

Live reef fish displaying physiological evidence of cyanide poisoning are still traded in the EU marine aquarium industry

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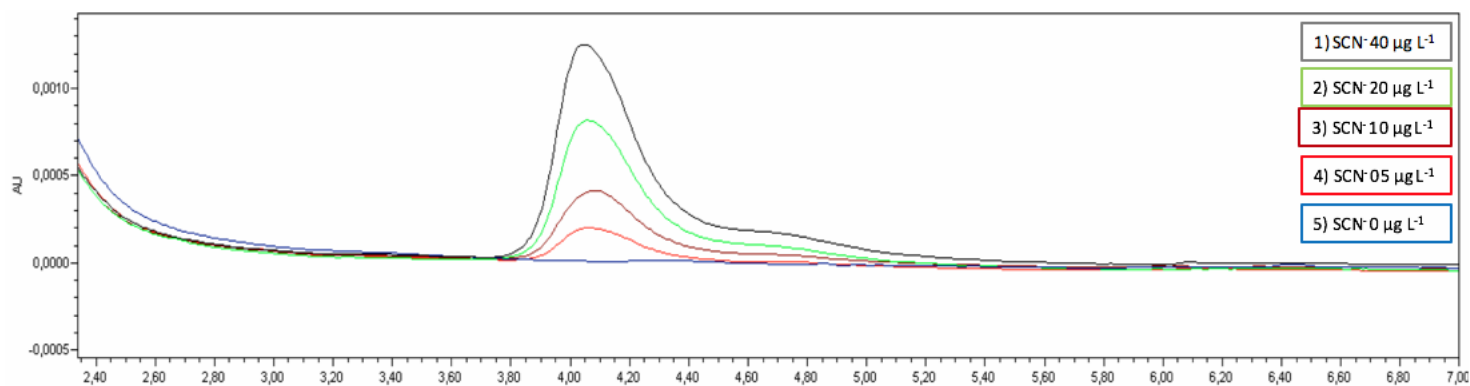
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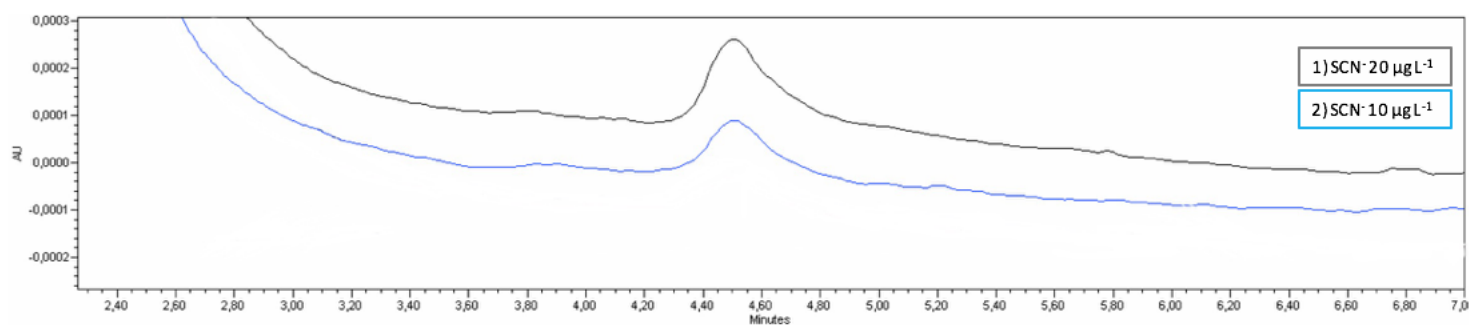
Supplementary Figure S1 - Chromatograms performed using HPLC to screen for the presence of SCN^- in seawater used to depurate live reef fish traded for marine aquariums. All details concerning each chromatogram are summarized in Supplementary Table S1.

Chromatograms of SCN^- Standard Solutions

S1a

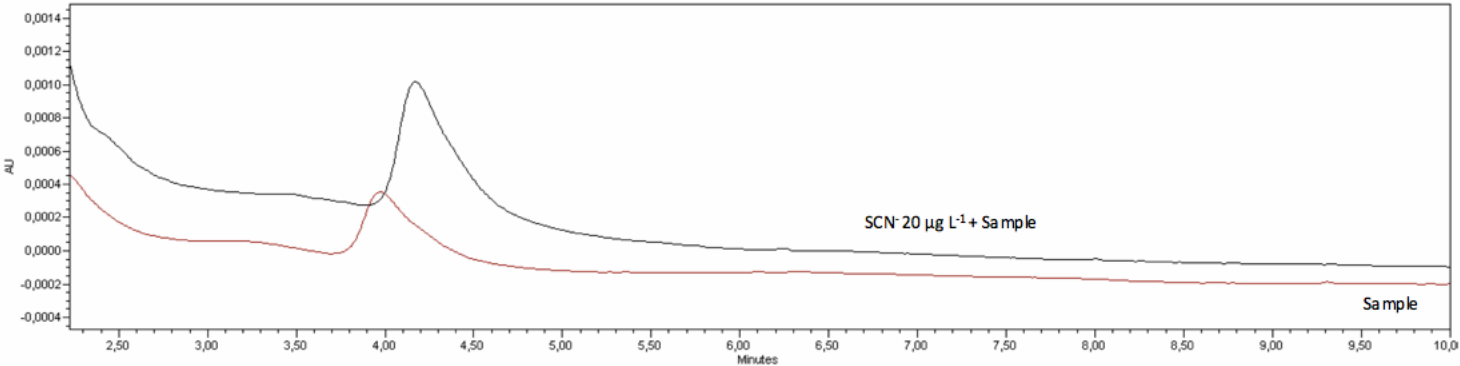


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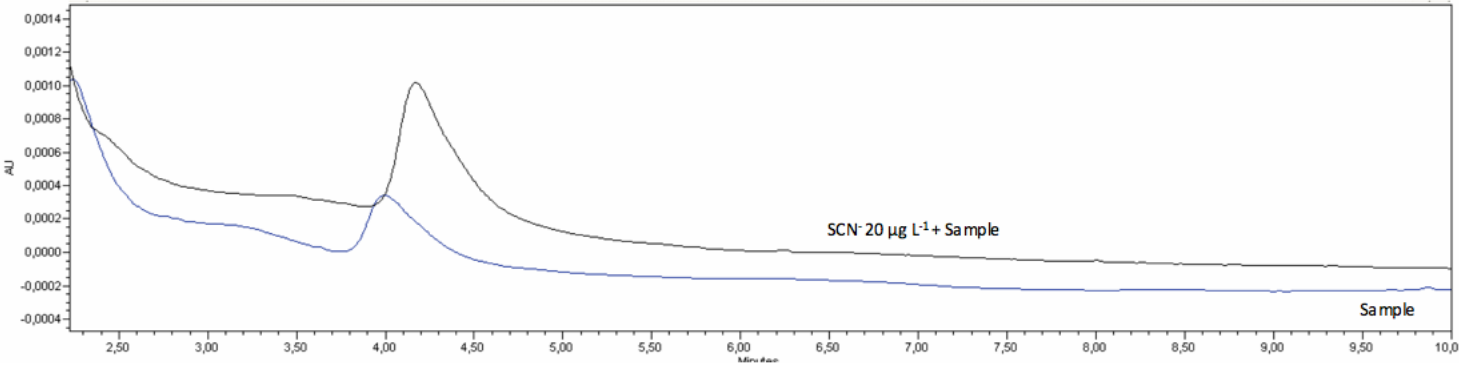


Chromatograms tested positive for SCN⁻

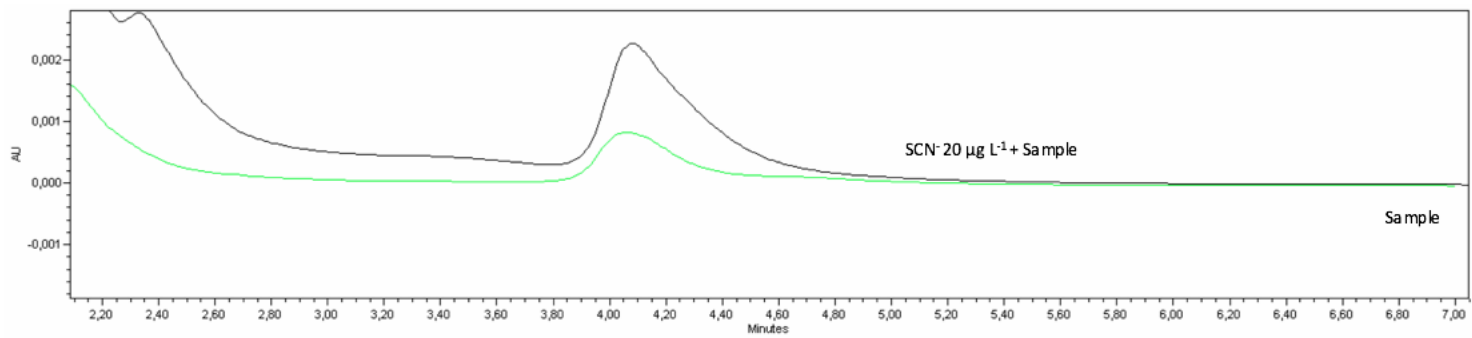
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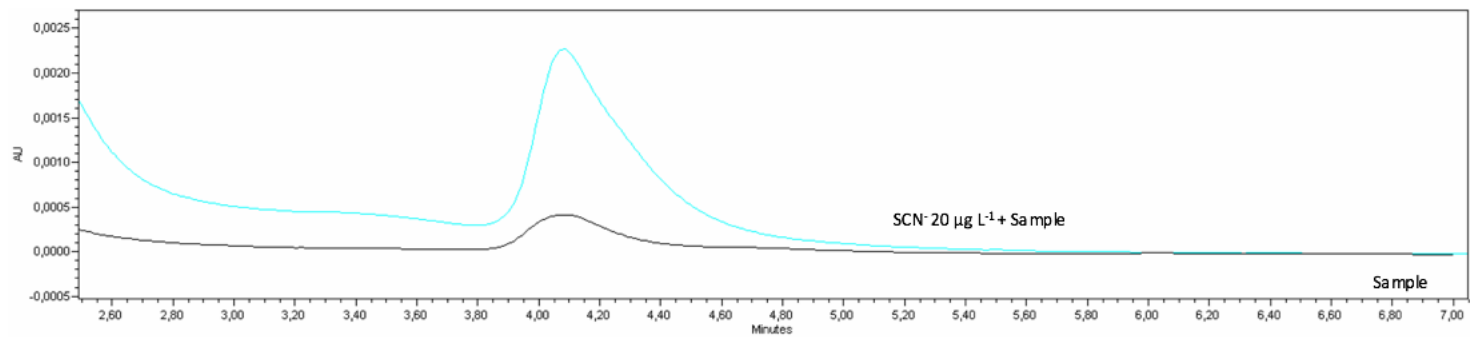
S1d



S1e

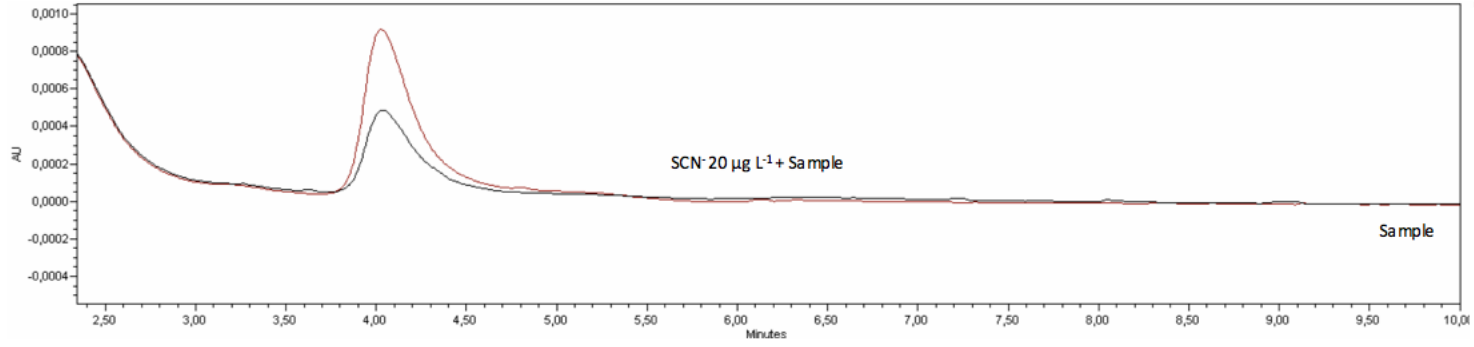


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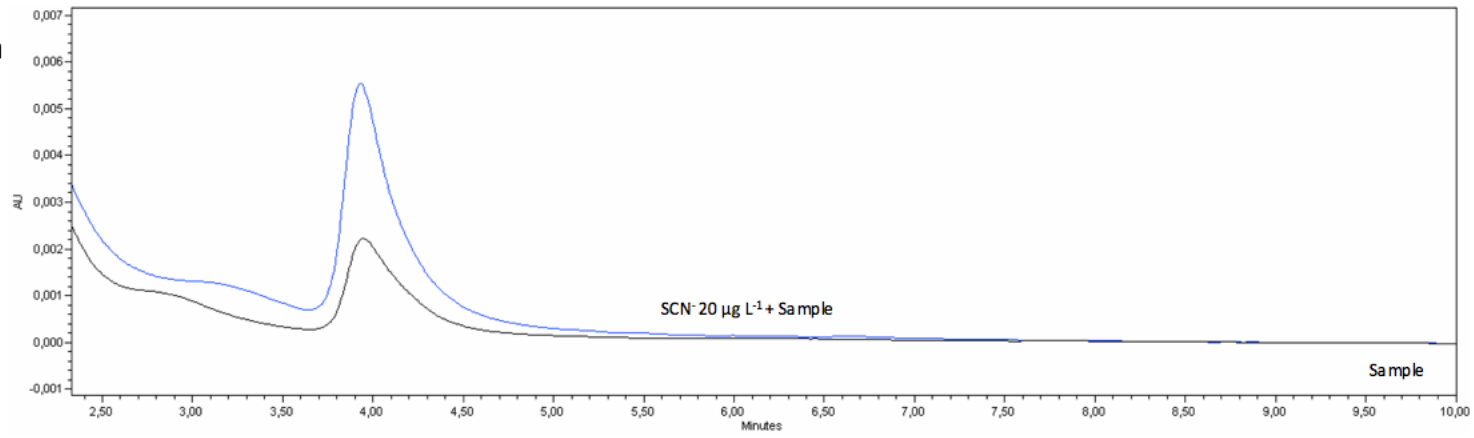




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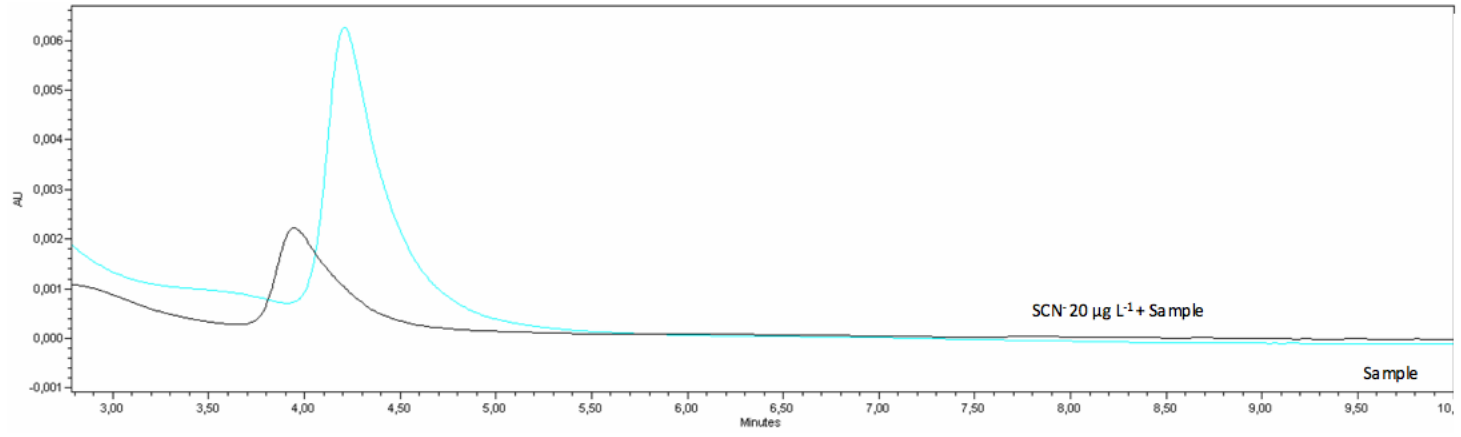


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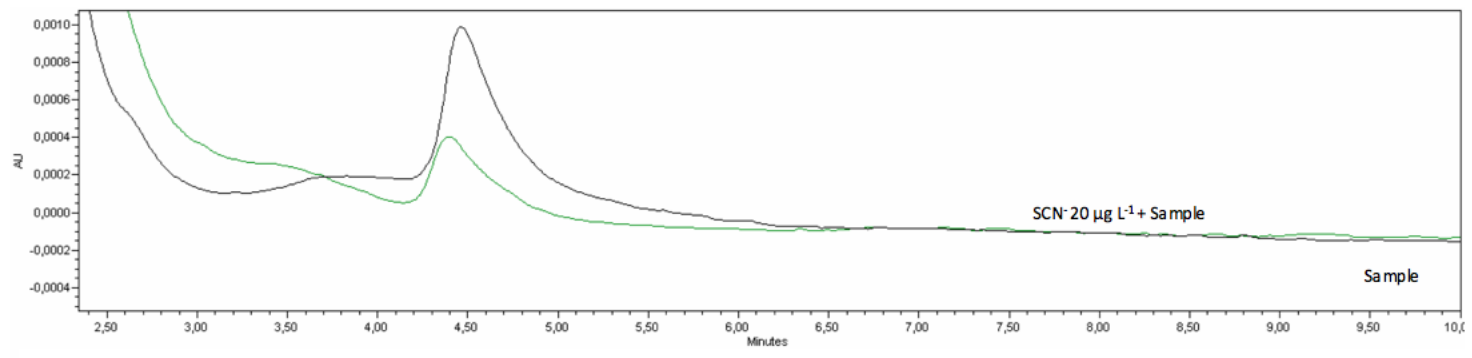




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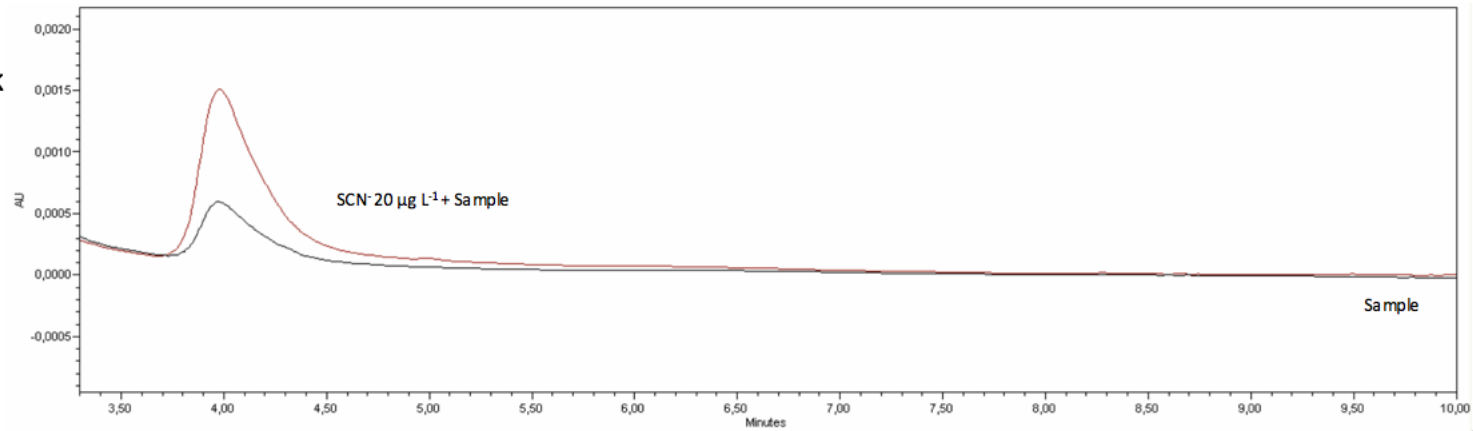


