1/4	571	5.3	0.93
		7	M	immature	600	5.8	0.97
July 16 2018		1	F	1/3	657	5.5	0.84
		2	M	1/8	494	4.7	0.95
		3	M	immature	406	3.11	0.77
		4	M	immature	599	6.02	1.01
		6	M	immature	769	7.45	0.97
Aug 27 2018		2	F	1/3	931	8.3	0.89
		3	M	immature	832	7.49	0.90
		4	F	immature	512	4.2	0.82
		5	F	1/3	600	6.7	1.12
		6	M	1/3	669	5.15	0.77
Sept 4 2018		1	M	immature	315	3.7	1.17
		2	M	immature	310	3.28	1.06
		4	F	1/4	267	2.8	1.05
		5	M	1/4	349	2.98	0.85
		6	M	immature	289	2.6	0.90
		7	F	1/3	262	2.28	0.87
		8	M	1/3	261	2.47	0.95
		9	F	1/3	196	1.9	0.97

		11	M	immature	250	2.07	0.83
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4 **Table S2. Experimental conditions for HPLC and LC-MS**

<b>Thermo <math>\mu</math>HPLC</b>	<b>Agilent <math>\mu</math>HPLC</b>	<b>Agilent LC-MS</b>
<b>Column:</b> Phenomenex, Synergi Hydro RP, 50x2 mm, 2.5 $\mu$ m	<b>Column:</b> Agilent Poroshell EC-18, 50x3 mm, 2.7 $\mu$ m	<b>ESI:</b> capillary, tube lens, cone, and extractor at 3000, 0.1, 65 and 3.7 V, respectively; capillary voltage and temperature: 2kV and 325°C, respectively. N <sub>2</sub> sheath 30 psi, N <sub>2</sub> auxiliary 10 psi. CID at 20 and 30 eV (N <sub>2</sub> ) with 2 scans at each CID. Product ion scans only. Mass range for products: 50-275 <i>m/z</i> . Desolvation temperature: 350°C, 11L/ min flow.
<b>Flow rate:</b> 0.750 mL/min Injection volume: 50 $\mu$ L	<b>Flow Rate:</b> 0.4 mL/min Injection volume: 1 $\mu$ L	
<b>Program:</b> 8.1 min Gradient	<b>Program:</b> 3 min Isocratic	
<b>Mobile phases:</b> A: 0.5% ACN, 20 mM NH <sub>4</sub> OAc in water, pH 5.0 B: 95% ACN, 10 mM NH <sub>4</sub> OAc in water 0-3.0 min 0% B; 3.0-3.1 min 15% B; 3.1-6.1 min 25% B; 7.1 min 0% B	<b>Mobile phases:</b> A: 4% ACN, 1% Acetic acid in water, pH 4.0. B: 99% ACN, 1% Acetic acid in water	
<b>DAD:</b> 245 and 266 nm Acetamiprid and N-Desmethyl metabolite @245 nm IMI and 5-hydroxy metabolite @ 266 nm	<b>DAD:</b> 245 and 266 nm Acetamiprid and N-Desmethyl metabolite @245 nm IMI and 5-hydroxy metabolite @ 266 nm	

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6 **Table S3.  $\mu$ HPLC and LC-MS Components**

<b><math>\mu</math>HPLC</b>	<b>LC-MS</b>
Thermo-Dionex Ultimate 3000	Agilent 1200 $\mu$ HPLC
Solvent delivery system	Binary solvent delivery system
Refrigerated autosampler (6 °C)	Degasser
Heated column compartment (40 °C)	Refrigerated autosampler (20 °C)
Diode array detector (DAD); 245 & 266 nm	Heated column compartment (30 °C)
	Diode array detector (DAD); 245 & 266 nm
	Agilent 1200 LC coupled to a 6410 series triple-quadrupole MS

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8 **Table S4. Physical and experimental properties of available standards for chemical**  
9 **identification.**

