Supplemental Information

Identification of Furan Metabolites Derived from Cysteine-*cis*-2-Butene-1,4-Dial-Lysine Crosslinks

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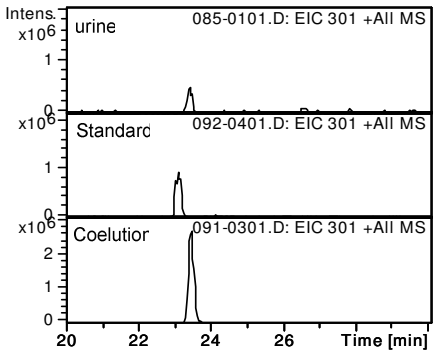
A.



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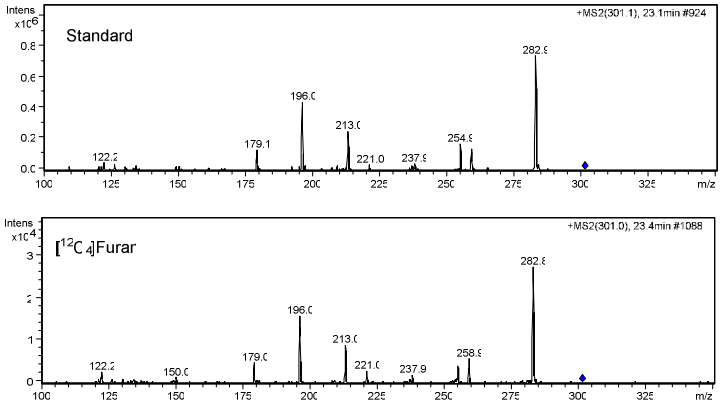
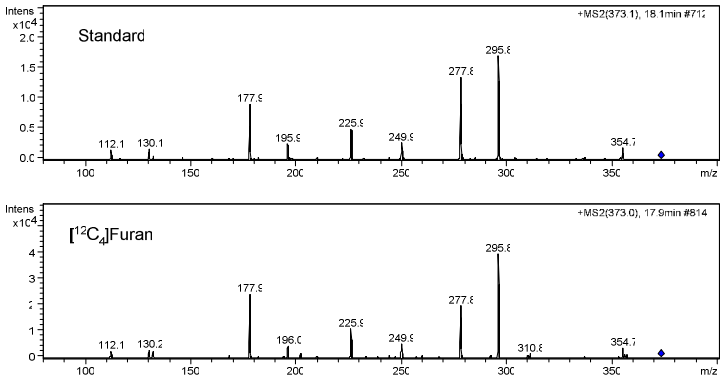


Supplemental Figure 1.

A. Extracted ion current at 301 Da demonstrating co-elution of the urinary metabolite with *S*-[1-(5-acetylamino-5-carboxypentyl)-1*H*-pyrrol-3-yl]methanethiol sulfoxide (**8**). LC-MS/MS analysis was performed on a Synergi column according to HPLC Method 5.
B. The mass spectra for synthetic **8** and furan metabolite **8**.

A.