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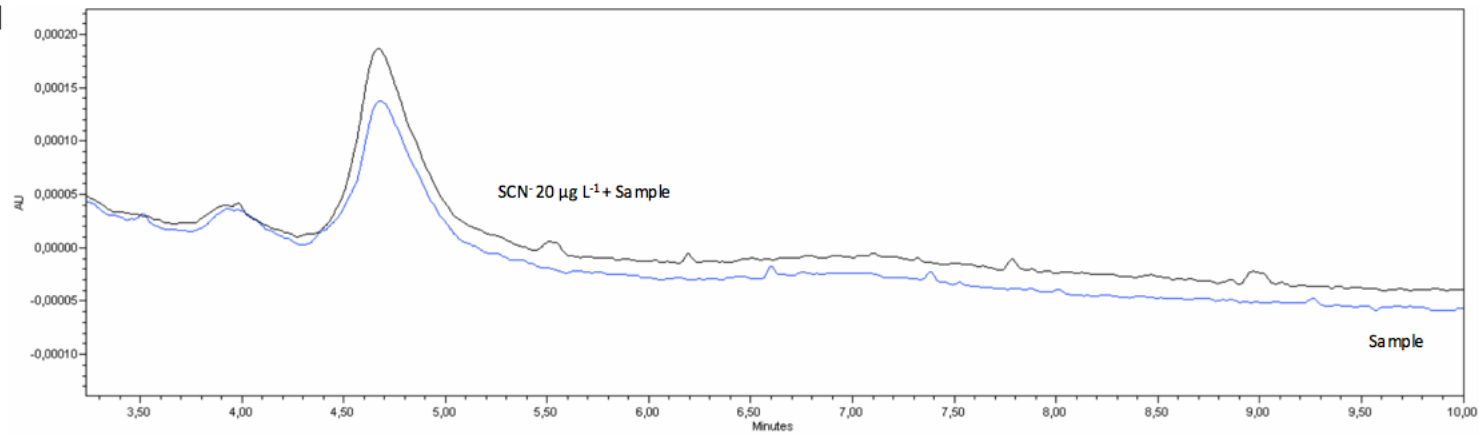




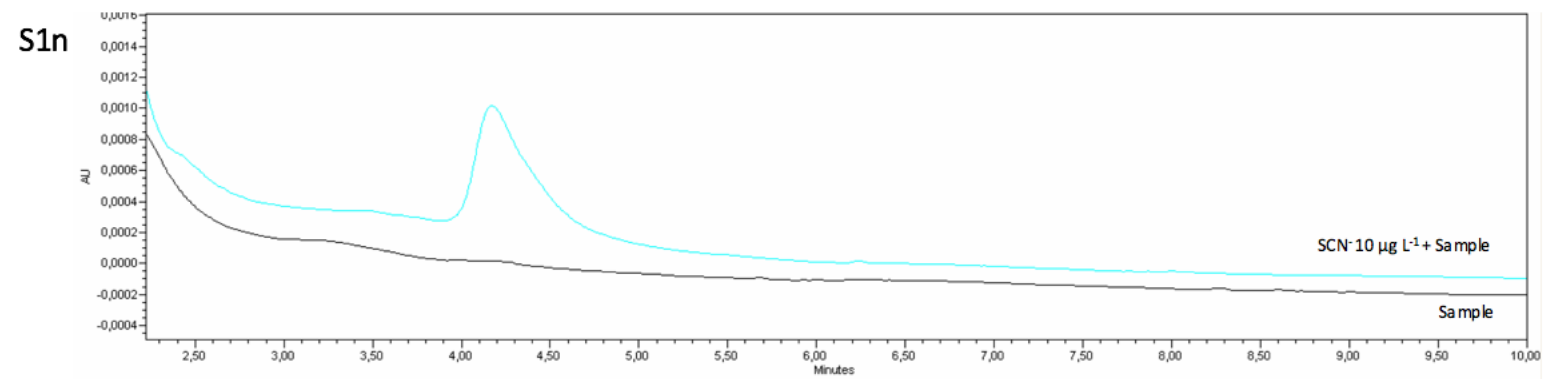
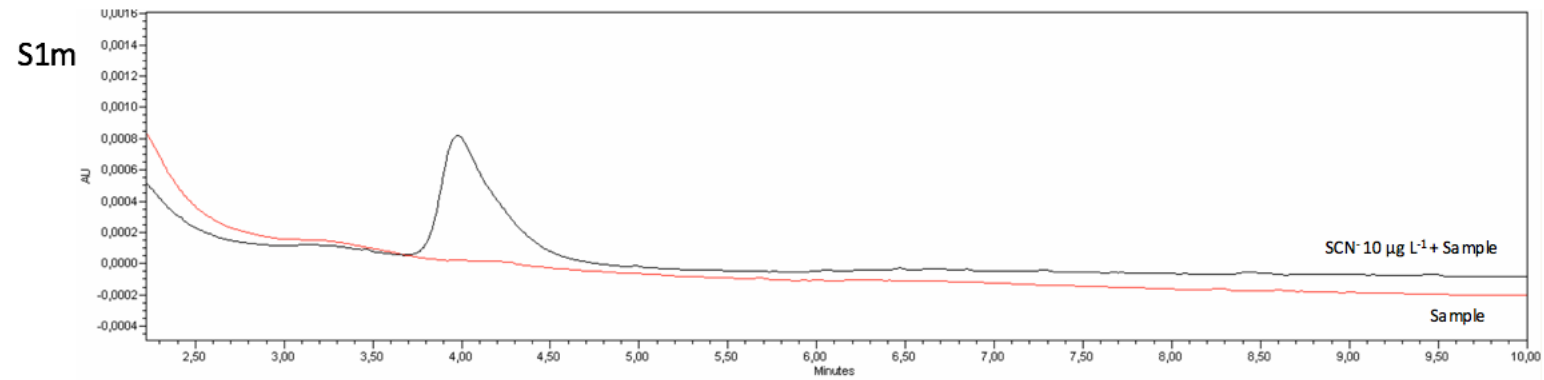
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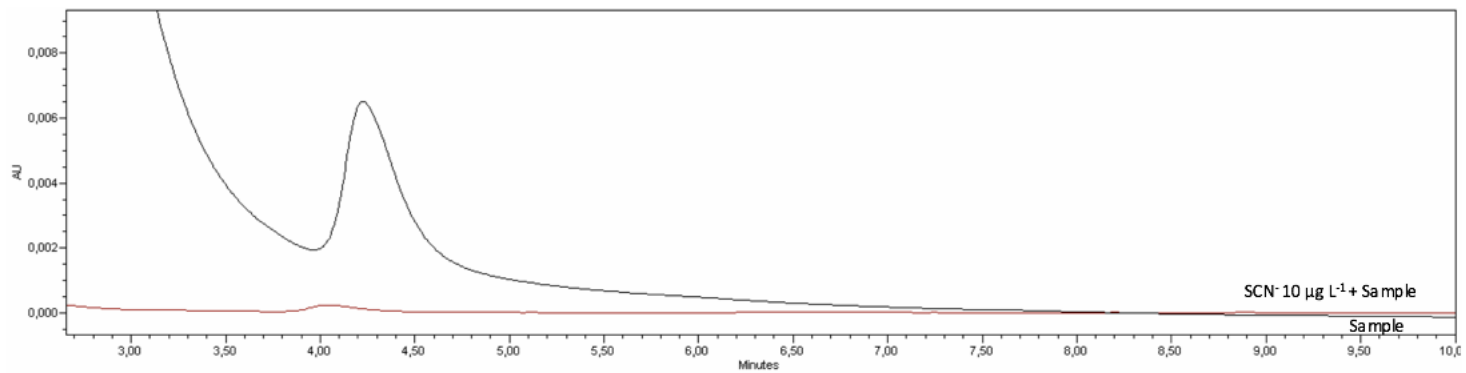
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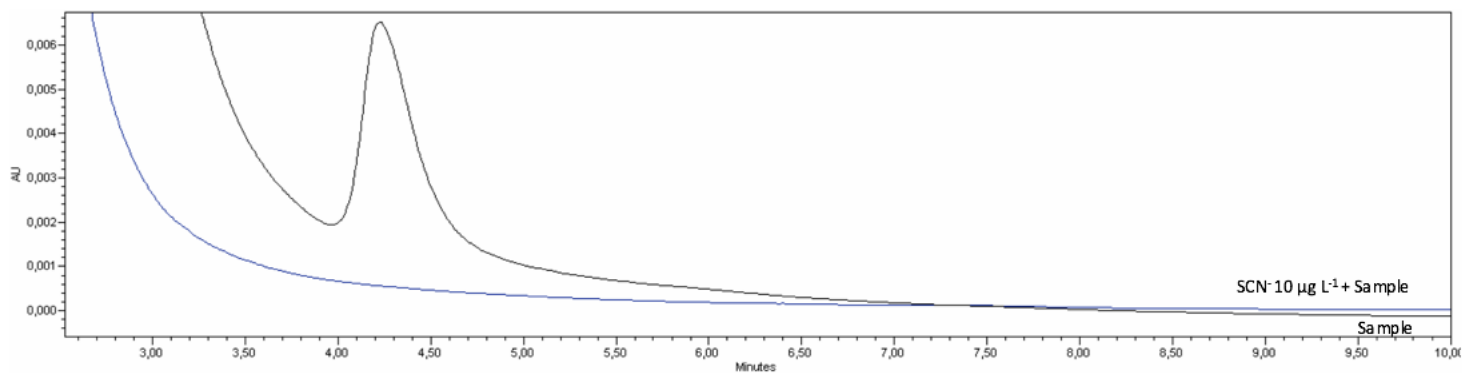
Chromatograms tested negative for SCN^- * For concentrations above $\text{SCN}^- 10 \mu\text{g L}^{-1}$