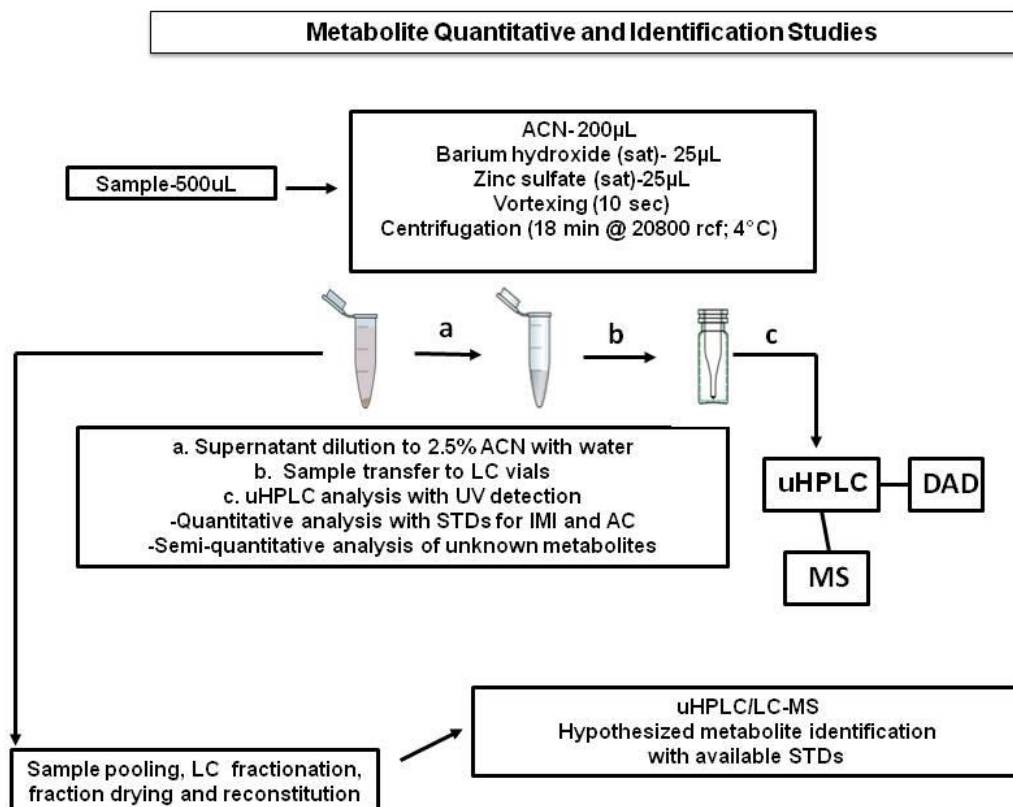
Chemical Name	Acronym	Classification	LogKow	Absorbance Max (nm)	Retention Time (R <sub>f</sub> ) min
Acetamiprid	AC	Parent	2.55	245	5.01
Acetamiprid-N-desmethyl	AC-1	Metabolite	2.34	224	4.68
6-chloronicotinic acid	AC-2	Metabolite	1.33	245	2.37
N-cyano-N'-methyl-ethanimidamide	AC-3	Metabolite	1.17	224	1.18
Imidacloprid	IMI	Parent	2.21	266	4.73
5-hydroxy-Imidacloprid	IMI-1	Metabolite	2.01	266	4.36
Imidacloprid Olefin	IMI-2	Metabolite	1.82	266	4.19

10 *LogKow calculated from EPIWEBv4.1*

11 *Data was obtained from Chemspider and experimental analysis*

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13 **Figure S1: Analytical approach used for the quantification and identification of the main**  
14 **metabolites of Imidacloprid (IMI) and Acetamiprid (AC) formed after in *in vitro* chemical**  
15 **exposure of RBT and rat liver microsomes and RBT liver slices.**