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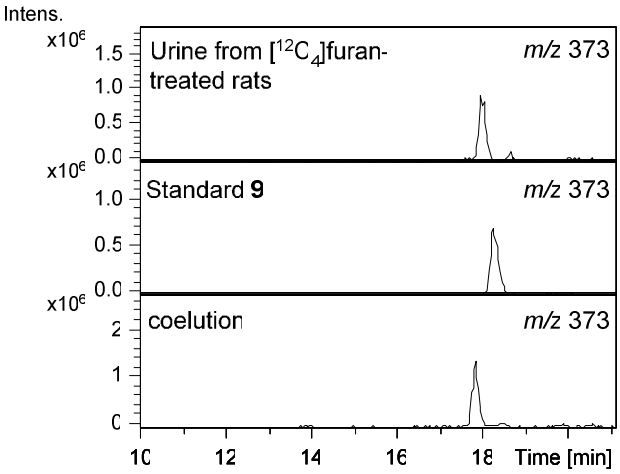
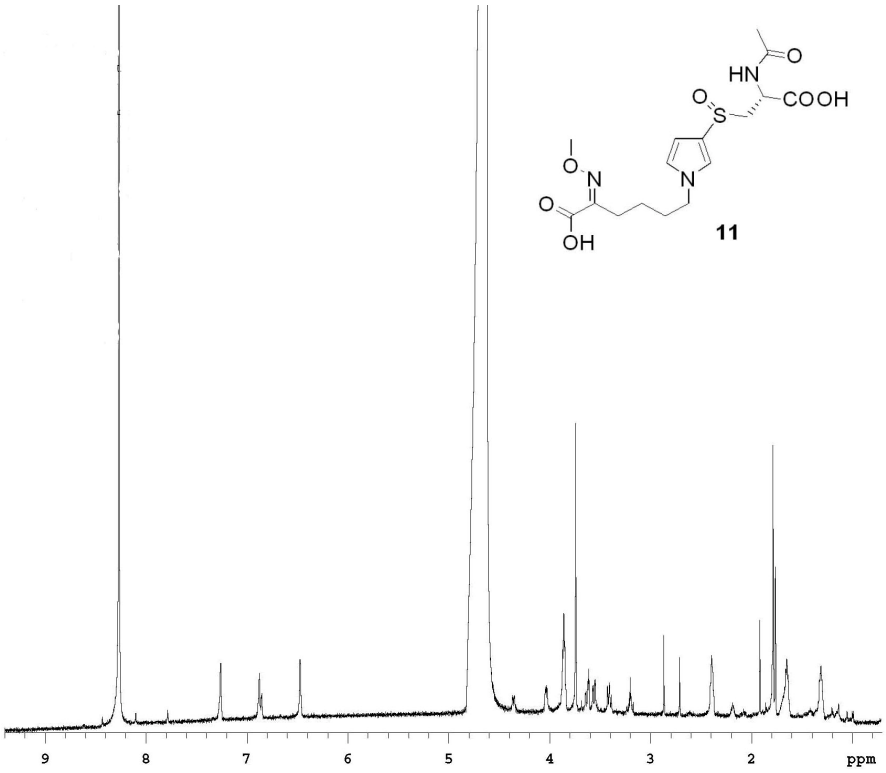


Supplemental Figure 2.

A. The mass spectra corresponding to synthetic **9** and furan metabolite **9**.

B. Extracted ion current at 373 Da demonstrating co-elution of the urinary metabolite with *N*-acetyl-*S*-[1-(5-oxo-5-carboxypentyl)-1*H*-pyrrol-3-yl]-L-cysteine sulfoxide (**9**). LC-MS/MS analysis was performed on a Synergi column according to HPLC Method 5.

A.