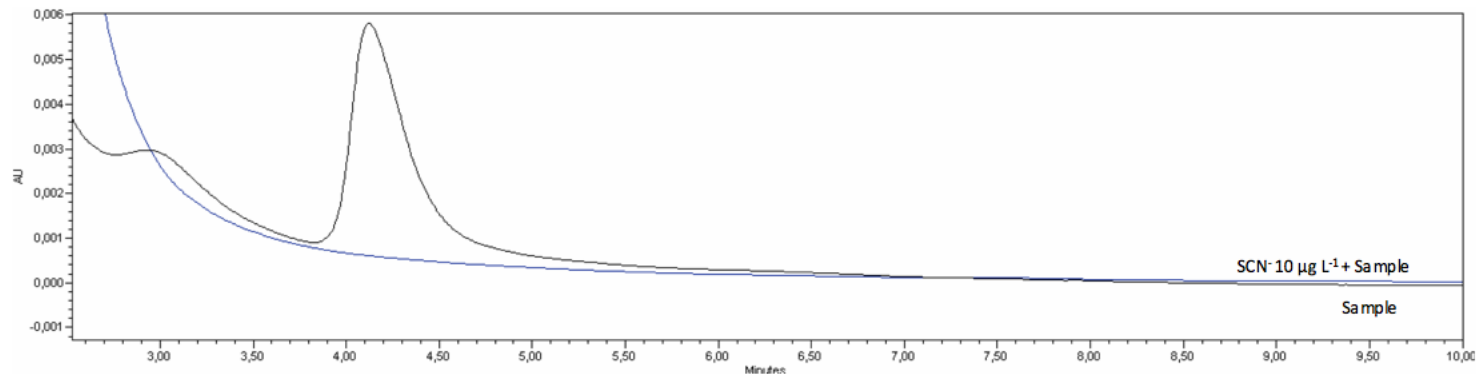
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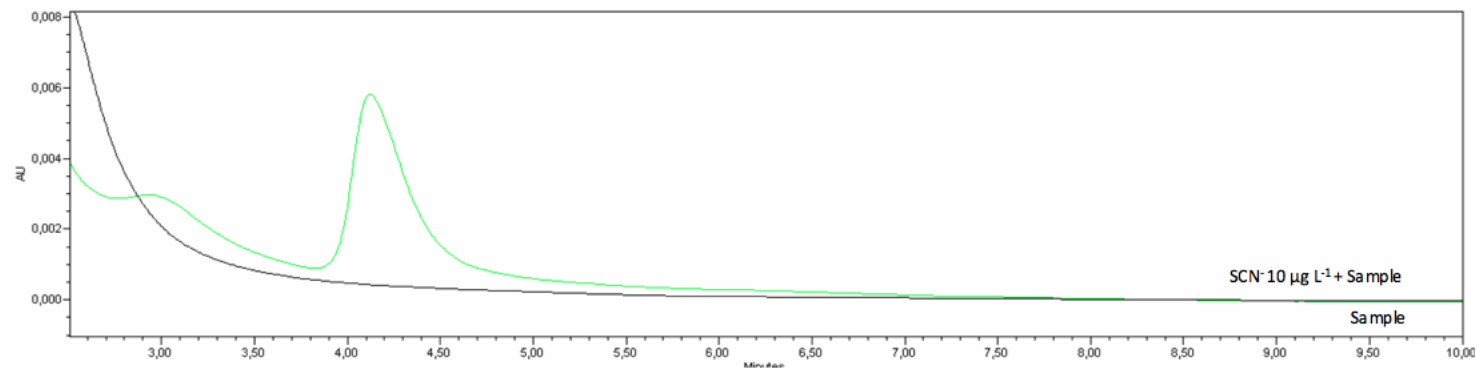
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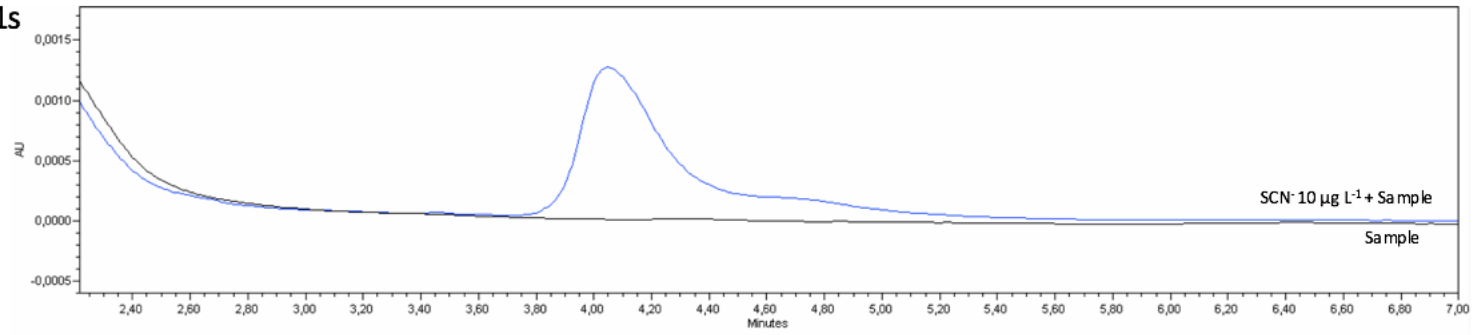
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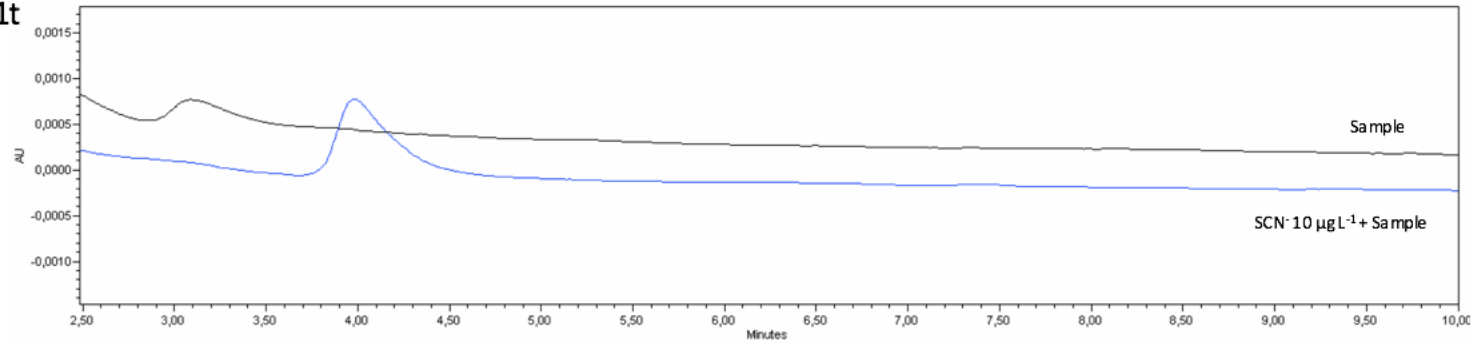
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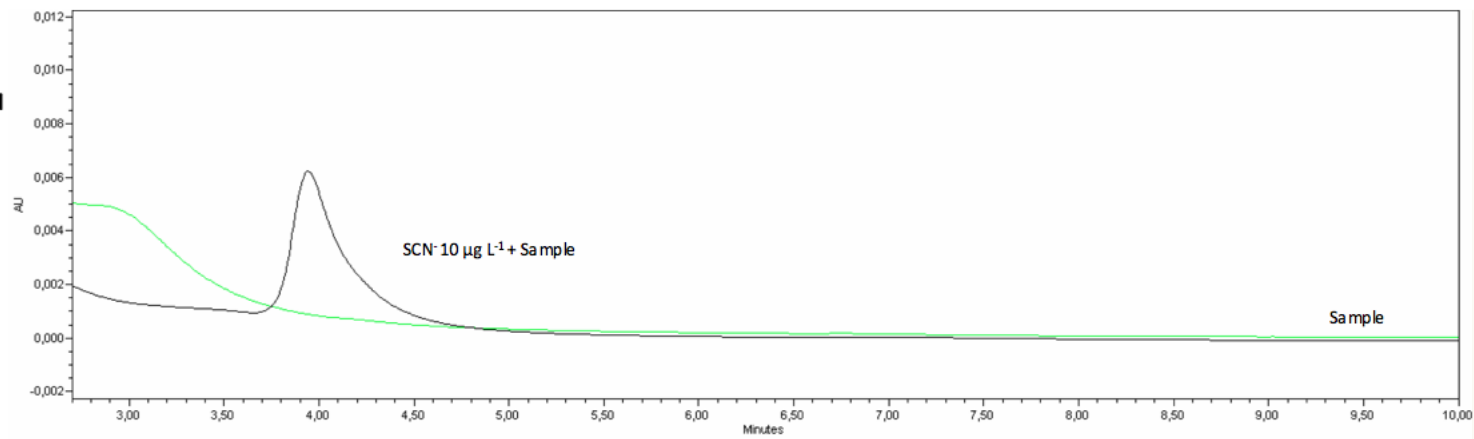
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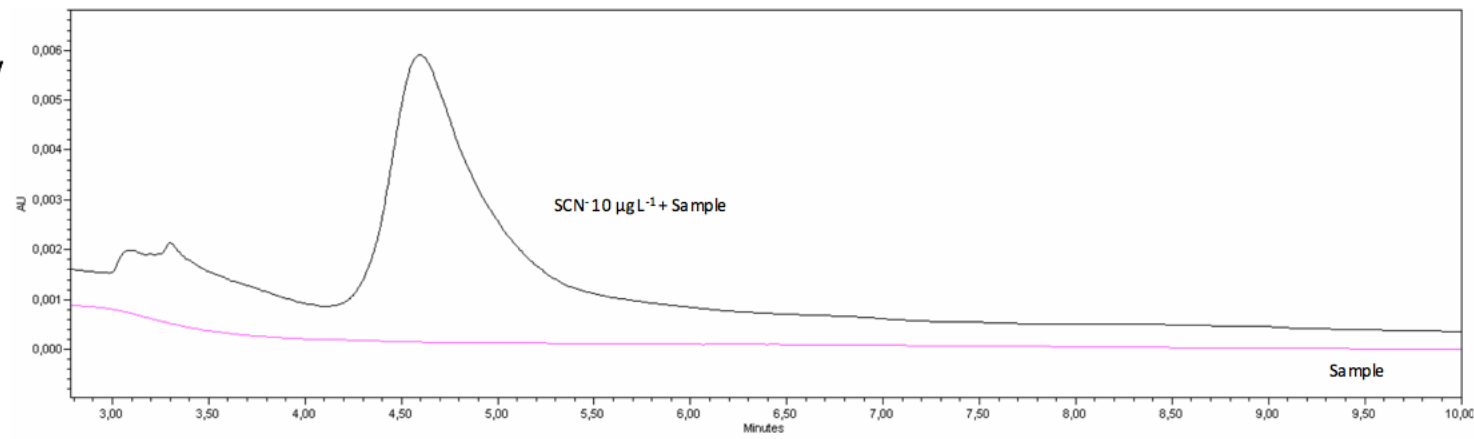
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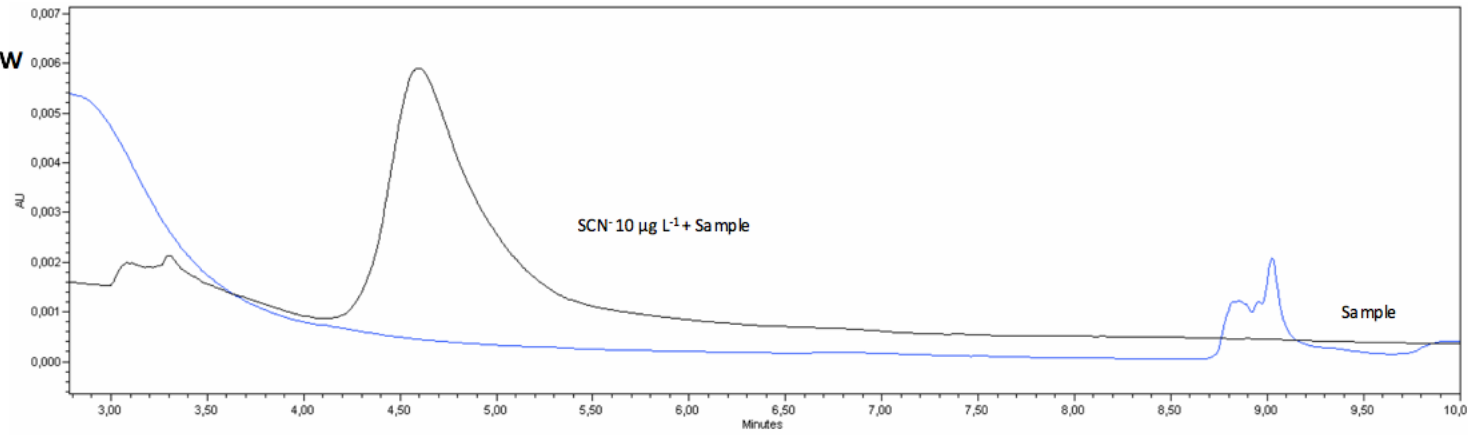
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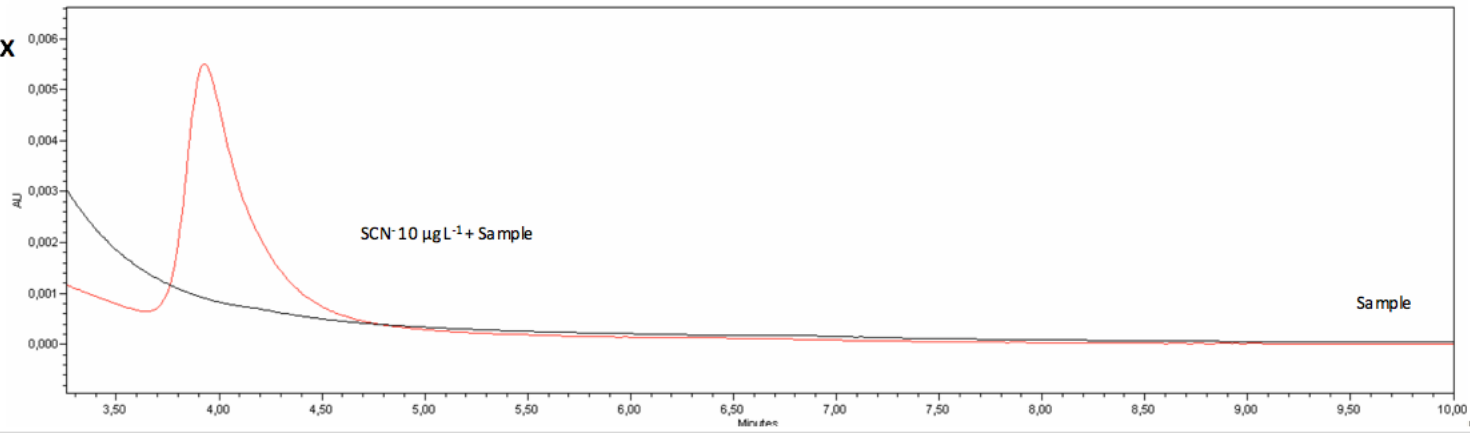
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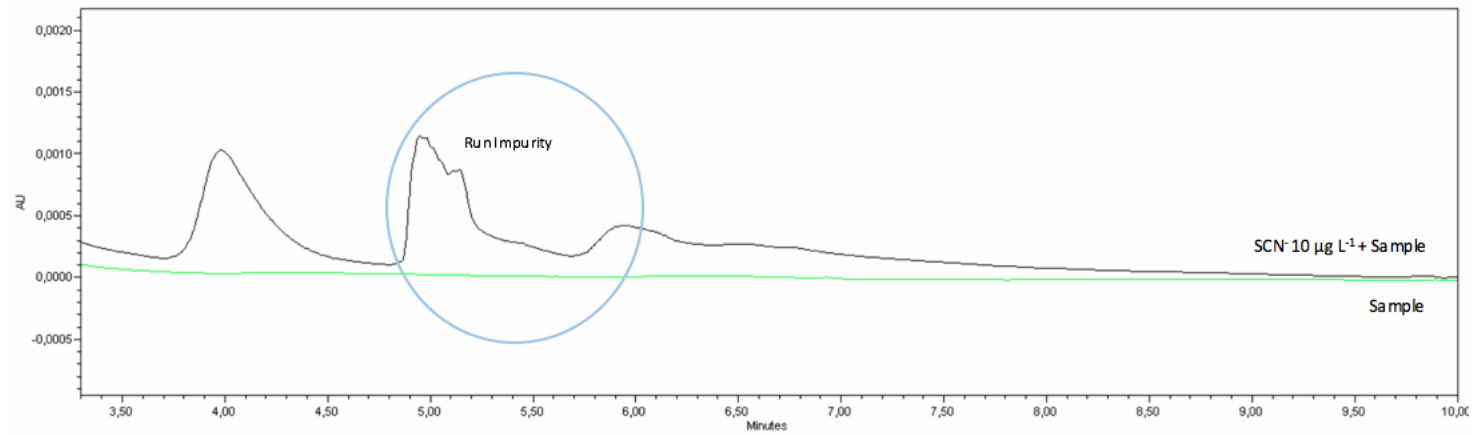
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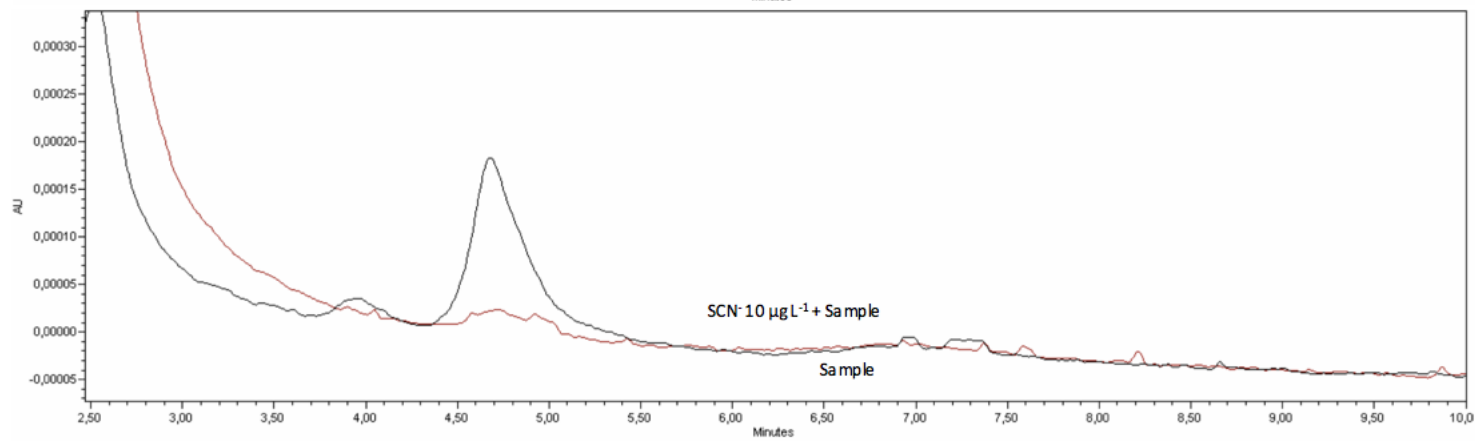
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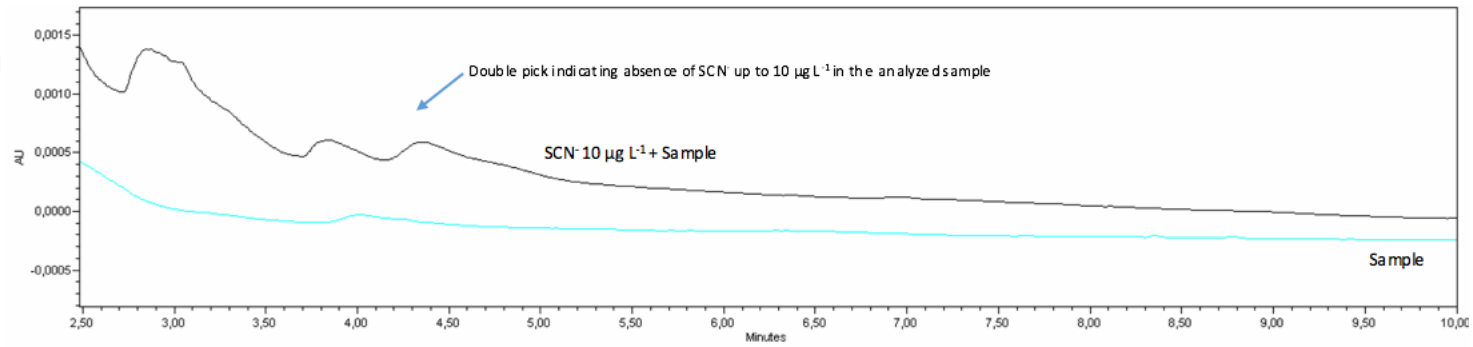
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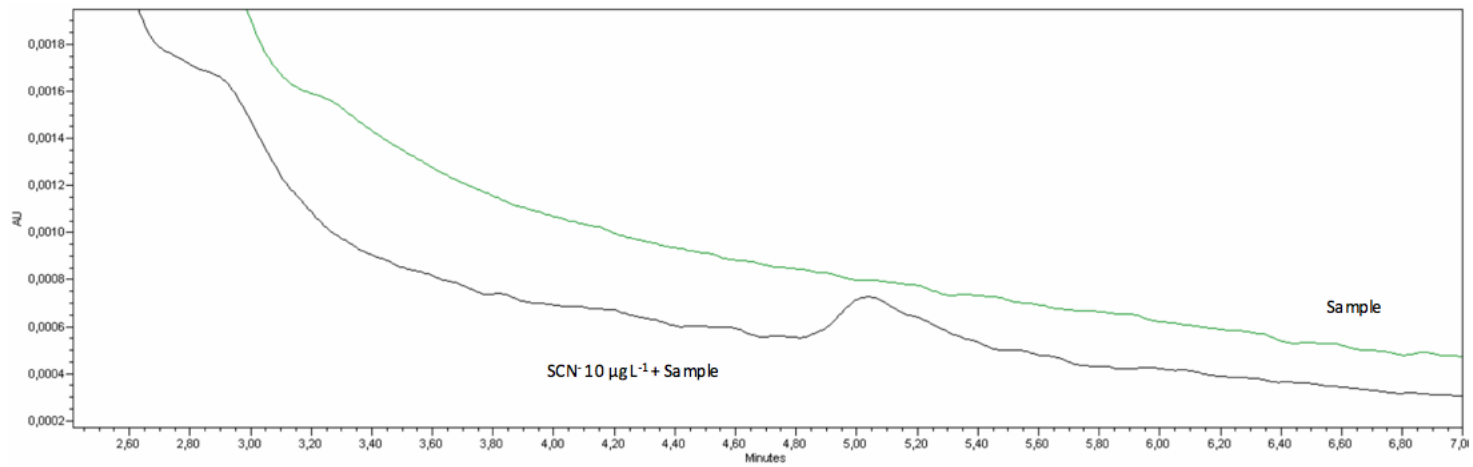
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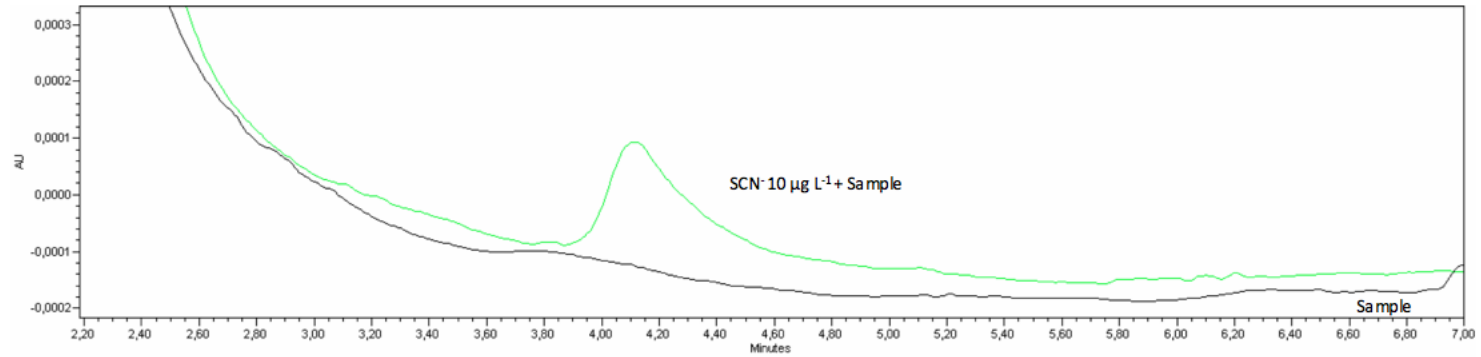
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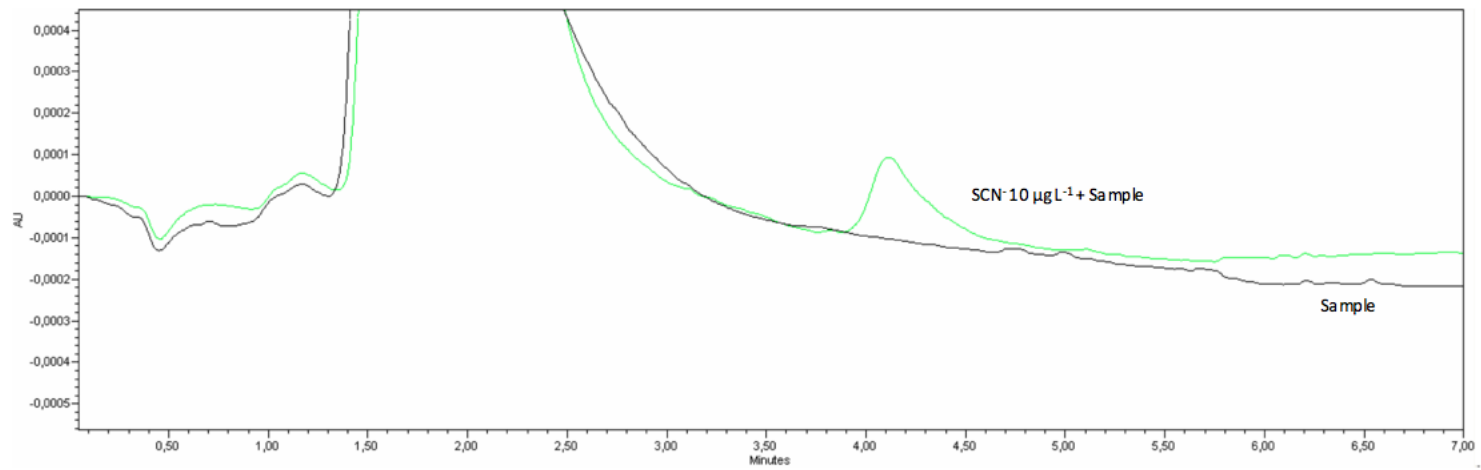
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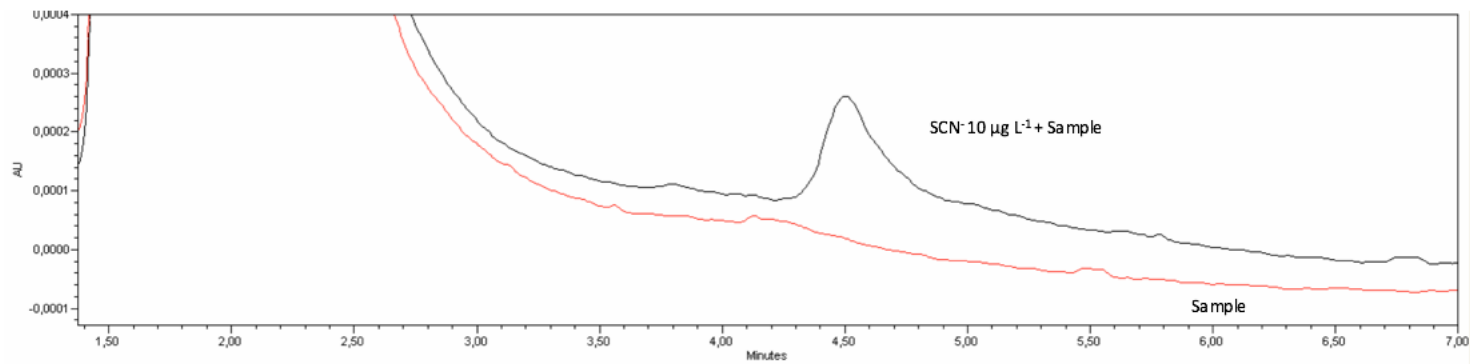


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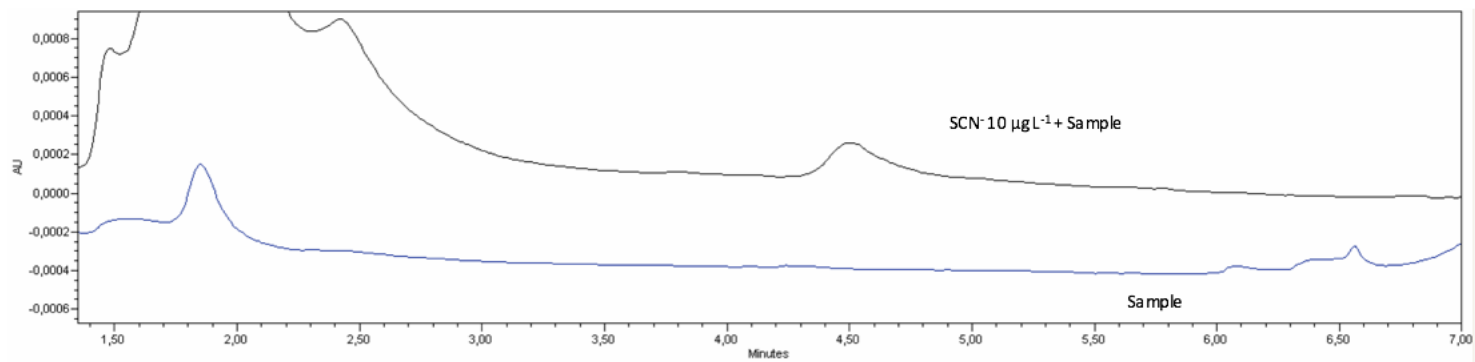