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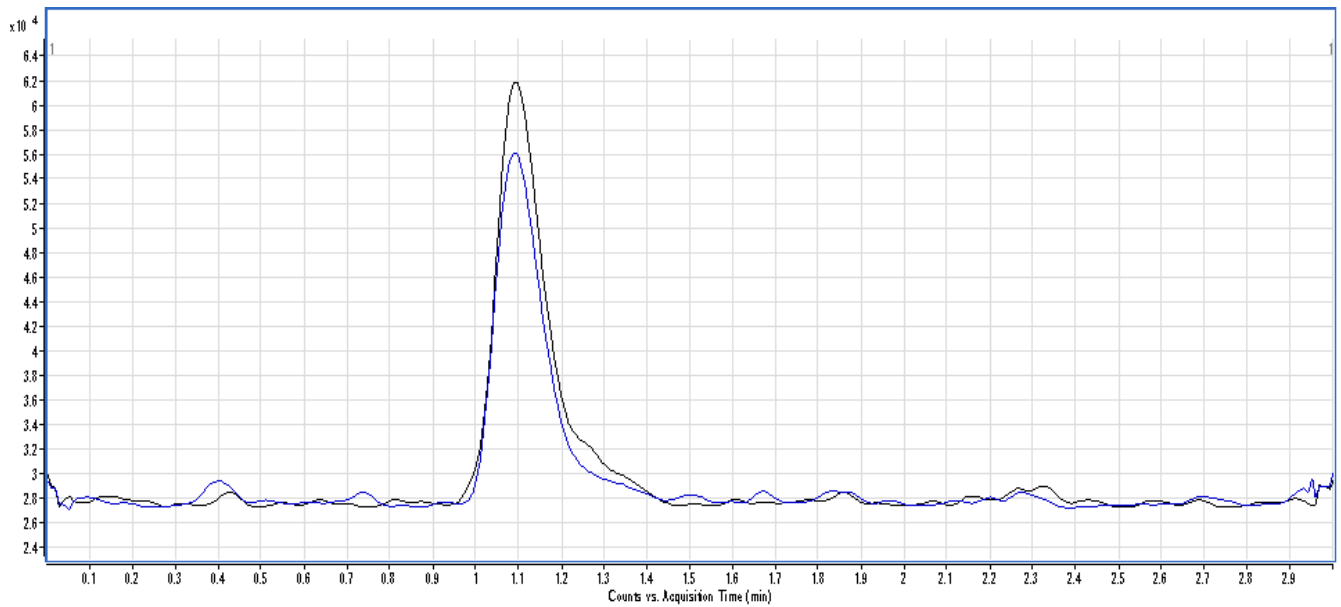
18 **Figure S2: LC-MS Identification of IMI Metabolite**

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20 **A: 4% ACN, 1% Acetic acid/water, pH 4.0**  
 21 **B: 99% ACN, 1% Acetic acid/water; 0.4 mL /min**  
 22 **5-hydroxy-IMI Standard 50µM; (upper trace)**  
 23 **IMI-1 Fractionated Unknown: (lower trace)**

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25 **Counts vs. Acquisition Time (min)**