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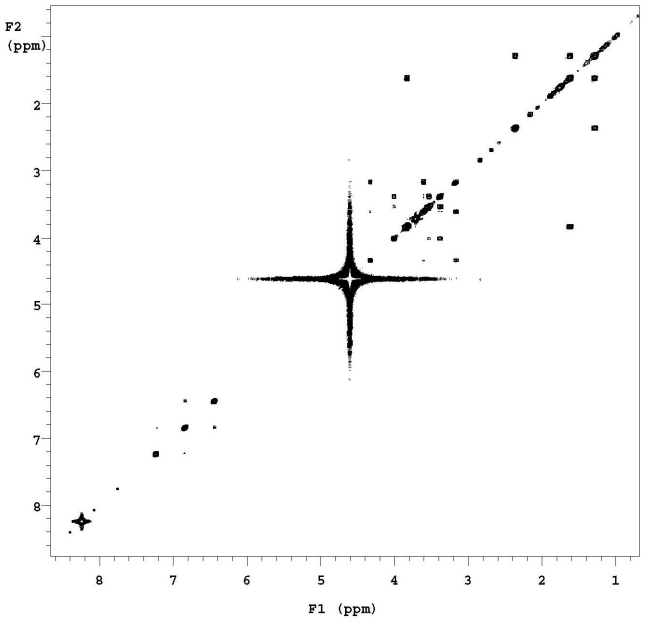
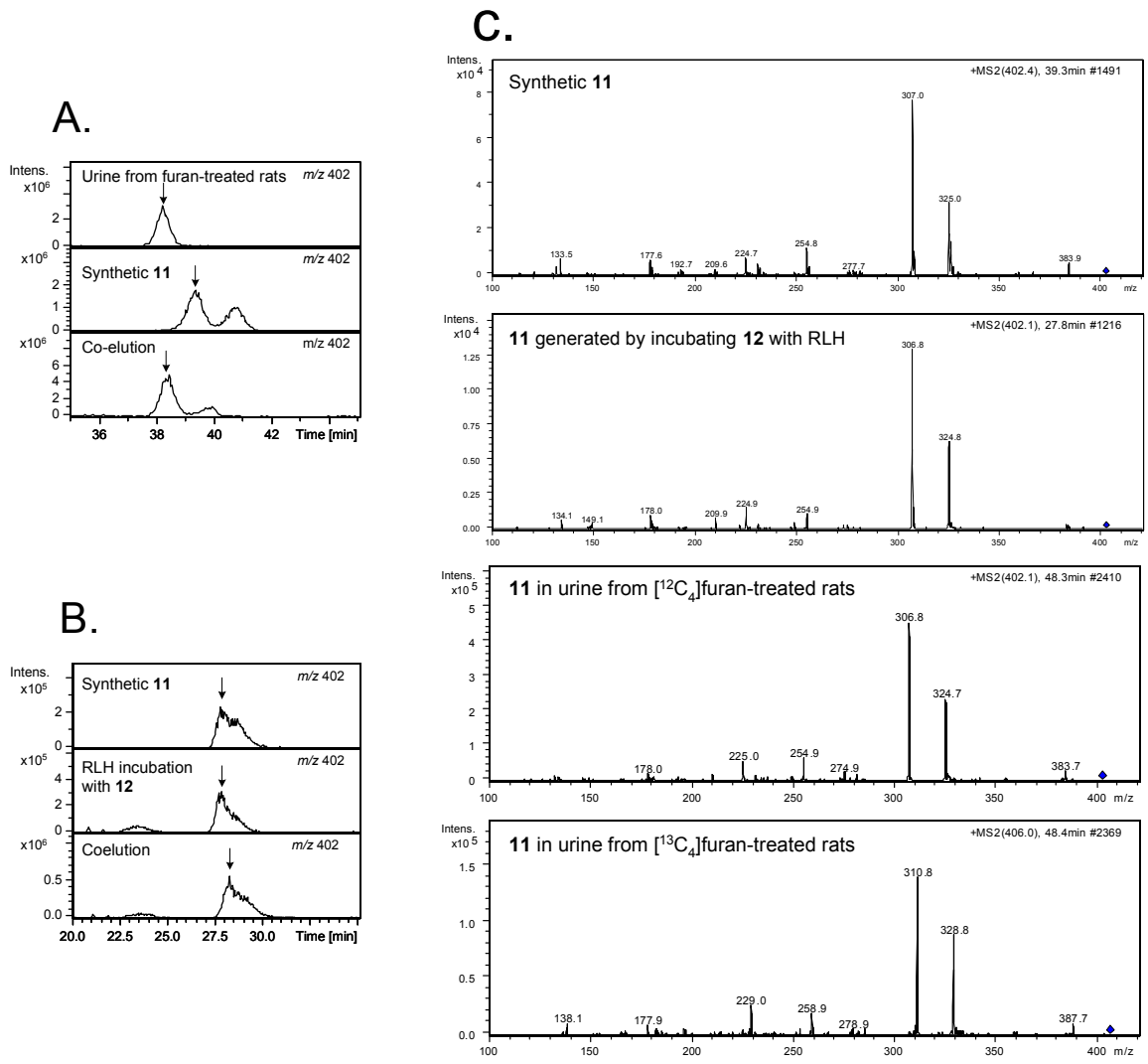


Supplemental Figure 3.