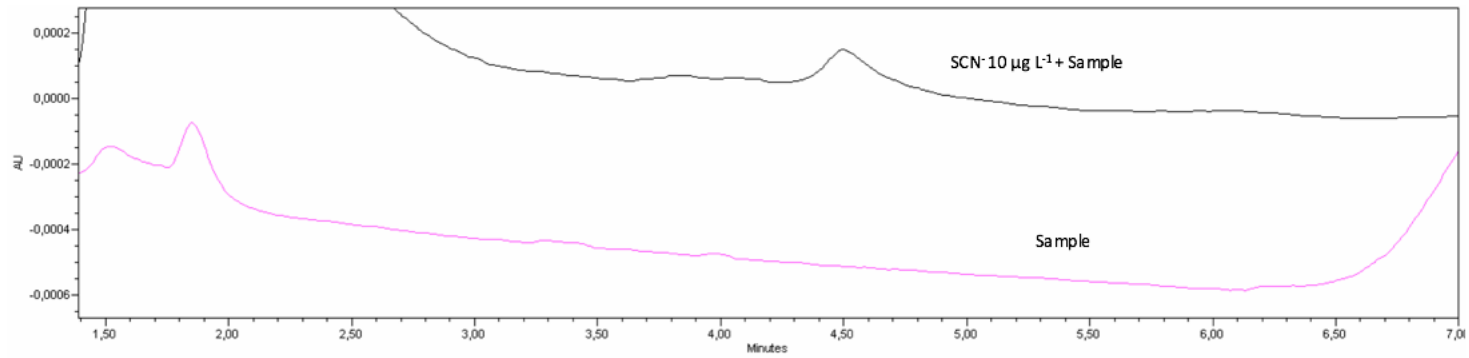
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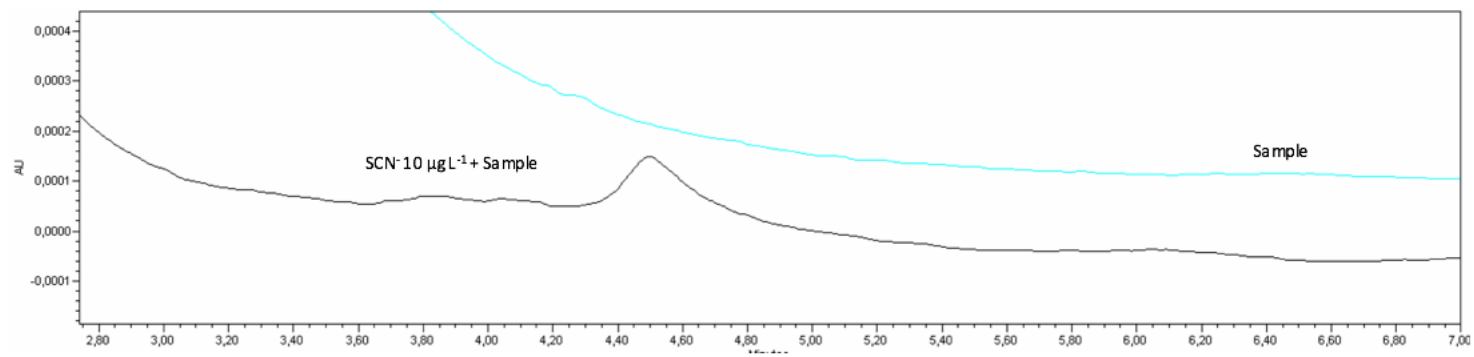
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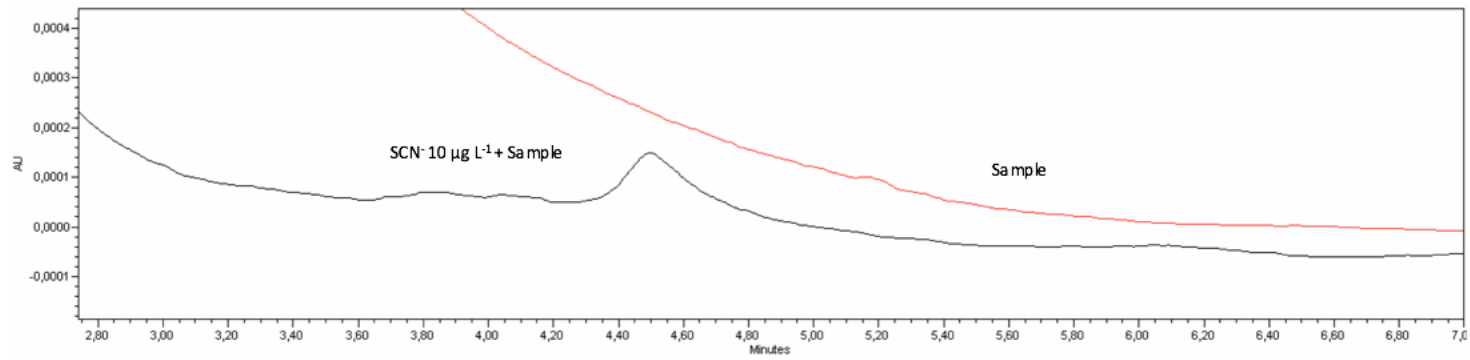
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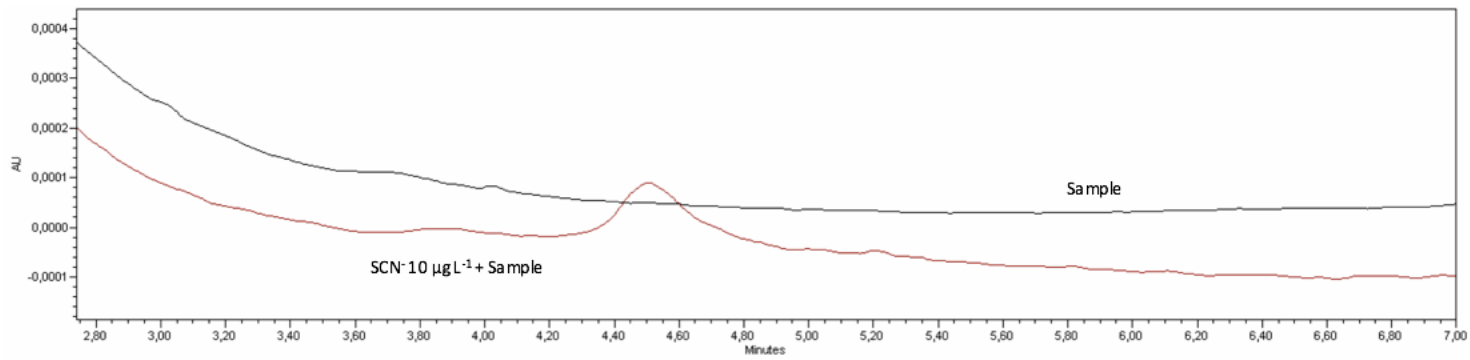
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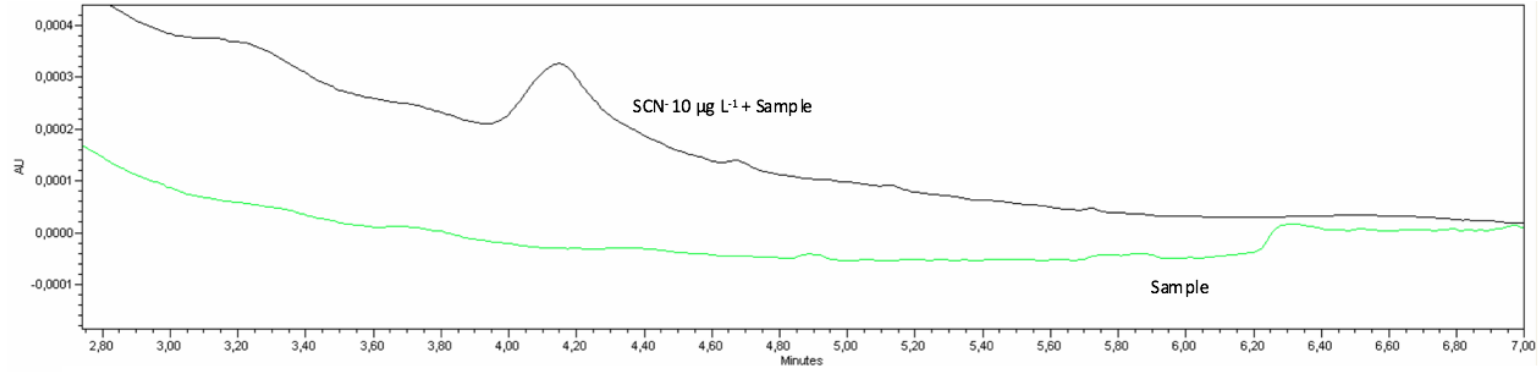
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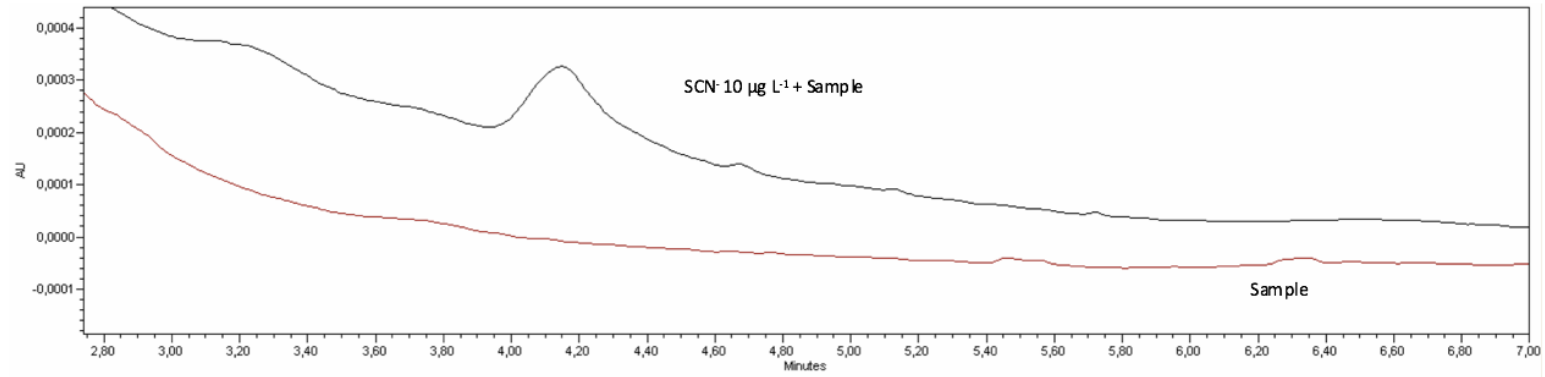
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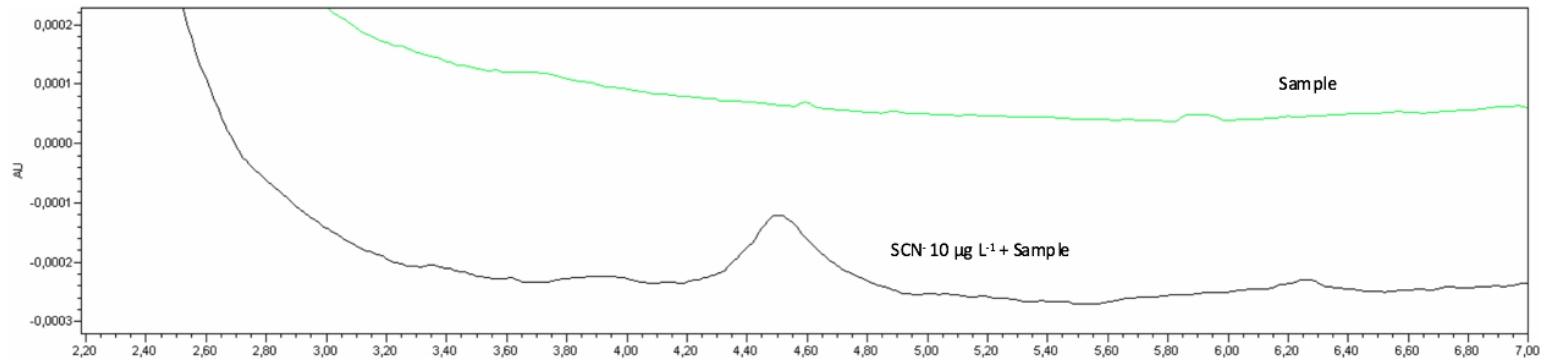


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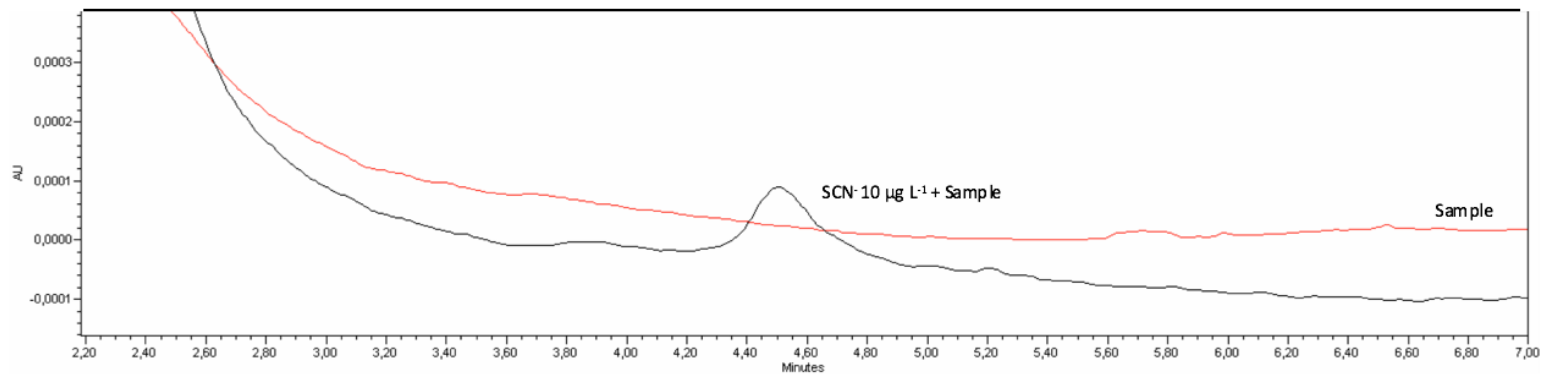