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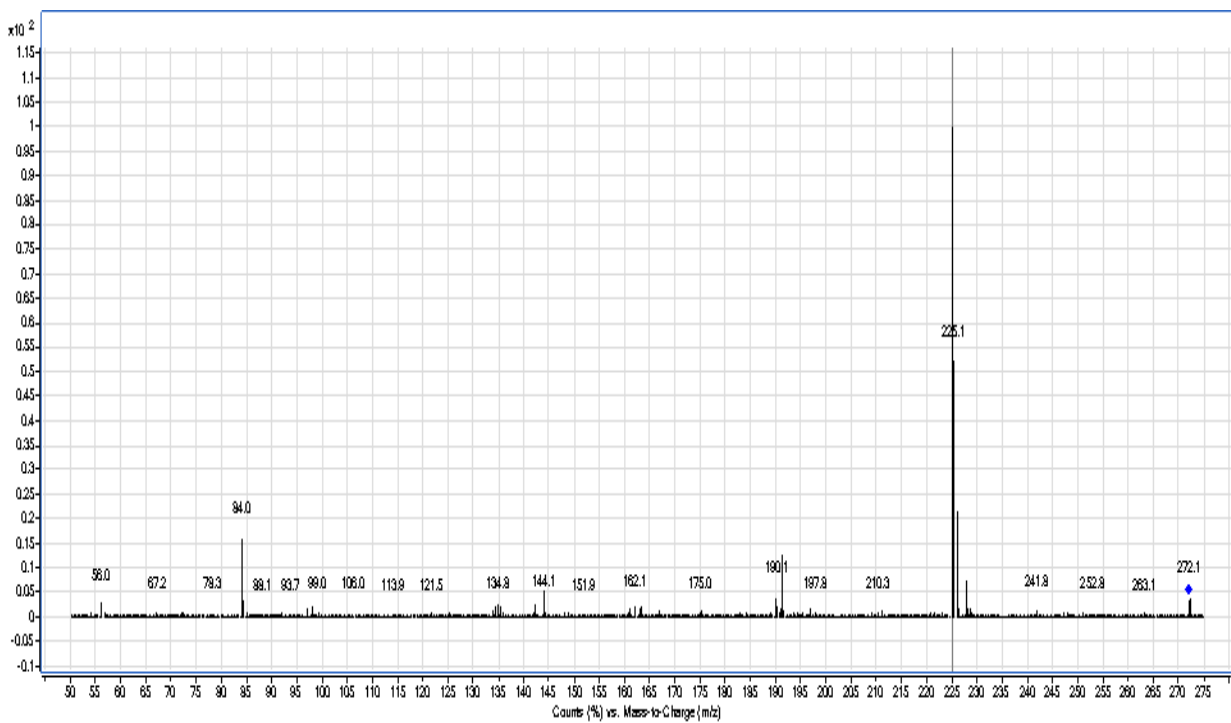
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**5-hydroxy-IMI (IMI-1) Standard  
LC-MS/MS product ion mass spectrum of  $m/z$  272.1 ( $[M+H]^+$ )**

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31 **Counts (%) vs. Mass-to-Charge ( $m/z$ )**



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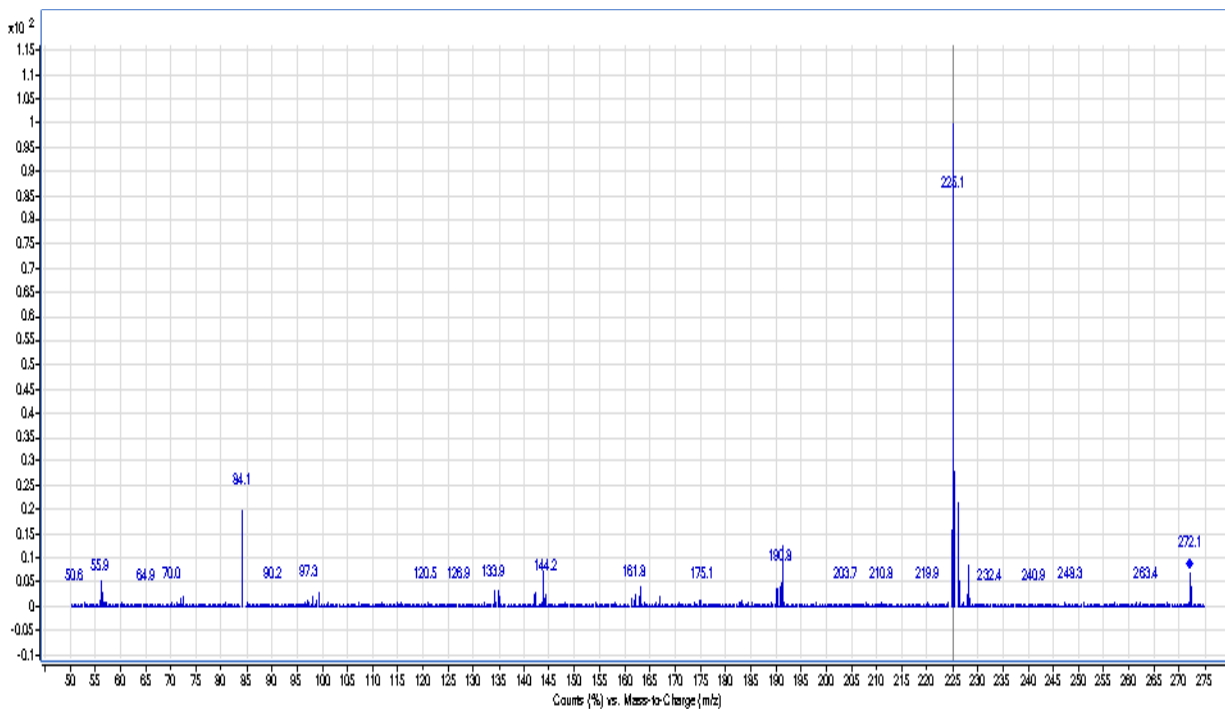
**IMI-1 Fractionated Unknown**