A. ^{1}H NMR spectrum of synthetic *N*-acetyl-*S*-[1-(5-methoxyimino-5-carboxypentyl)-1*H*-pyrrol-3-yl]-L-cysteine sulfoxide (**11**)

B. COSY NMR of **11**

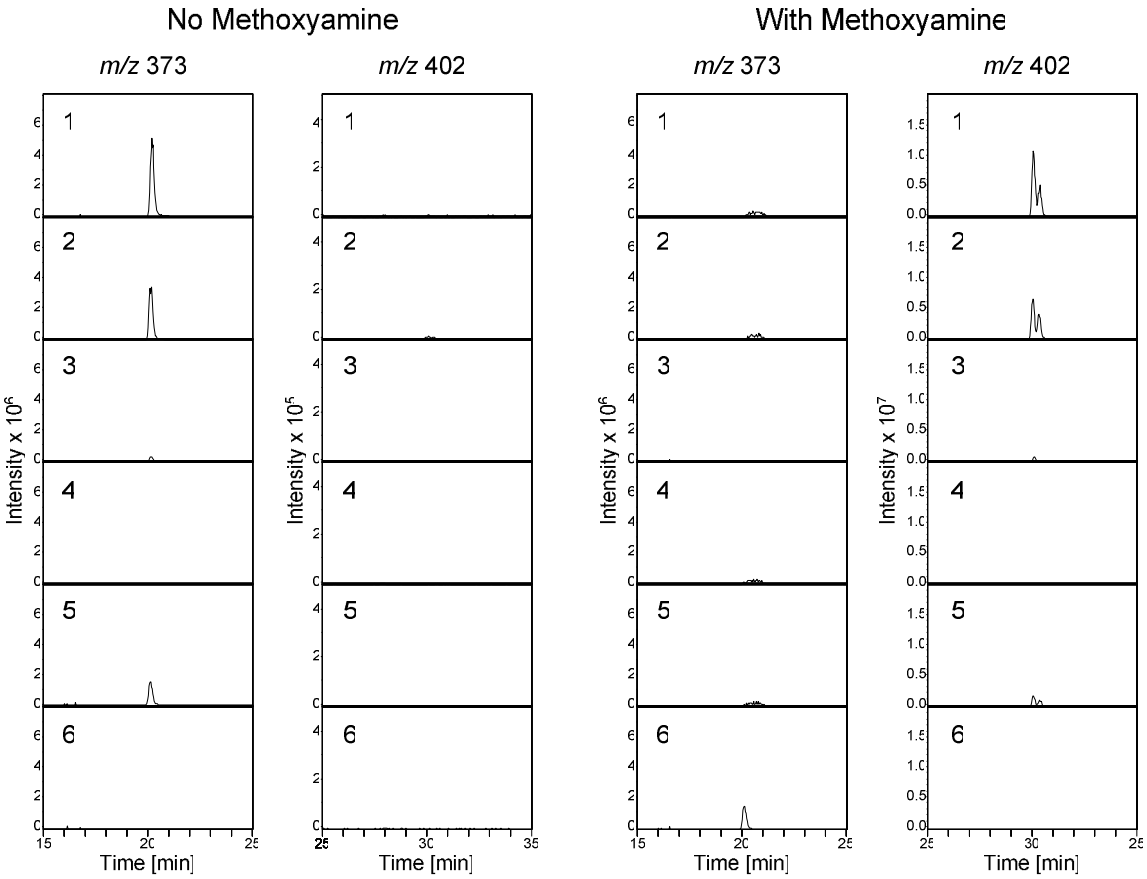


Supplemental Figure 4.

A. Extracted ion current at 402 Da showing co-elution of *N*-acetyl-*S*-[1-(5-methoxyimino-5-carboxypentyl)-1*H*-pyrrol-3-yl]-L-cysteine sulfoxide (**11**) with urinary metabolite **9** following reaction with methoxyamine. LC-MS/MS analysis was performed on a Zorbax column eluted according to HPLC Method 3.