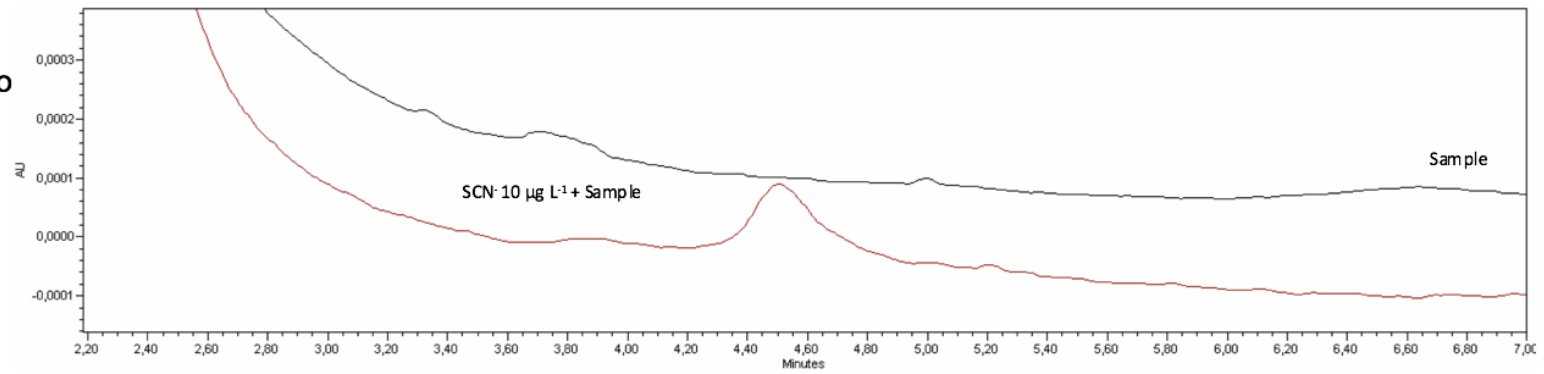
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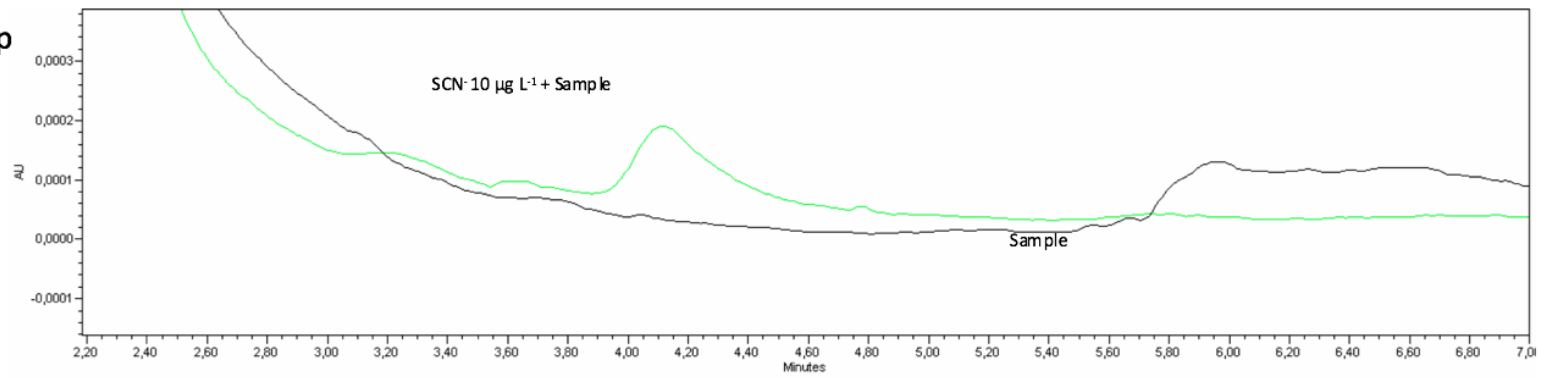
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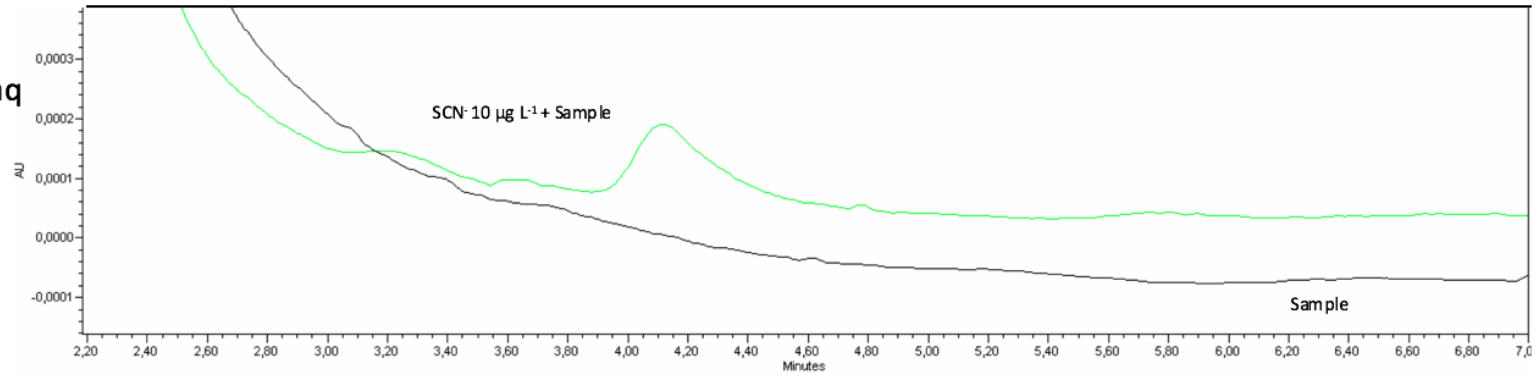
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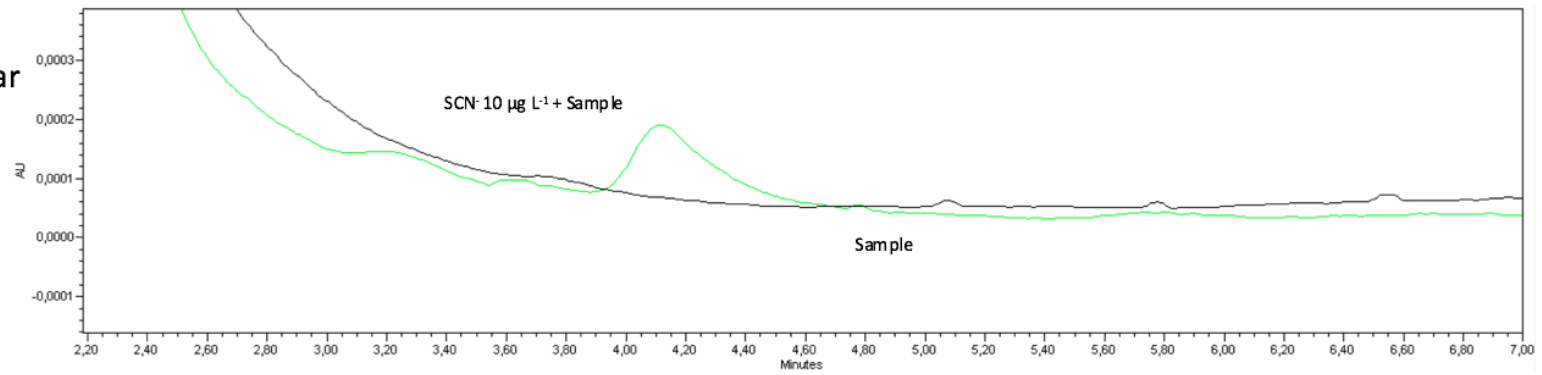
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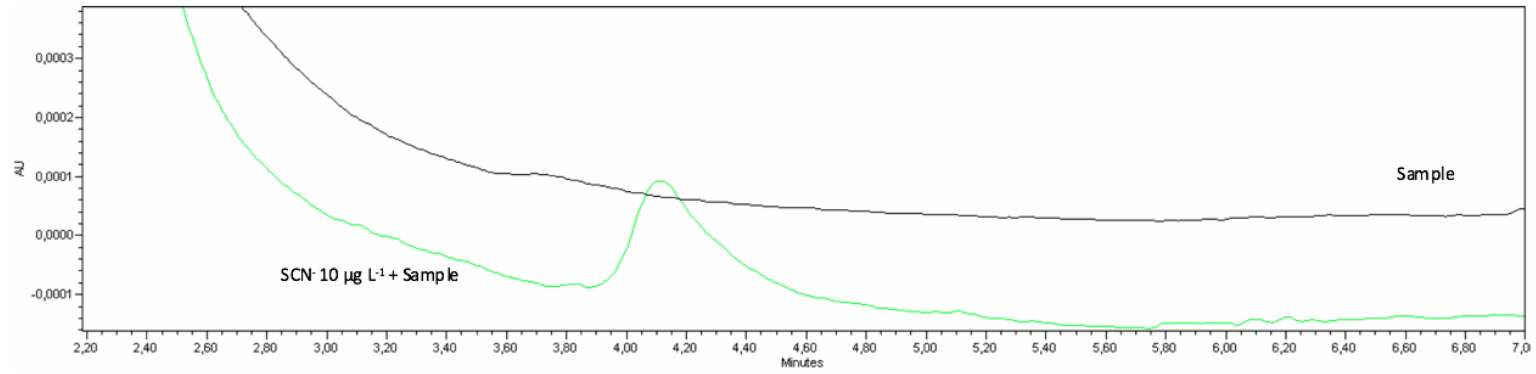
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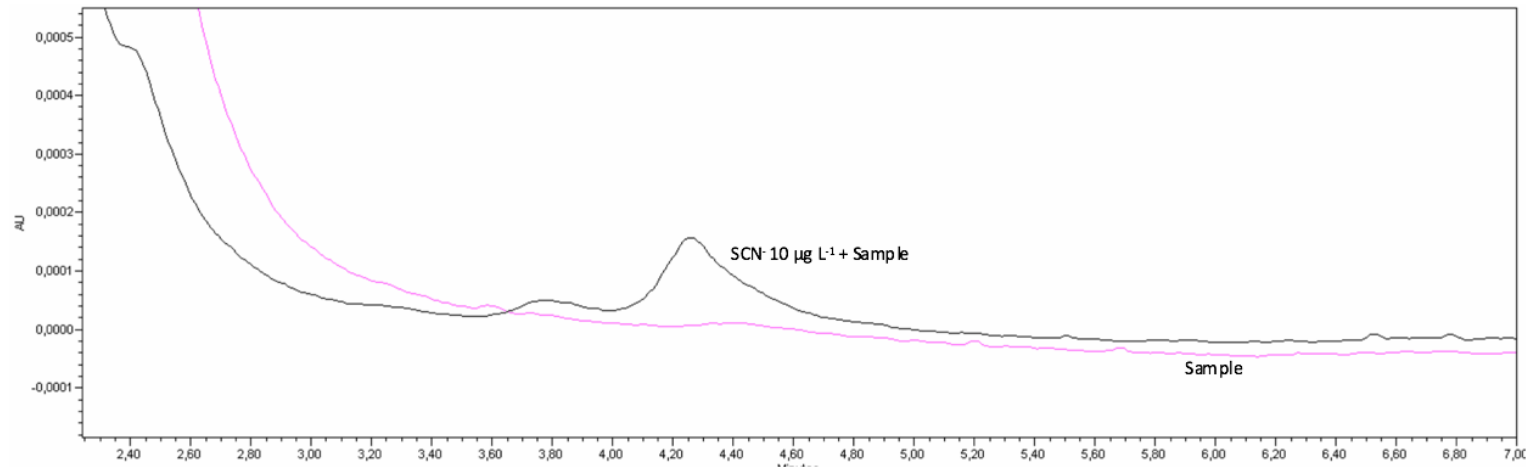
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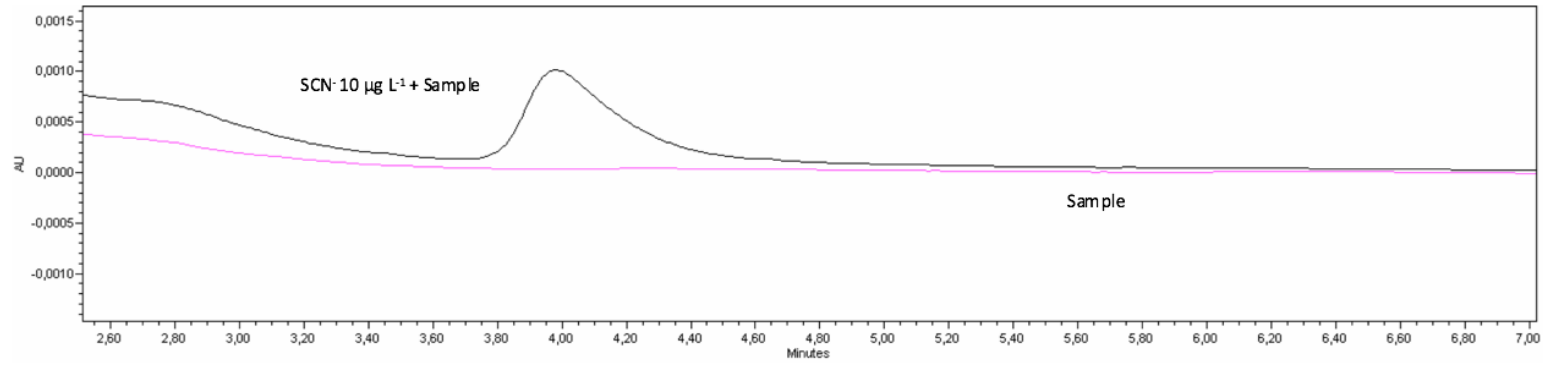
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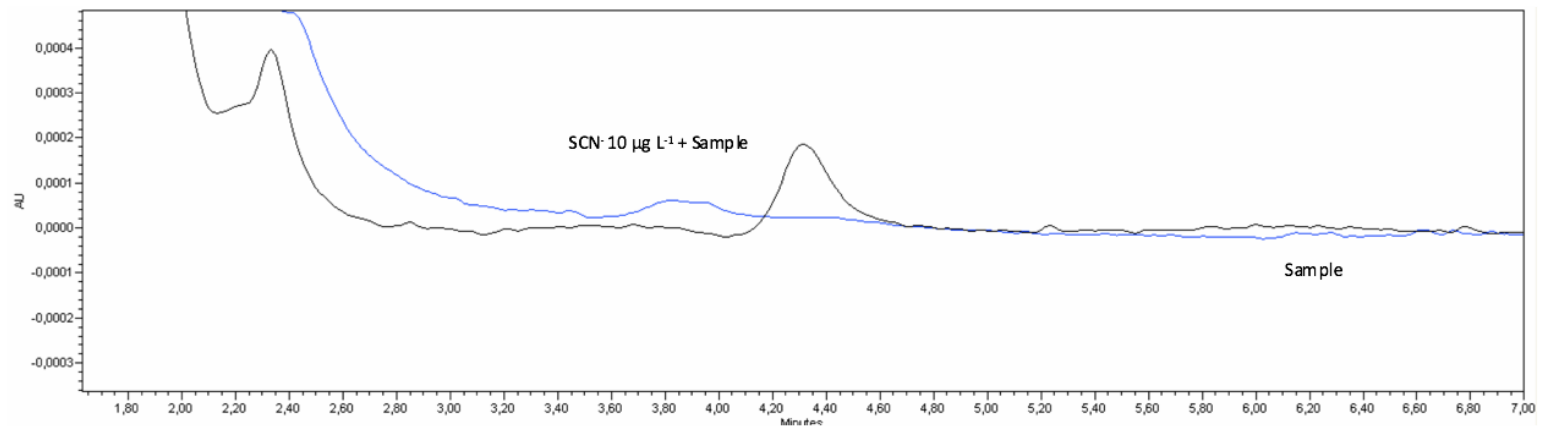
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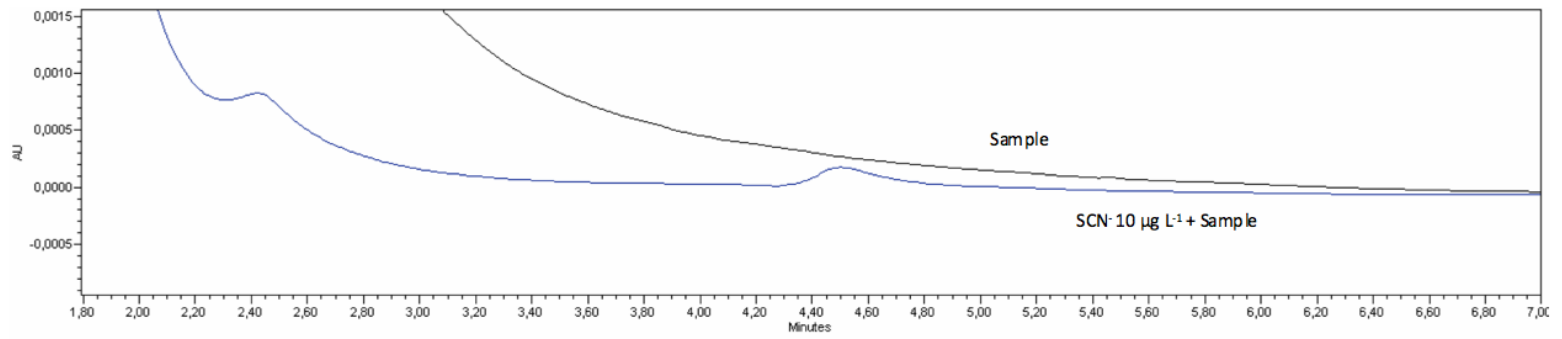


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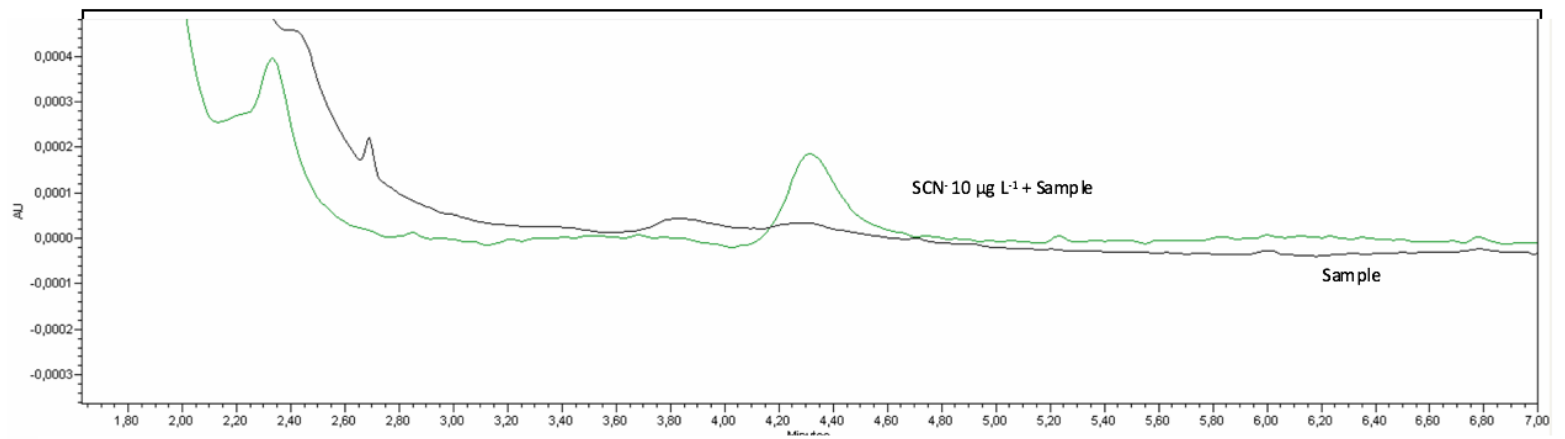




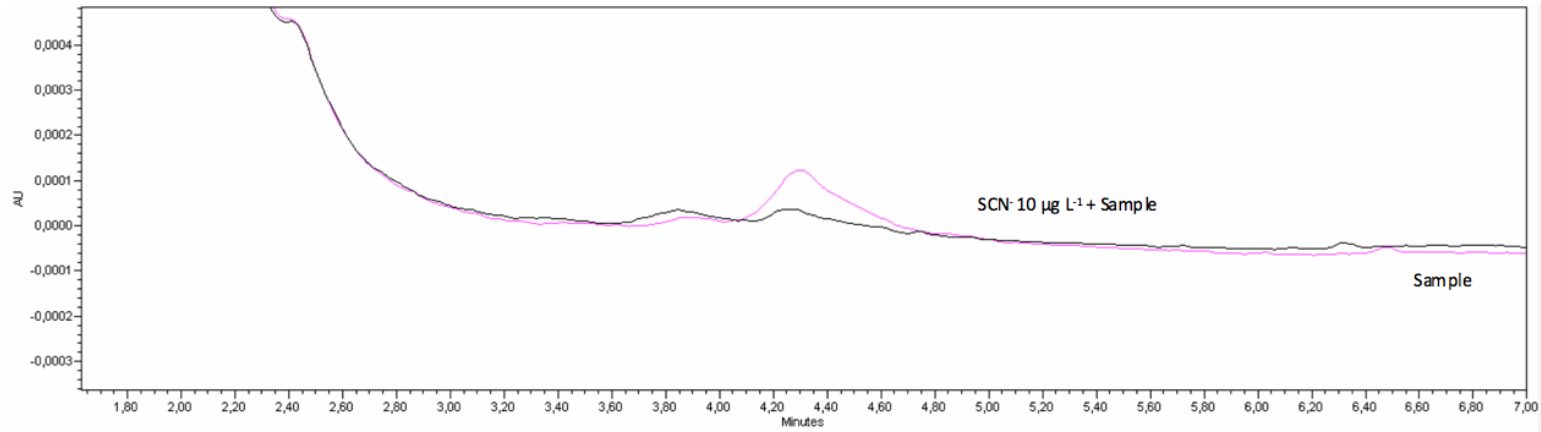
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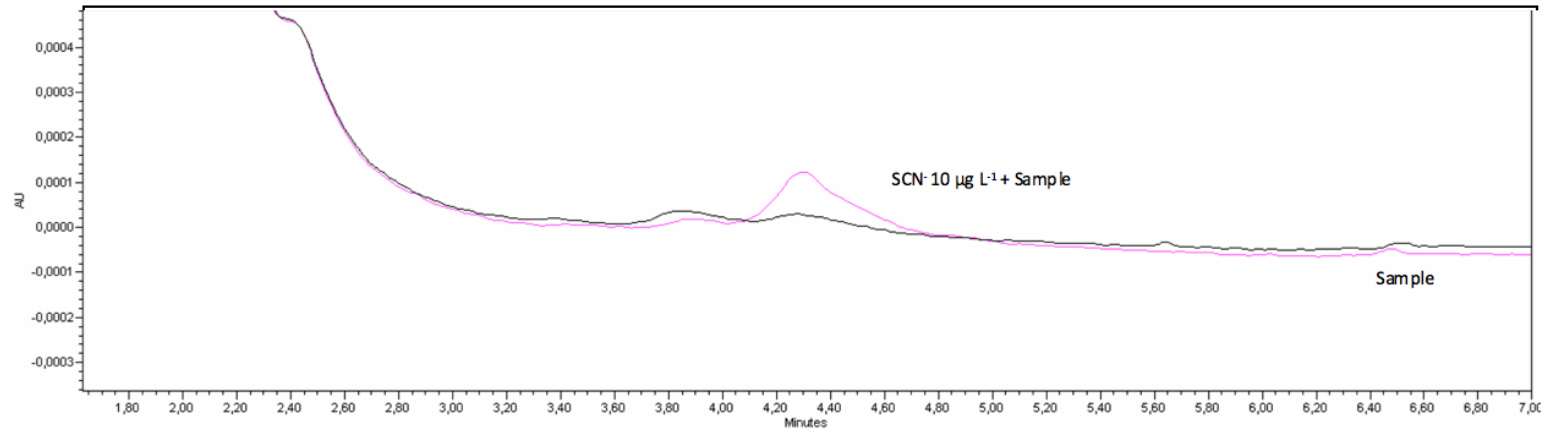
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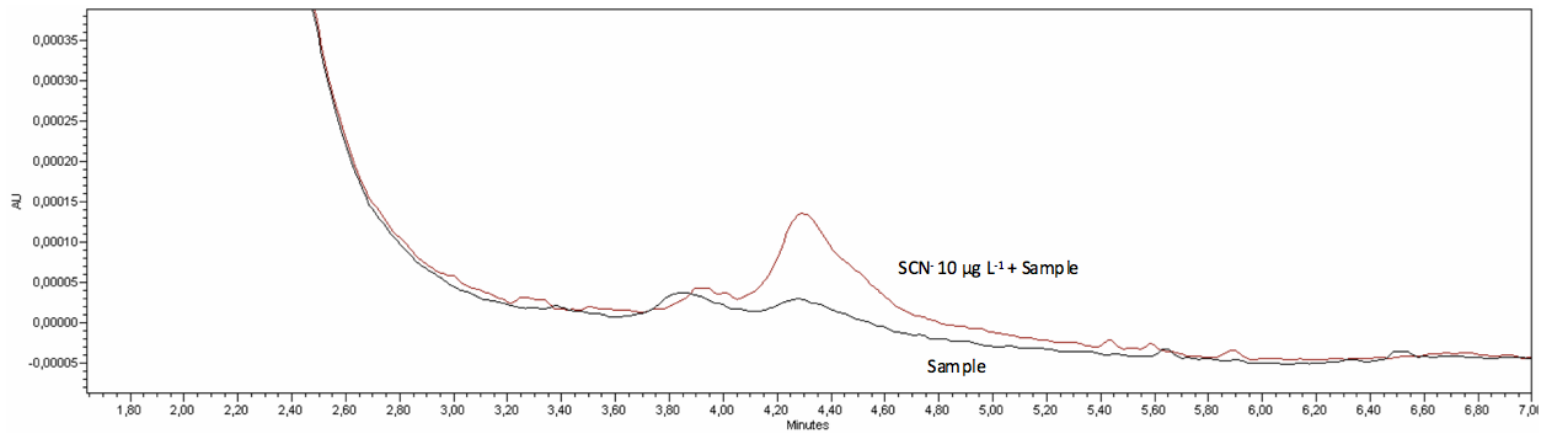