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LC-MS/MS product ion mass spectrum of  $m/z$  272.1 ( $[M+H]^+$ )

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36 Counts (%) vs. Mass-to-Charge ( $m/z$ )



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39 **Figure S3: LC-MS Identification of AC Metabolite**

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LC-MS Full Scan; 3 min isocratic LC program

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A: 4% ACN, 1% Acetic acid/water, pH 4.0

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B: 99% ACN, 1% Acetic acid/water; 0.4 mL/min

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Acetamidrid-N-Desmethyl Standard 50 $\mu$ M; (upper trace)

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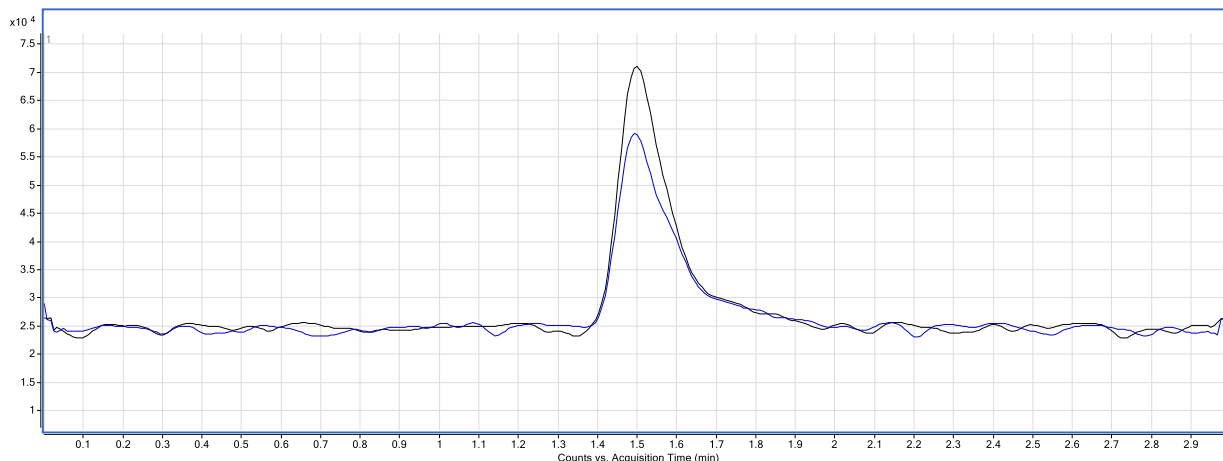
AC-1 Fractionated Unknown: (lower trace)

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48 Counts vs. Acquisition Time (min)