B. Extracted ion current at 402 Da showing co-elution of synthetic **11** with the RLH generated metabolite of *N*-acetyl-*S*-[1-(5-amino-5-carboxypentyl)-1*H*-pyrrol-3-yl]-L-cysteine sulfoxide (**12**) following reaction with methoxyamine. LC-MS/MS analysis was performed on a Luna column eluted according to HPLC Method 6.

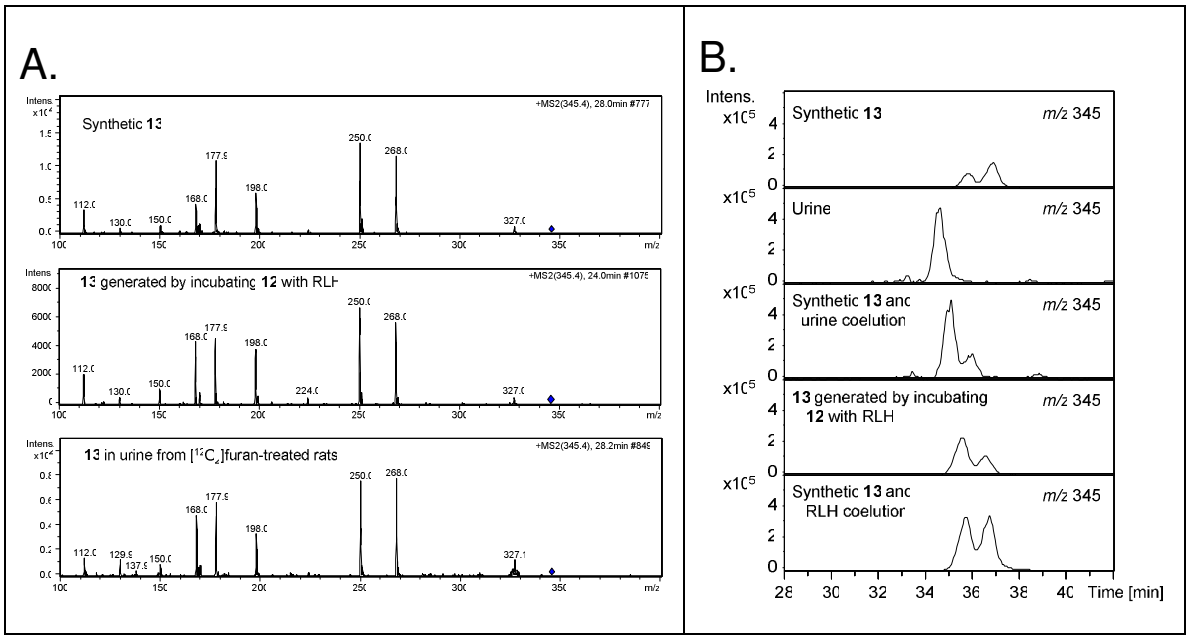
C. The mass spectra of synthetic **11** and **11** formed when supernatant from RLH incubations of **12** or urine from furan-treated rats are reacted with methoxyamine. RLH = rat liver homogenate.



Condition	substrate	RLH	PLP+AKGA
1	+	+	+
2	+	-	+
3	+	+	-
4	-	+	+
5	+	Boiled	+
6	+	Boiled	-

Supplemental Figure 5.