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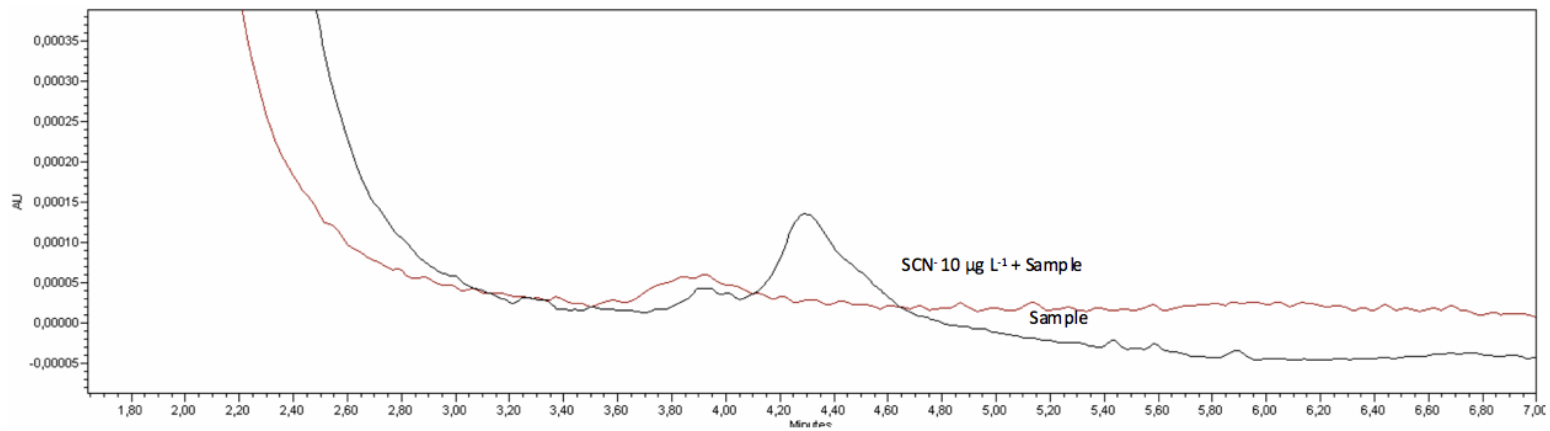




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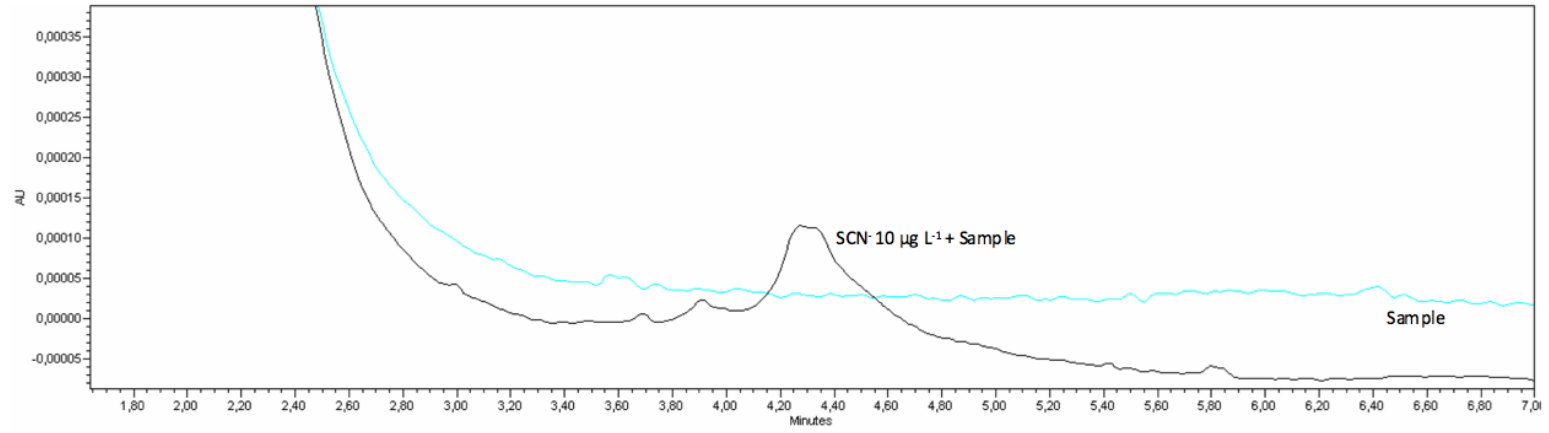


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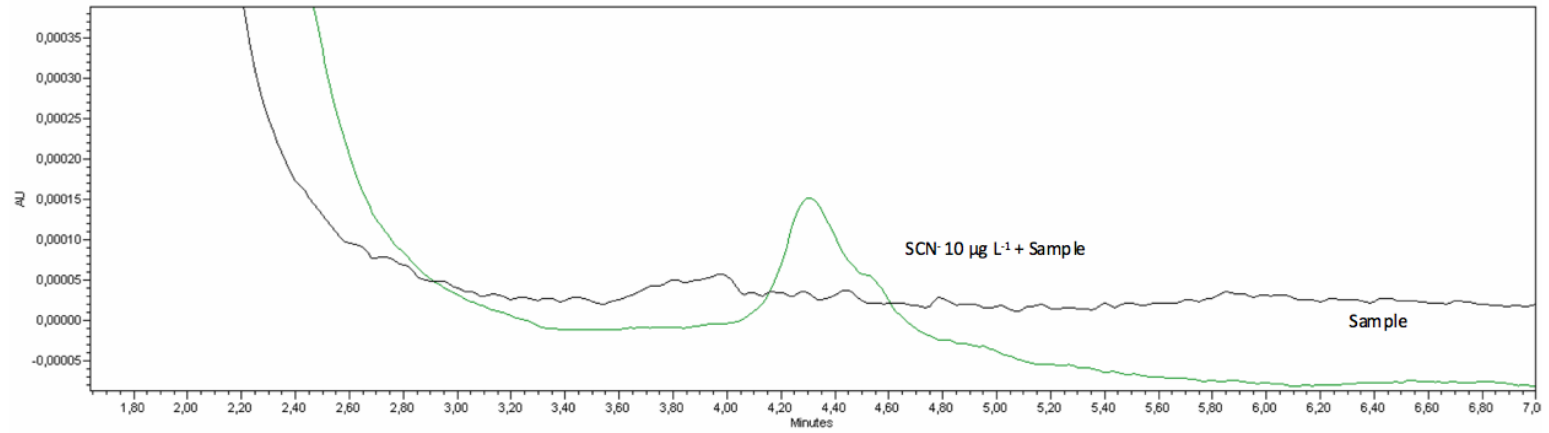




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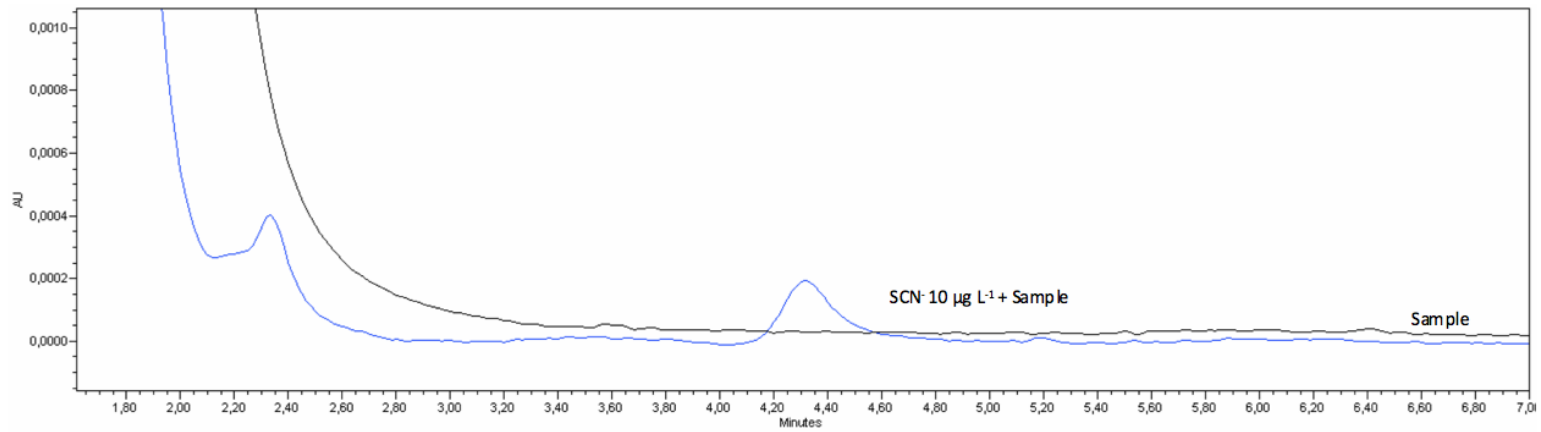


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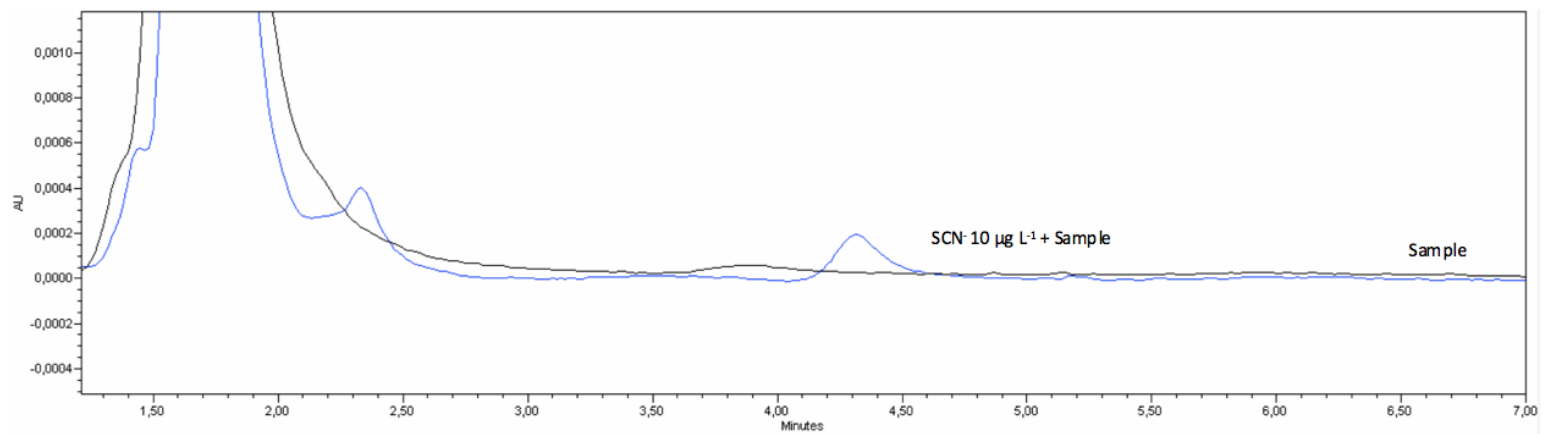




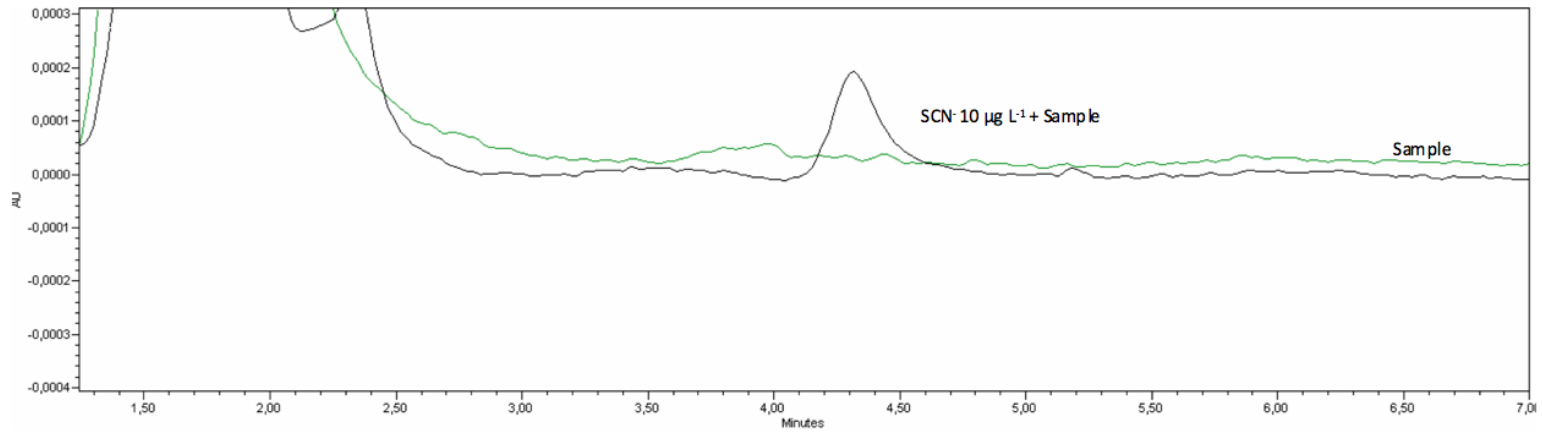
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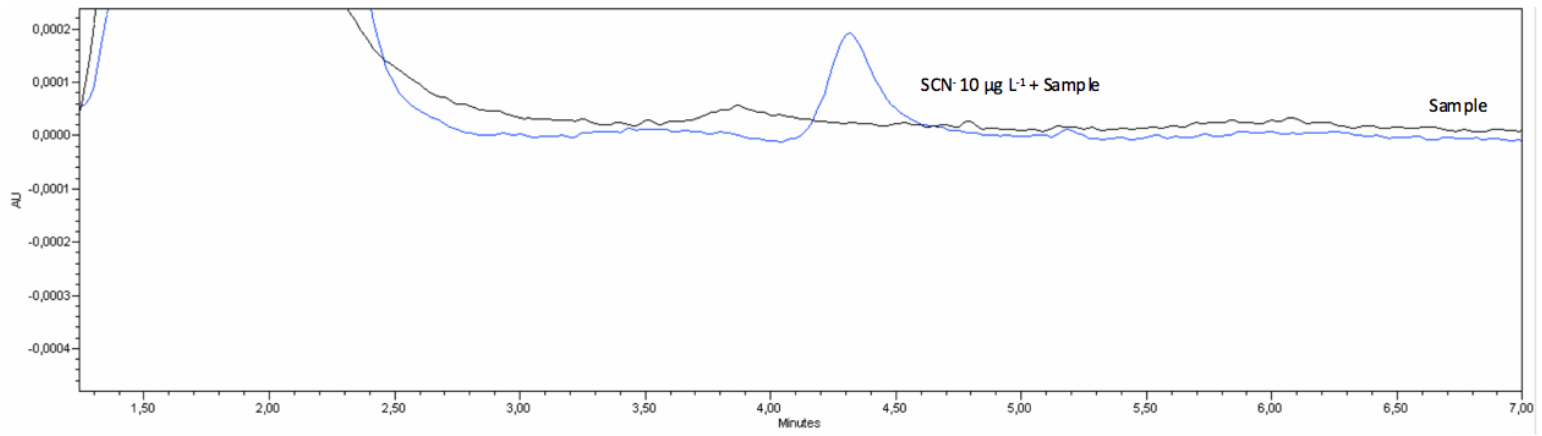
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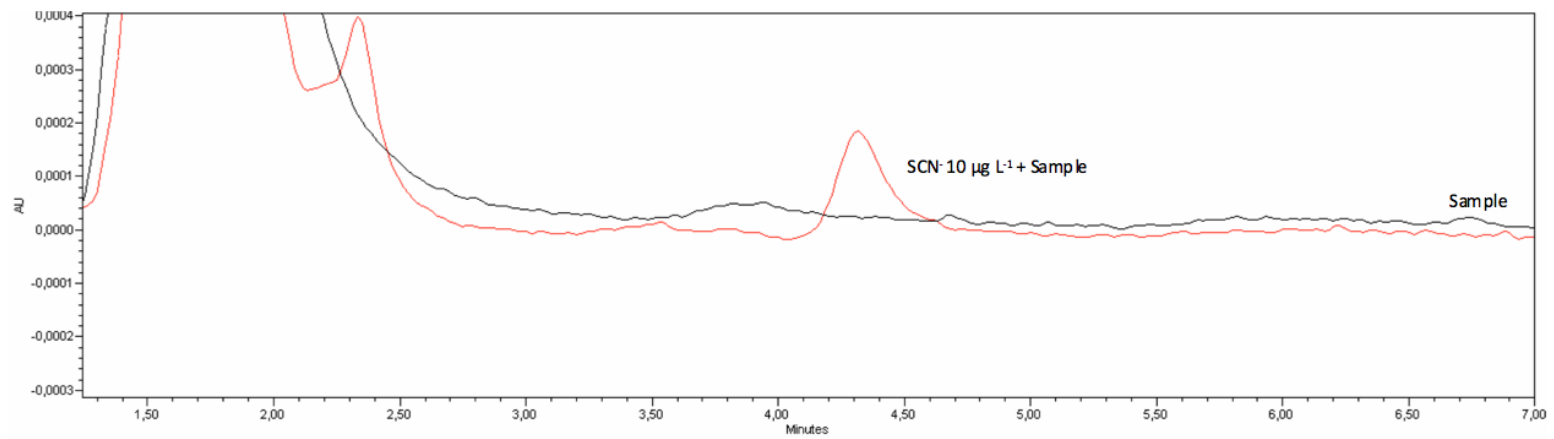


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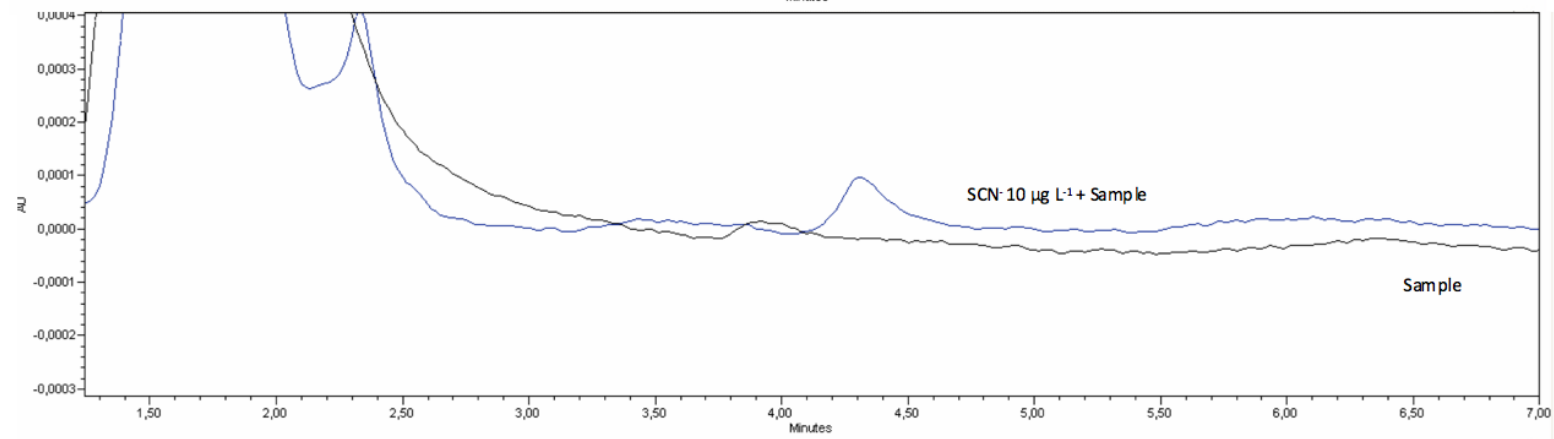