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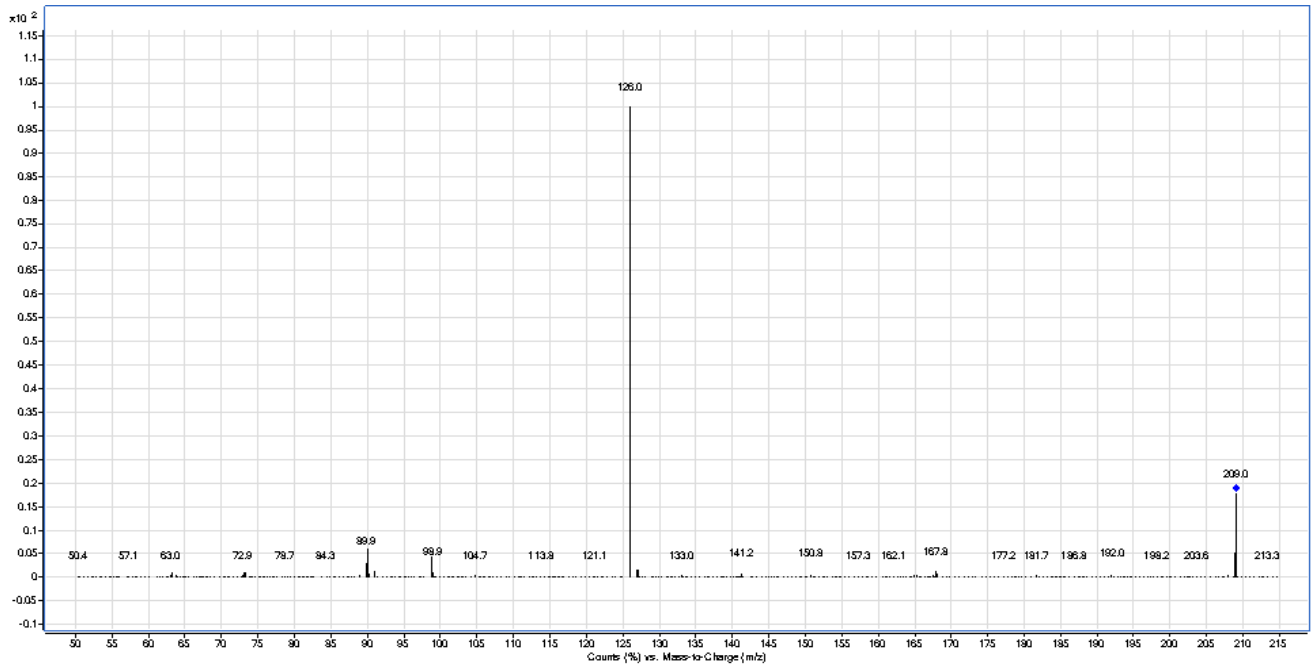
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Acetamidiprid-N-Desmethyl Standard  
LC-MS/MS product ion mass spectrum of  $m/z$  209.1 ( $[M+H]^+$ )

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56 Counts (%) vs. Mass-to-Charge ( $m/z$ )