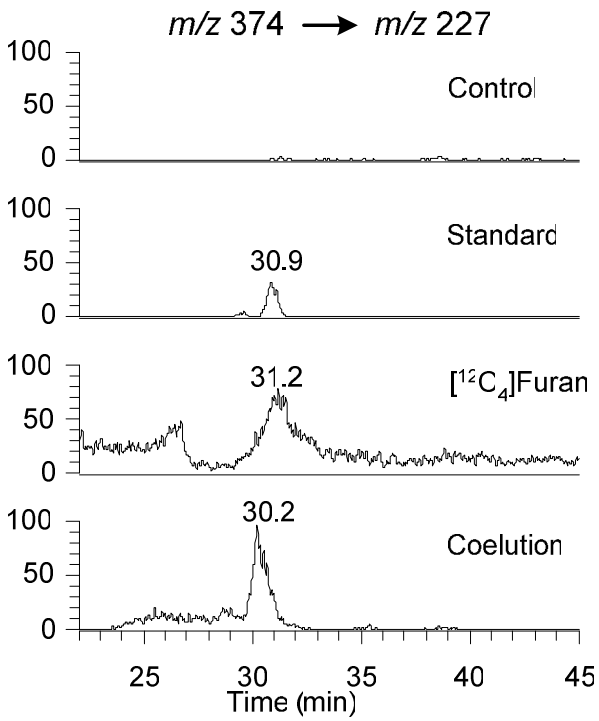
Extracted mass chromatograms obtained at *m/z* 373 and 402 for the incubation of **12** with rat liver homogenate employing HPLC method 5. RLH = rat liver homogenate; PLP = pyridoxal 5'-phosphate; and AKGA = α-ketoglutaric acid.



Supplemental Figure 6.

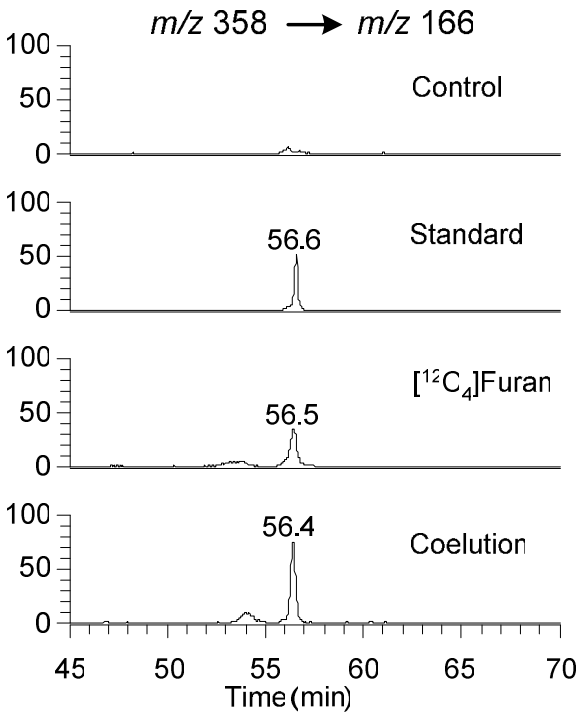
A. The mass spectra of *N*-acetyl-*S*-[1-(4-carboxybutyl)-1*H*-pyrrol-3-yl]-L-cysteine sulfoxide (**13**), the metabolite formed in RLH incubations of **12** and the urinary metabolite.

B. Extracted ion current at 345 Da demonstrating co-elution of the urinary metabolite and RLH metabolite of **12** with synthetic **13**. LC-MS/MS analysis was performed on a Zorbax column according to HPLC Method 3. The synthesis of **13** generated two diastereomers. Only one of these diastereomers is observed in urine of furan-treated rats. The diastereomeric distribution of **13** in the RLH incubations reflects the diastereomeric distribution of the starting compound **12** which is different from that of synthetic **13**.