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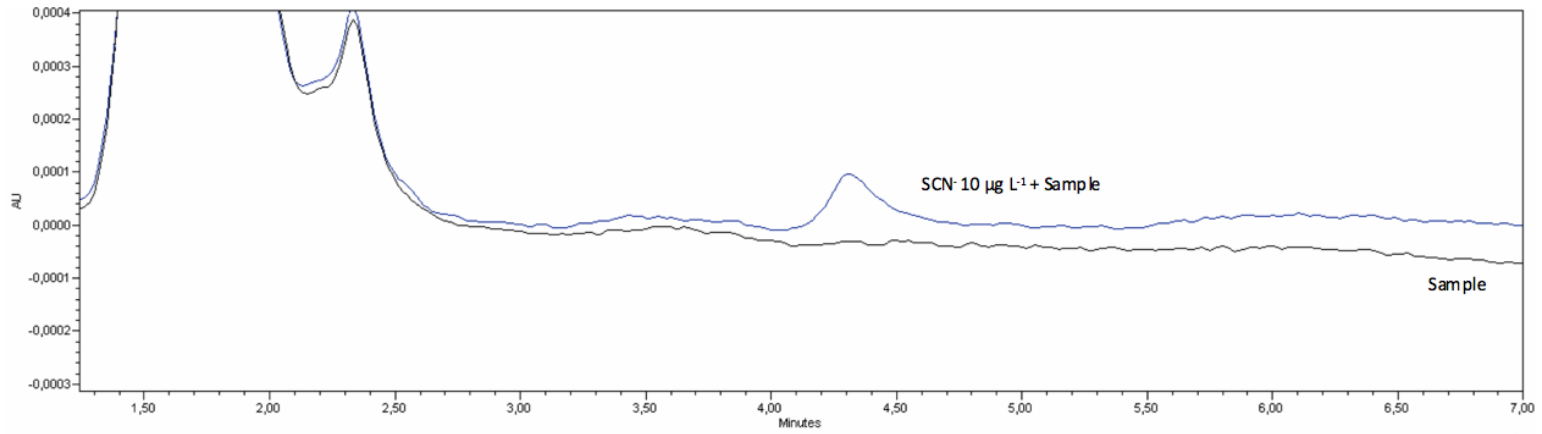


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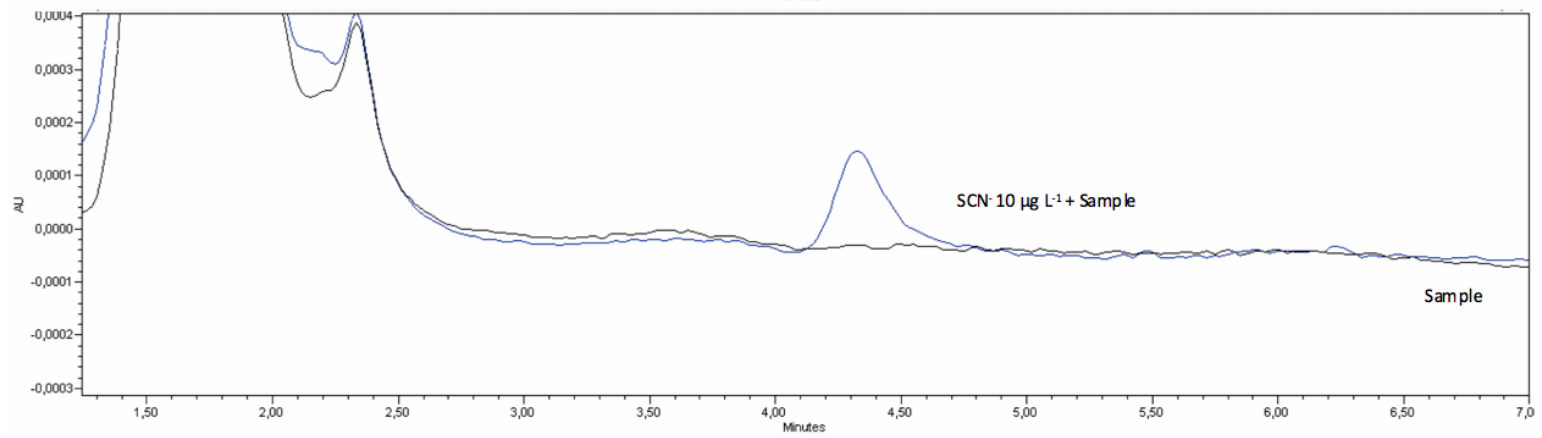




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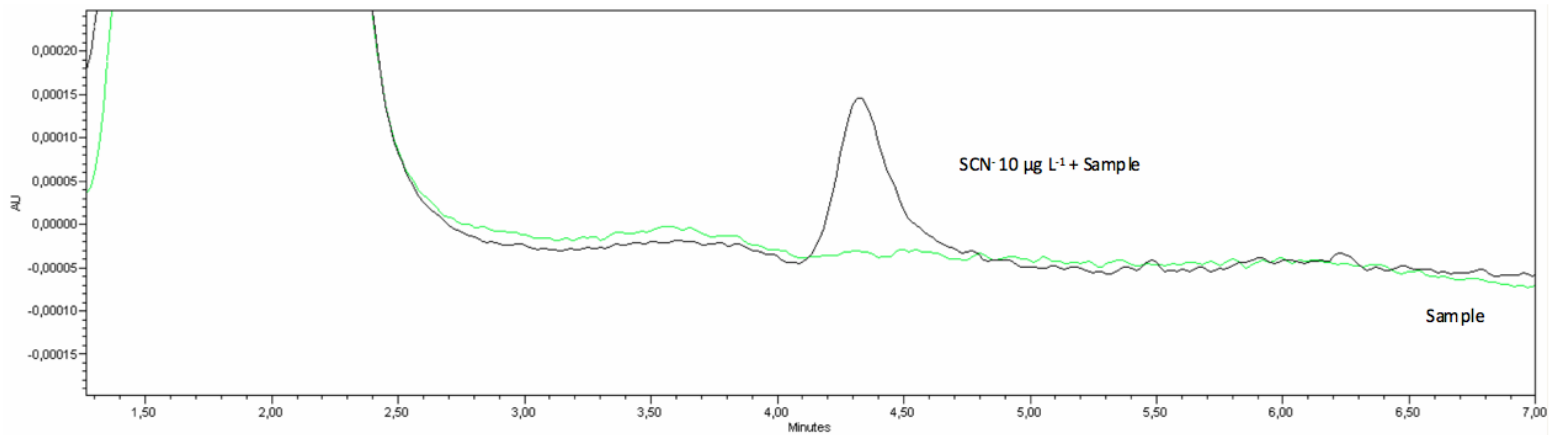


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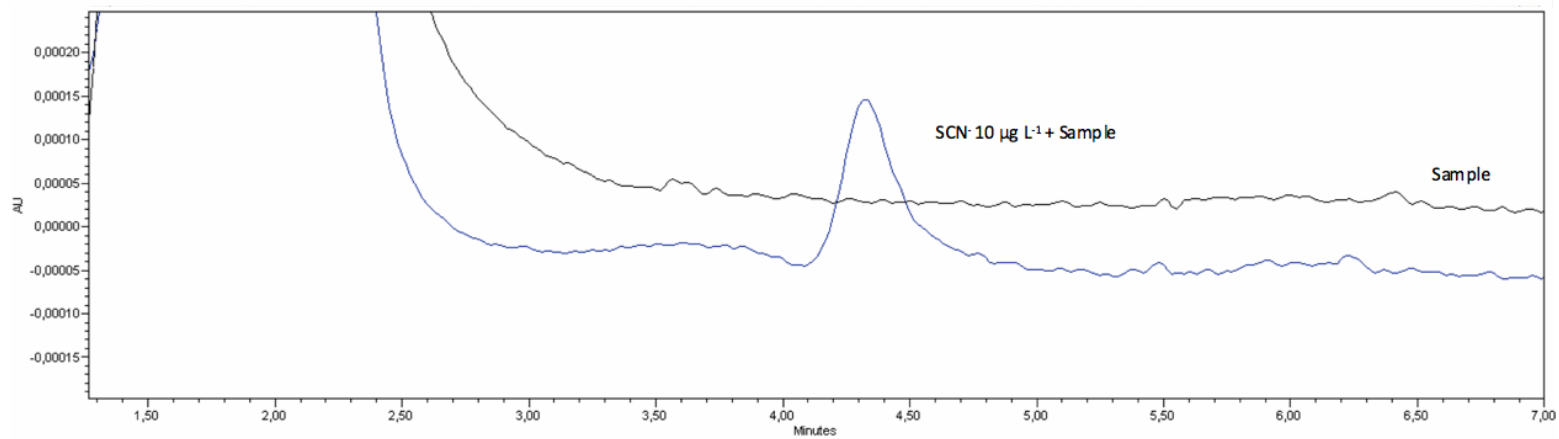




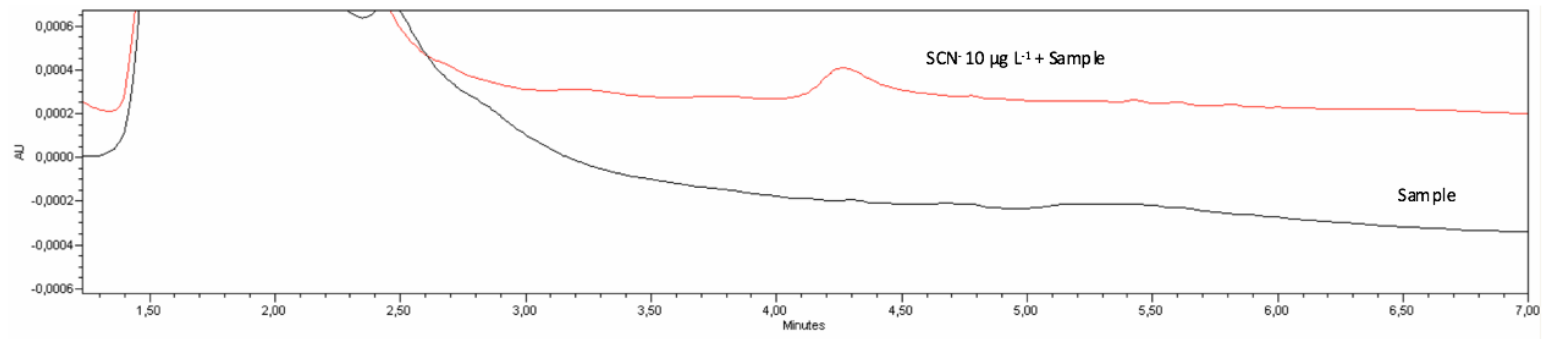
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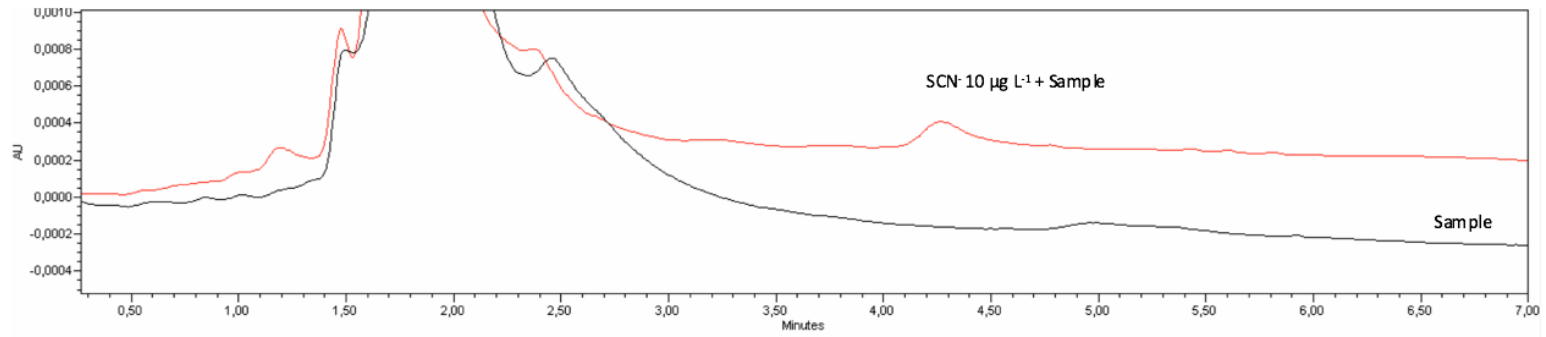
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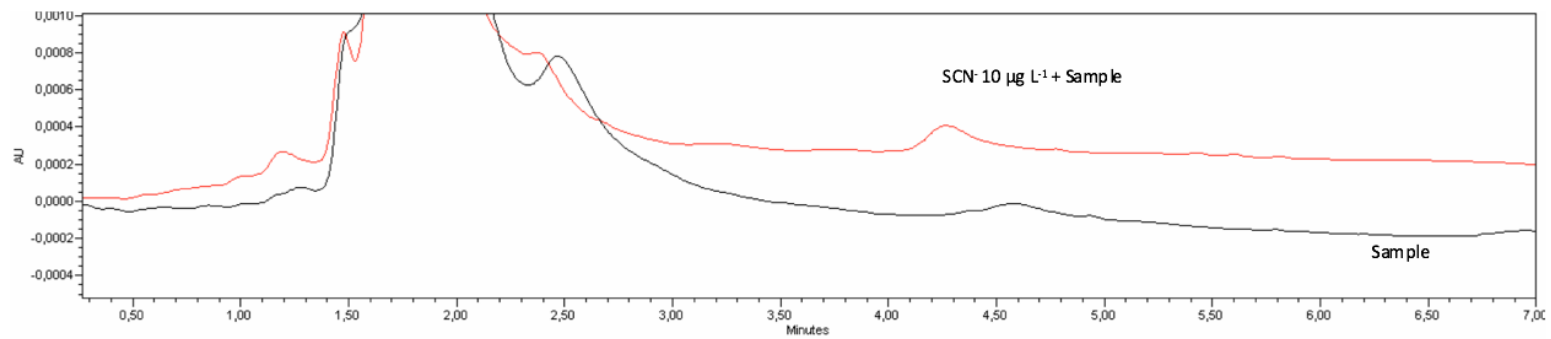


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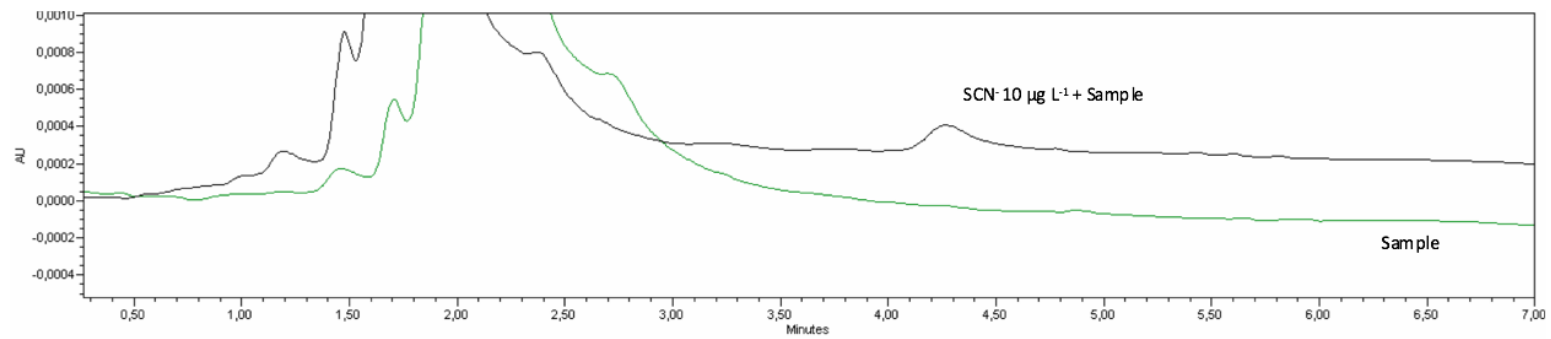




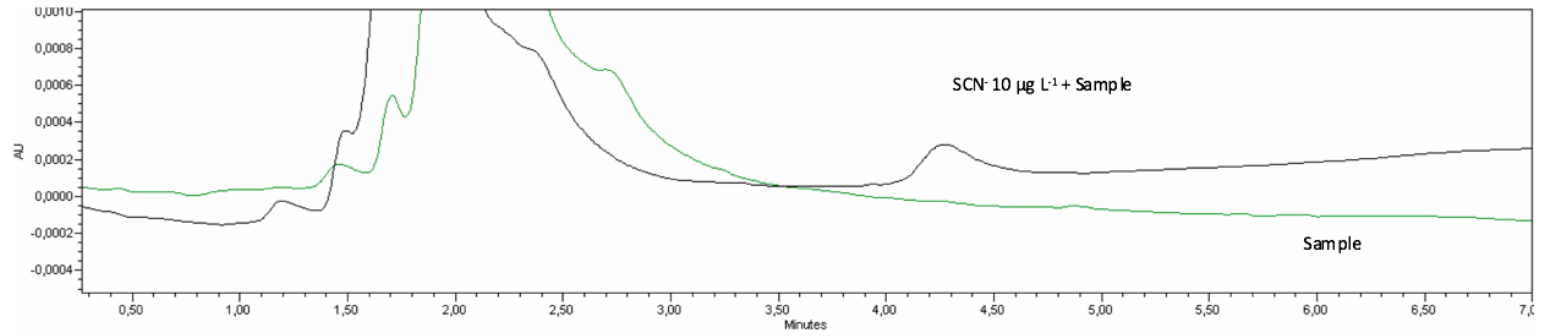
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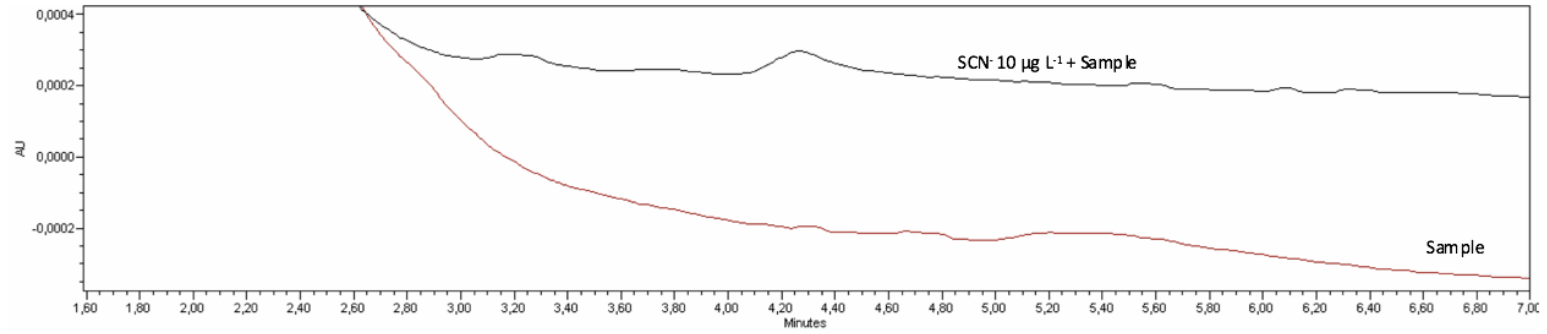
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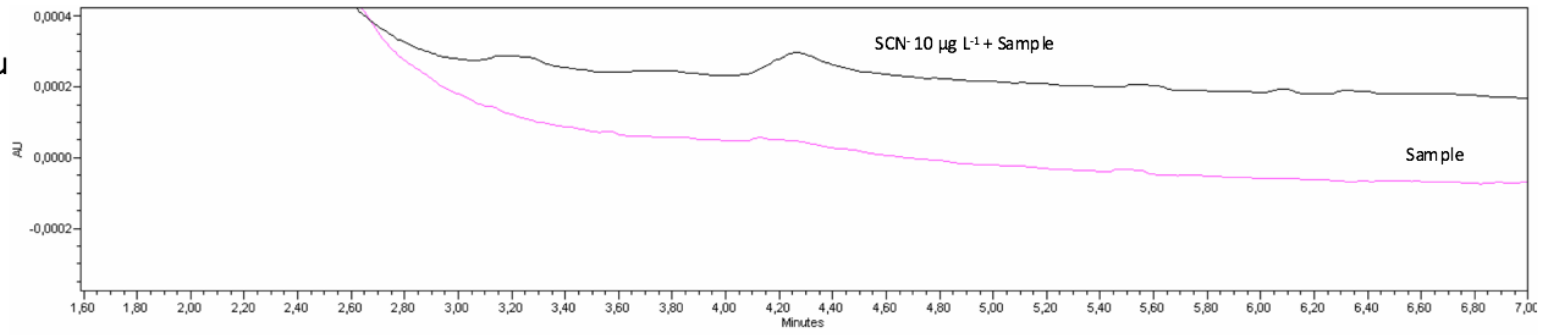
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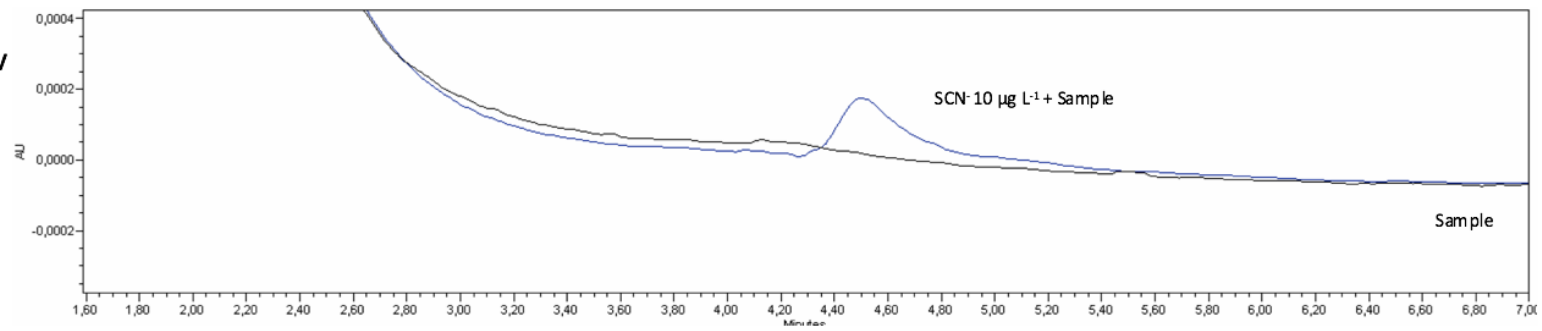
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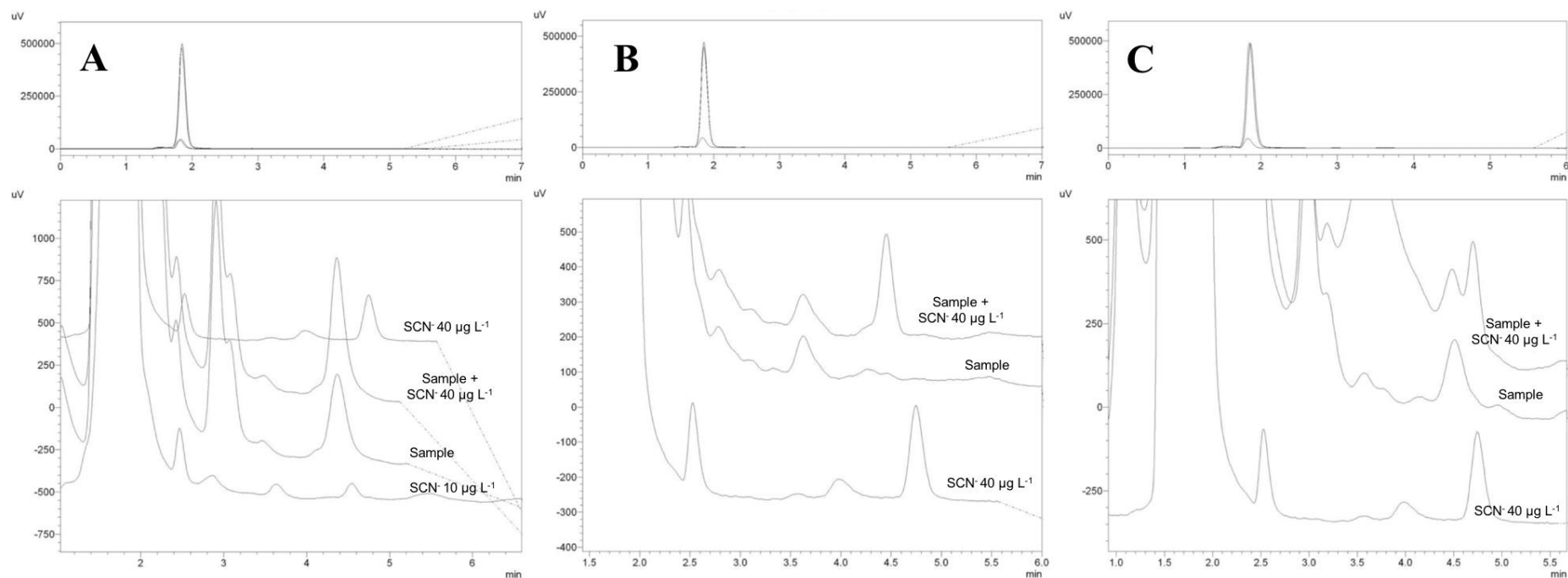
S1bu