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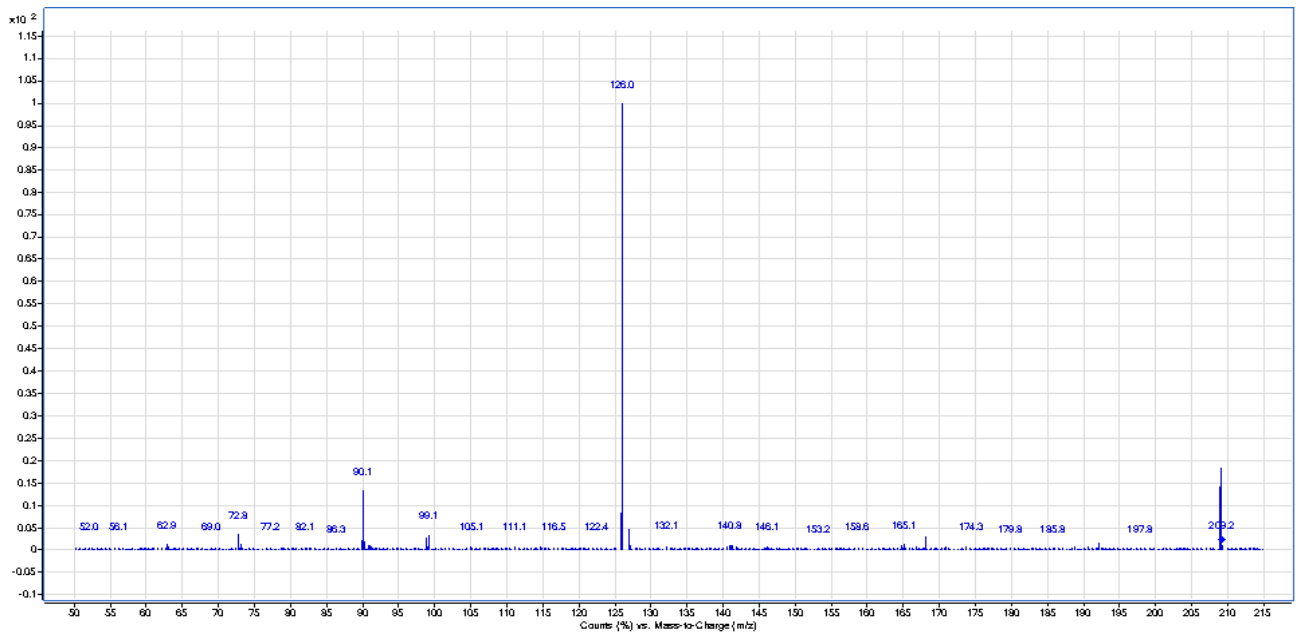
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AC-1 Fractionated Unknown  
LC-MS/MS product ion mass spectrum of  $m/z$  209.2 ( $[M+H]^+$ )

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62 Counts (%) vs. Mass-to-Charge ( $m/z$ )



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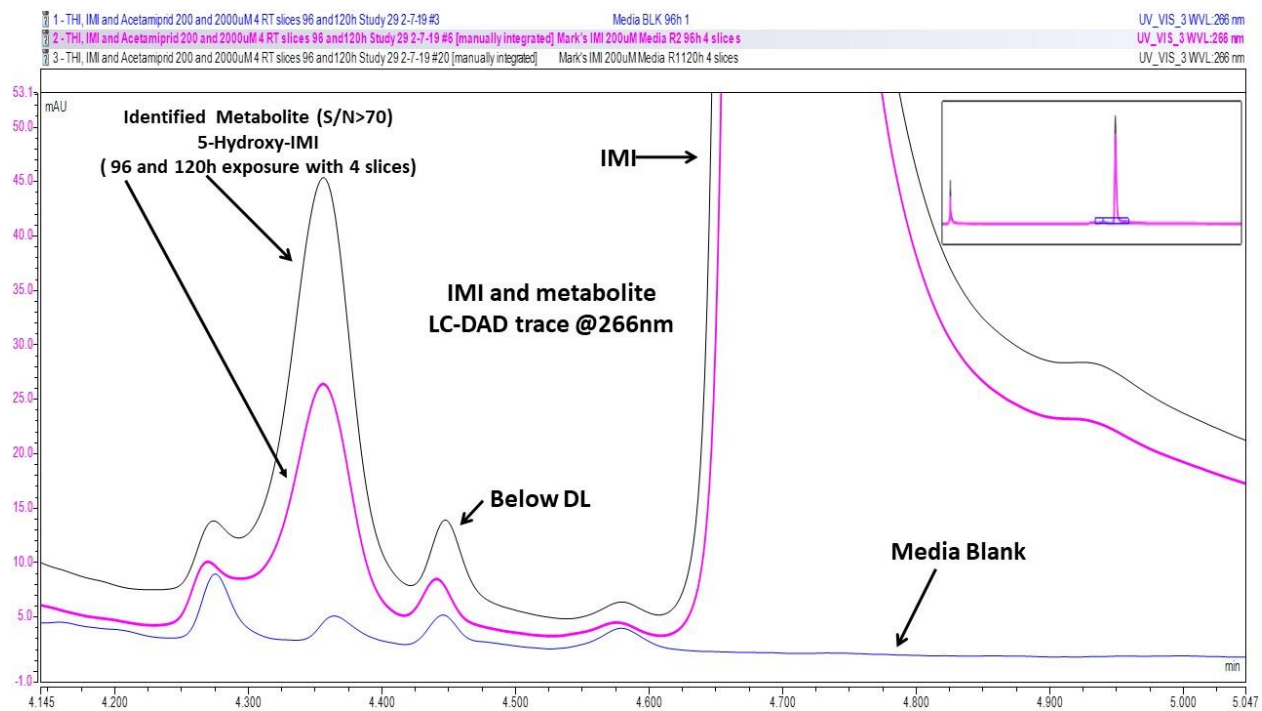
LC-MS Full Scan; 3 min isocratic LC program