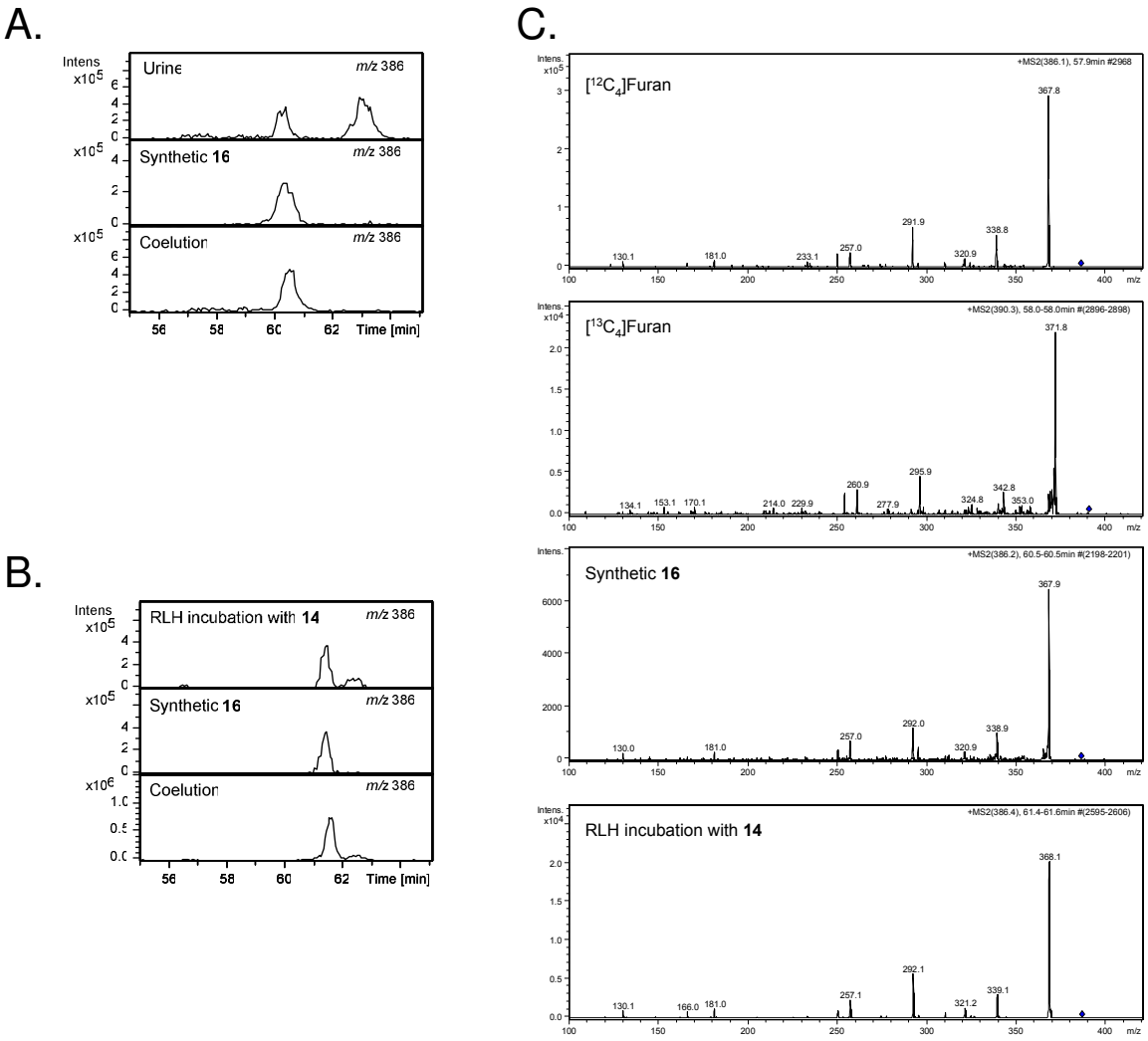
Supplemental Figure 7

LC-ESI-MS/MS analysis demonstrating coelution of the synthetic standard *N*-acetyl-*S*-[1-(5-amino-5-carboxypentyl)-1*H*-pyrrol-3-yl]-L-cysteine sulfoxide (**12**) with urinary metabolite **12** by selected reaction monitoring of *m/z* 374 → *m/z* 277 by LC-ESI-MS/MS analysis on a Synergi column according to HPLC Method 3.



Supplemental Figure 8

LC/ESI-MS/MS analysis demonstrating coelution of the synthetic standard *N*-acetyl-*S*-[1-(5-amino-5-carboxypentyl)-1*H*-pyrrol-3-yl]-L-cysteine (**14**) with urinary metabolite **14** by selected reaction monitoring of *m/z* 358 → *m/z* 166 by LC-ESI-MS/MS analysis on a Synergi column according to HPLC Method 3. This transition was chosen for the co-elution experiment since it provided more intense signals than *m/z* 358 → *m/z* 229.



Supplemental Figure 9.