S1bv



Supplementary Figure S2 – Chromatograms performed using HPLC to screen for the presence of SCN^- in seawater used to depurate blue damsels *Chrysiptera cyanea* (family Pomacentridae) of similar size during 24 h. A) Chromatograms representing a sample displaying potential levels of SCN^- (sample), with identification being confirmed through the spiking of the sample with $40 \mu\text{g L}^{-1}$ of SCN^- ($\text{SCN}^- 40 \mu\text{g L}^{-1}$) and detecting a proportional increase in the peak of SCN^- present in the sampled spiked with $40 \mu\text{g L}^{-1}$ of SCN^- (Sample + $\text{SCN}^- 40 \mu\text{g L}^{-1}$); B) Chromatograms representing a sample displaying no detectable level of SCN^- (sample), with the absence of SCN^- being confirmed through the spiking of the sample with $40 \mu\text{g L}^{-1}$ of SCN^- ($\text{SCN}^- 40 \mu\text{g L}^{-1}$) and the sole detection of the peak referring to concentration of SCN^- added to the sample (Sample + $\text{SCN}^- 40 \mu\text{g L}^{-1}$); C) Chromatograms representing a sample displaying potential levels of SCN^- (sample), with the absence of SCN^- being confirmed through the spiking of the sample with $40 \mu\text{g L}^{-1}$ of SCN^- ($\text{SCN}^- 40 \mu\text{g L}^{-1}$) and the detection of two peaks referring to the concentration of SCN^- added to the sample and the ion initially detected that was not confirmed as SCN^- (Sample + $\text{SCN}^- 40 \mu\text{g L}^{-1}$). Retention times recorded for the SCN^- were always between 4.2 to 4.8 minutes, with shifts in retention times for SCN^- peaks represented in the figure being due to the stripping of PEG (Polyethyleneglycol - $\text{H}(\text{OCH}_2\text{CH}_2)_n\text{OH}$) used to modify the C30 column employed for HPLC analysis.



Supplementary Table S1 – HPLC chromatogram of standard solutions and all live reef fish screened for thiocyanate (SCN⁻) excretion after being depurated for 24 h in synthetic seawater along with their family, scientific and common names. Scientific names of fish species and families are reported according to WoRMS²⁰, while common names are reported according to Michael²¹.

Standart Solutions	
Base Line	Retention Time
SCN ⁻ 40 µg L ⁻¹	4,0952
SCN ⁻ 20 µg L ⁻¹	4,1013
SCN ⁻ 10 µg L ⁻¹	4,1592
SCN ⁻ 5 µg L ⁻¹	4,1491
SCN ⁻ 20 µg L ⁻¹	4,5013
SCN ⁻ 10 µg L ⁻¹	4,5503
Average	4,2594
Max value	4,5503
Min value	4,0952
Supplementary figure S1a and S1b	

Supplementary Table S1 (continued)

Chromatograms tested positive for SCN ⁻ *(Detection Limit = 10 µg L ⁻¹ SCN ⁻)								
Family	Common name	Species	Common name	Supplementary figure	Base Line	Retention Time	Base line	Retention Time
Acanthuridae	Surgeonfishes	<i>Acanthurus lineatus</i>	Lined surgeonfish	S1c	SCN ⁻ 20 µg L ⁻¹ + Sample	4,1821	Sample	3,9716
		<i>Zebrasoma scopas</i>	Brown tang	S1g	SCN ⁻ 20 µg L ⁻¹ + Sample	4,0323	Sample	4,0321
Chaetodontidae	Butterflyfishes	<i>Chelmon rostratus</i>	Copperbanded butterflyfish	S1h	SCN ⁻ 20 µg L ⁻¹ + Sample	4,0010	Sample	3,9563
		<i>Chelmon rostratus</i>		S1i	SCN ⁻ 20 µg L ⁻¹ + Sample	4,2162	Sample	4,0100
		<i>Chelmon rostratus</i>		S1j	SCN ⁻ 20 µg L ⁻¹ + Sample	4,7456	Sample	4,3798
Monacanthidae	Foolfish	<i>Acreichthys tomentosus</i>	Bristle-tail file-fish	S1l	SCN ⁻ 20 µg L ⁻¹ + Sample	4,6770	Sample	4,6928
Pomacentridae	Damselfishes	<i>Chrysiptera cyanea</i>	Blue damselfish	S2A	SCN ⁻ 40 µg L ⁻¹ + Sample	4,5234	Sample	4,5765
Pomacanthidae	Angelfishes	<i>Centropyge ferrugata</i>	Rusty angelfish	S1d	SCN ⁻ 20 µg L ⁻¹ + Sample	4,1753	Sample	4,0924
		<i>Centropyge ferrugata</i>		S1e	SCN ⁻ 20 µg L ⁻¹ + Sample	4,1286	Sample	4,1174
		<i>Centropyge ferrugata</i>		S1f	SCN ⁻ 20 µg L ⁻¹ + Sample	4,1985	Sample	4,1291
Siganidae	Rabbitfishes	<i>Siganus vulpinus</i>	Foxface rabbitfish	S1k	SCN ⁻ 20 µg L ⁻¹ + Sample	4,009	Sample	4,1110
Average						4,2372	Average	4,1881
Max value						4,6770	Max value	4,6928
Min value						4,0010	Min value	3,9563

Supplementary Table S1 (continued)

Chromatograms tested negative for SCN ⁻ * (For concentrations above 10 µg L ⁻¹ SCN ⁻)						
Family	Common name	Species	Common name	Supplementary figure	Base Line	Retention Time
Acanthuridae	Surgeonfishes	<i>Acanthurus lineatus</i>	Lined surgeonfish	S1m	SCN 10 µg L ⁻¹ + Sample	3,9964
		<i>Acanthurus lineatus</i>		S1n	SCN 10 µg L ⁻¹ + Sample	4,1693
		<i>Zebrasoma scopas</i>	Brown tang	S1s	SCN 10 µg L ⁻¹ + Sample	4,0598
		<i>Zebrasoma scopas</i>		S1t	SCN 10 µg L ⁻¹ + Sample	3,9321
		<i>Zebrasoma scopas</i>		S1u	SCN 10 µg L ⁻¹ + Sample	3,9479
		<i>Acanthurus pyroferus</i>	Chocolate surgeonfish	S1am	SCN 10 µg L ⁻¹ + Sample	4,5911
		<i>Acanthurus tennentii</i>	Doubleband surgeonfish	S1an	SCN 10 µg L ⁻¹ + Sample	4,4776
		<i>Zebrasoma veliferum</i>	Sailfin tang	S1ao	SCN 10 µg L ⁻¹ + Sample	4,4312
Apogonidae	Cardinalfishes	<i>Pterapogon kauderni</i>	Banggai cardinalfish	S1ap	SCN 10 µg L ⁻¹ + Sample	4,1345
		<i>Sphaeramia nematoptera</i>	Pajama cardinalfish	S1aq	SCN 10 µg L ⁻¹ + Sample	4,1444
Balistidae	Triggerfishes	<i>Odonus niger</i>	Niger triggerfish	S1ar	SCN 10 µg L ⁻¹ + Sample	4,1255
Blenniidae	Blennies	<i>Ecsenius bicolor</i>	Bicolor blenny	S1as	SCN 10 µg L ⁻¹ + Sample	4,1677
Callionymidae	Dragonets	<i>Synchiropus splendidus</i>	Green mandarinfish	S1at	SCN 10 µg L ⁻¹ + Sample	4,2987
Chaetodontidae	Butterflyfishes	<i>Chelmon rostratus</i>	Copperbanded butterflyfish	S1w	SCN 10 µg L ⁻¹ + Sample	3,9956
		<i>Chelmon rostratus</i>		S1y	SCN 10 µg L ⁻¹ + Sample	3,9897
		<i>Chaetodon auriga</i>	Threadfin butterflyfish	S1au	SCN 10 µg L ⁻¹ + Sample	4,0202
		<i>Heniochus.sp</i>	-	S1av	SCN 10 µg L ⁻¹ + Sample	4,3124
Labridae	Wrasses	<i>Labroides dimidiatus</i>	Bluestreak cleaner wrasse	S1aw	SCN 10 µg L ⁻¹ + Sample	4,5211
Lutjanidae	Snappers	<i>Lutjanus sebae</i>	Emperor snapper	S1ax	SCN 10 µg L ⁻¹ + Sample	4,3211
Microdesmidae	Dartfishes	<i>Nemateleotris magnifica</i>	Fire goby	S1v	SCN 10 µg L ⁻¹ + Sample	4,5984
		<i>Nemateleotris magnifica</i>		S1x	SCN 10 µg L ⁻¹ + Sample	4,6001
Ostraciidae	Cowfishes/Boxfishes	<i>Ostracion cubicus</i>	Cube boxfish	S1ay	SCN 10 µg L ⁻¹ + Sample	4,3031
		<i>Ostracion cubicus</i>		S1az	SCN 10 µg L ⁻¹ + Sample	4,3112
		<i>Ostracion cubicus</i>		S1ba	SCN 10 µg L ⁻¹ + Sample	4,3428

Supplementary Table S1 (continued)

Chromatograms tested negative for SCN^- * (For concentrations above $10 \mu\text{g L}^{-1} \text{SCN}^-$)						
Family	Common name	Species	Common name	Supplementary figure	Base Line	Retention Time
Pomacentridae	Damselishes	<i>Chrysiptera cyanea</i>	Blue damselfish	S2B	$\text{SCN}^- 40 \mu\text{g L}^{-1} + \text{Sample}$	4,6234
		<i>Chrysiptera cyanea</i>		S2C	$\text{SCN}^- 40 \mu\text{g L}^{-1} + \text{Sample}$	4,6109
		<i>Chrysiptera cyanea</i>		S1aa	$\text{SCN}^- 10 \mu\text{g L}^{-1} + \text{Sample}$	4,3210
		<i>Chrysiptera cyanea</i>		S1ab	$\text{SCN}^- 10 \mu\text{g L}^{-1} + \text{Sample}$	5,0090
		<i>Chrysiptera cyanea</i>		S1ac	$\text{SCN}^- 10 \mu\text{g L}^{-1} + \text{Sample}$	4,1230
		<i>Chrysiptera cyanea</i>		S1ad	$\text{SCN}^- 10 \mu\text{g L}^{-1} + \text{Sample}$	4,1807
		<i>Chrysiptera cyanea</i>		S1ae	$\text{SCN}^- 10 \mu\text{g L}^{-1} + \text{Sample}$	4,4098
		<i>Chrysiptera cyanea</i>		S1af	$\text{SCN}^- 10 \mu\text{g L}^{-1} + \text{Sample}$	4,5321
		<i>Chrysiptera cyanea</i>		S1ag	$\text{SCN}^- 10 \mu\text{g L}^{-1} + \text{Sample}$	4,4164
		<i>Chrysiptera cyanea</i>		S1ah	$\text{SCN}^- 10 \mu\text{g L}^{-1} + \text{Sample}$	4,5007
		<i>Chrysiptera cyanea</i>		S1ai	$\text{SCN}^- 10 \mu\text{g L}^{-1} + \text{Sample}$	4,5164
		<i>Chrysiptera cyanea</i>		S1aj	$\text{SCN}^- 10 \mu\text{g L}^{-1} + \text{Sample}$	4,5415
		<i>Amphiprion ocellaris</i>		Ocellaris anemonefish	S1be	$\text{SCN}^- 10 \mu\text{g L}^{-1} + \text{Sample}$
	<i>Amphiprion ocellaris</i>		S1bf	$\text{SCN}^- 10 \mu\text{g L}^{-1} + \text{Sample}$	4,3211	
	<i>Chromis nitida</i>	Barrier reef chromis	S1bg	$\text{SCN}^- 10 \mu\text{g L}^{-1} + \text{Sample}$	4,3121	
	<i>Chromis nitida</i>		S1bh	$\text{SCN}^- 10 \mu\text{g L}^{-1} + \text{Sample}$	4,2987	
	<i>Chromis viridis</i>		S1bi	$\text{SCN}^- 10 \mu\text{g L}^{-1} + \text{Sample}$	4,3228	
	<i>Chromis viridis</i>		S1bj	$\text{SCN}^- 10 \mu\text{g L}^{-1} + \text{Sample}$	4,3239	
	<i>Chrysiptera parasema</i>	Yellowtail blue damselfish	S1bk	$\text{SCN}^- 10 \mu\text{g L}^{-1} + \text{Sample}$	4,3183	
	<i>Chrysiptera parasema</i>		S1bl	$\text{SCN}^- 10 \mu\text{g L}^{-1} + \text{Sample}$	4,2976	
	<i>Chrysiptera parasema</i>		S1bm	$\text{SCN}^- 10 \mu\text{g L}^{-1} + \text{Sample}$	4,3928	
	<i>Chrysiptera parasema</i>		S1bn	$\text{SCN}^- 10 \mu\text{g L}^{-1} + \text{Sample}$	4,3865	
	<i>Dascyllus sp.</i>	-	S1bo	$\text{SCN}^- 10 \mu\text{g L}^{-1} + \text{Sample}$	4,2701	

Supplementary Table S1 (continued)

Chromatograms tested negative for SCN ⁻ * (For concentrations above 10 µg L ⁻¹ SCN ⁻)						
Family	Common name	Species	Common name	Supplementary figure	Base Line	Retention Time
Pomacanthidae	Angelfishes	<i>Pomacentrus alleni</i>	Allen's damselfish	S1o	SCN ⁻ 10 µg L ⁻¹ + Sample	4,2274
		<i>Pomacentrus alleni</i>		S1p	SCN ⁻ 10 µg L ⁻¹ + Sample	4,2297
		<i>Centropyge acanthops</i>	African flameback angelfish	S1q	SCN ⁻ 10 µg L ⁻¹ + Sample	4,1345
		<i>Pygoplites diacanthus</i>	Regal angelfishes	S1r	SCN ⁻ 10 µg L ⁻¹ + Sample	4,1445
		<i>Centropyge bispinosa</i>	Coral beauty	S1z	SCN ⁻ 10 µg L ⁻¹ + Sample	4,6817
		<i>Centropyge bicolor</i>	Bicolor angelfish	S1bb	SCN ⁻ 10 µg L ⁻¹ + Sample	4,2867
		<i>Centropyge bicolor</i>		S1bc	SCN ⁻ 10 µg L ⁻¹ + Sample	4,2952
		<i>Centropyge bicolor</i>		S1bd	SCN ⁻ 10 µg L ⁻¹ + Sample	4,3077
Scaridae	Parrotfishes	<i>Scarus quoyi</i>	Quoy's parrotfish	S1bp	SCN ⁻ 10 µg L ⁻¹ + Sample	4,2551
Serranidae	Anthias	<i>Pseudanthias squamipinnis</i>	Lyretail anthias	S1bq	SCN ⁻ 10 µg L ⁻¹ + Sample	4,3210
		<i>Pseudanthias squamipinnis</i>		S1br	SCN ⁻ 10 µg L ⁻¹ + Sample	4,3190
		<i>Pseudanthias squamipinnis</i>		S1bs	SCN ⁻ 10 µg L ⁻¹ + Sample	4,2700
Siganidae	Rabbitfishes	<i>Siganus vulpinus</i>	Foxface rabbitfish	S1bt	SCN ⁻ 10 µg L ⁻¹ + Sample	4,3525
		<i>Siganus vulpinus</i>		S1bu	SCN ⁻ 10 µg L ⁻¹ + Sample	4,2123
Syngnathidae	Pipefishes	<i>Corythoichthys intestinalis</i>	Messmate pipefish	S1ak	SCN ⁻ 10 µg L ⁻¹ + Sample	4,2409
Tetraodontidae	Pufferfishes	<i>Arothron hispidus</i>	Whitespotted	S1al	SCN ⁻ 10 µg L ⁻¹ + Sample	4,1767
		<i>Canthigaster valentini</i>	Saddled toby	S1bv	SCN ⁻ 10 µg L ⁻¹ + Sample	4,5102
					Average (from all negatives)	4,3122
					Max value	5,0090
					Min value	3,9321

Supplementary Table S2 – Synthetic seawater volume employed to depurate selected fish according to their total length (measured from the tip of the snout to the tip of the longer lobe of the caudal fin).

Fish total length (TL) (mm)	Seawater volume (ml)
TL < 1,5	500
1,5 < TL > 3	1000
3 < TL > 5	1500
5 < TL > 10	2000
10 < TL > 20	5000