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**Figure S4: LC-DAD trace for liver slice media following 96 and 120 hour**

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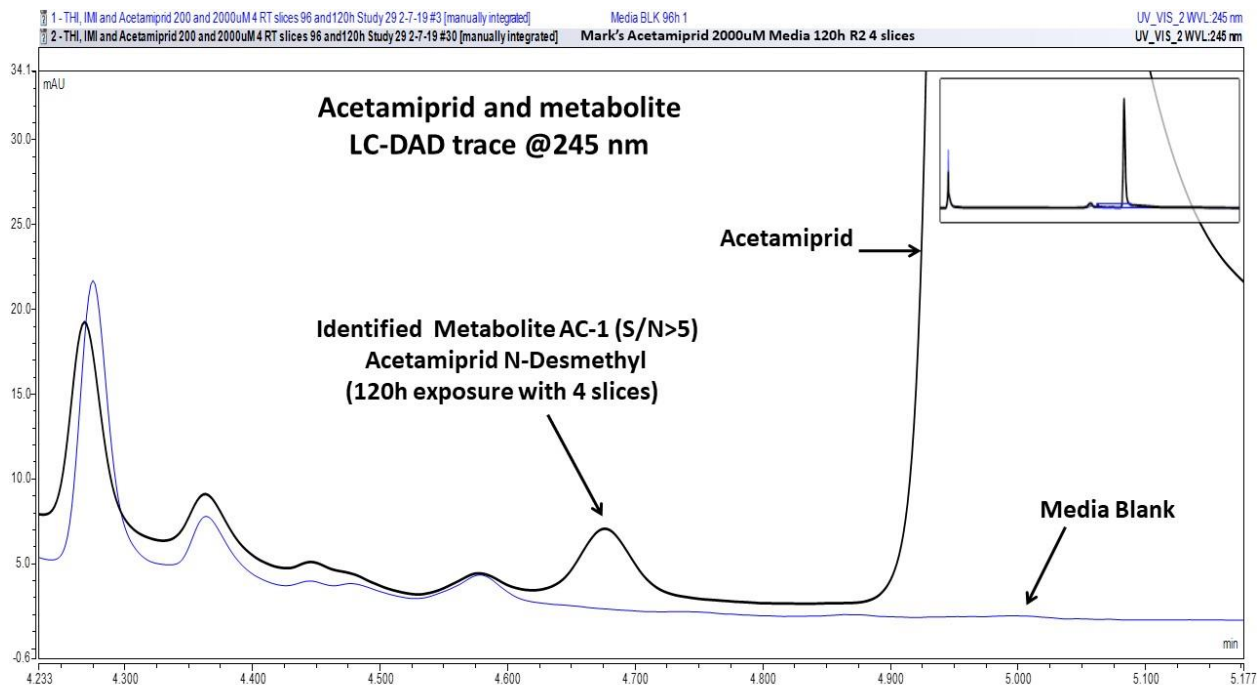
**exposure to 200  $\mu$ M IMI versus a media blank.**



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70 **Figure S5: LC-DAD trace for liver slice media following a 120 hour exposure**  
71 **to 2000  $\mu$ M AC versus a media blank.**



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