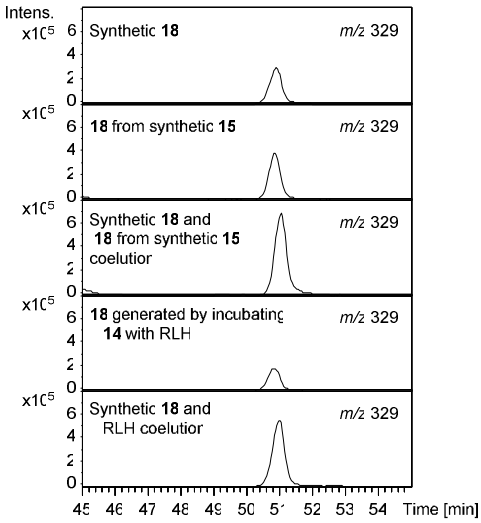
A. Extracted ion current at 386 Da showing co-elution of *N*-acetyl-*S*-[1-(5-methoxyimino-5-carboxypentyl)-1*H*-pyrrol-3-yl]-L-cysteine (**16**) with urinary metabolite *N*-acetyl-*S*-[1-(5-oxo-5-carboxypentyl)-1*H*-pyrrol-3-yl]-L-cysteine (**15)** following reaction with methoxyamine. LC-MS/MS analysis was performed on a Synergi column eluted according to HPLC Method 3.

B. Extracted ion current at 386 Da showing co-elution of synthetic **16** with the RLH generated metabolite of **14** following reaction with methoxyamine. LC-MS/MS analysis was performed on a Synergi column eluted according to HPLC Method 3.

C. The mass spectra of synthetic **16** and **16** formed when urine from furan-treated rats or supernatant from RLH incubations of **14** are reacted with methoxyamine.

RLH = rat liver homogenate.

A.



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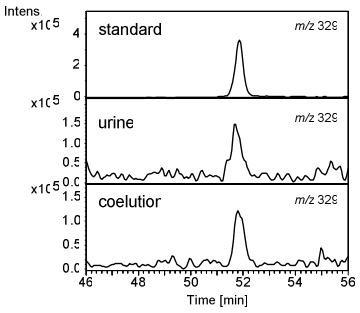
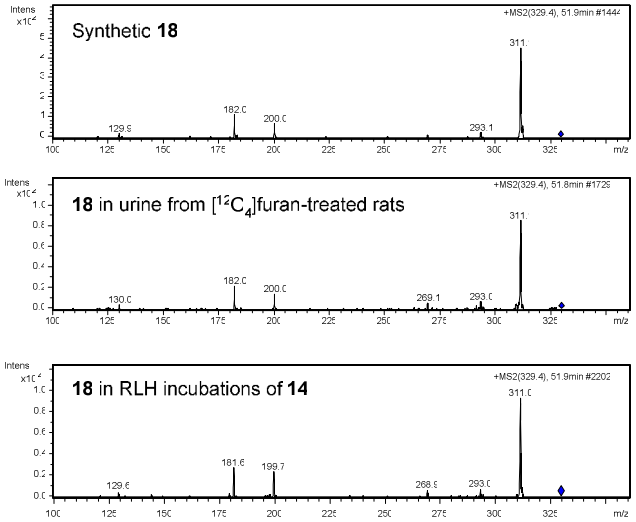


C.



Supplemental Figure 10.

A. Extracted ion current at 329 Da showing co-elution of *N*-acetyl-*S*-[1-(4-carboxybut-yl)-1*H*-pyrrol-3-yl]-L-cysteine (**18**) with urinary metabolite **18**. LC-MS/MS analysis was performed on a Zorbax column eluted according to HPLC Method 3.

B. Extracted ion current at 329 Da showing co-elution of synthetic **18** with **18** generated from synthetic **15** or **15** formed in the incubation of **14** with RLH. LC-MS/MS analysis was performed on a Zorbax column eluted according to HPLC Method 3

RLH = rat liver homogenate.

C. The mass spectra of synthetic **18** and **18** present in urine from furan-treated rats or supernatant from RLH incubations of **14**.