



# LCMS Detection and Quantitation of a Small Anion, Thiocyanate, Using Ion-Pair Chromatography

## Authors

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## Introduction

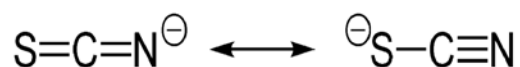
Thiocyanate is a small anion that is a byproduct of cyanide metabolism. The detection of small anions and cations can be difficult to achieve using HPLC-LC-MS-MS. The low molecular weight and high polarity results in a chromatogram that can be noisy with no retention in reversed phase HPLC. Additionally, with only 2 bonds, it is difficult to obtain fragmentation ions for thiocyanate. Derivatization techniques can be used to increase retention and reduce noise but require more time and effort than ion-pairing. The ion-pairing of thiocyanate with triethylamine results in a fast and simple solution.

A second challenge in dealing with thiocyanate is incurred levels in plasma, because thiocyanate is derived from many sources such as cigarettes, food or even car exhaust. Accordingly, a technique to deal with incurred levels is also required.

## Challenges with Thiocyanate

### 1ST PROBLEM – SMALL RESONATING MOLECULE

- Too much noise at M-1 ion, mol wt=58
- No retention reverse phase HPLC
- No substantial fragment ion for MS/MS



Mol. Wt. = 58



## Challenges with Thiocyanate (continued)

### 1st Solution – ADD TEA

#### Method - Protein Precipitation with TEA added

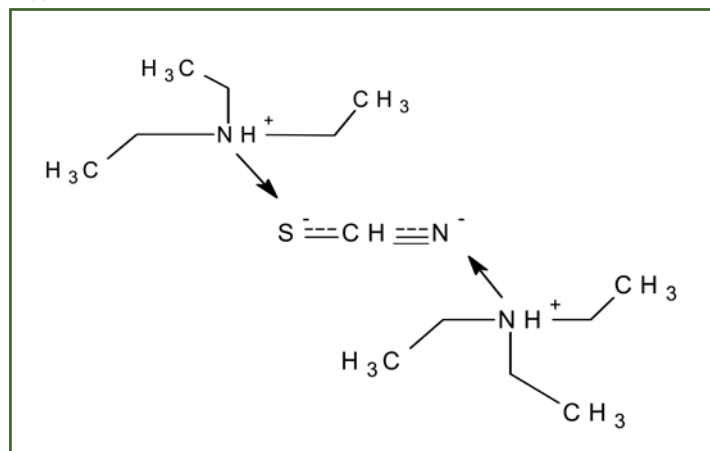
- 50  $\mu$ L human plasma
- 50  $\mu$ L of labeled Internal Standard- SC<sup>13</sup> N<sup>15</sup>
- 50  $\mu$ L ACN:TEA solution 80:20, v,v
- 350  $\mu$ L ACN
- Vortex, centrifuge, remove a 100  $\mu$ L aliquot and dilute to 1mL with water
- Inject on C18 column into API4000™ in MS/MS mode

**Final TEA concentration is 0.2 % per volume**

## Results

Creation of a complexed mass as: TEA + SCN + TEA  
 $102 + 58 + 102 = 262$  m/z  
 (See Figure 1)

FIGURE 1



CID fragment is loss of: TEA + SCN  
 $262 - (102 + 58) = 102$  m/z

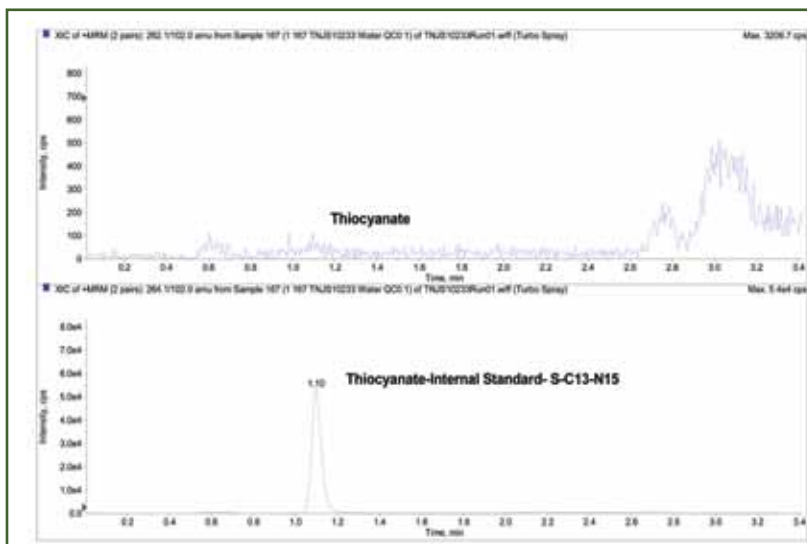


## Results (continued)

MS/MS transition 262 m/z – 102 m/z

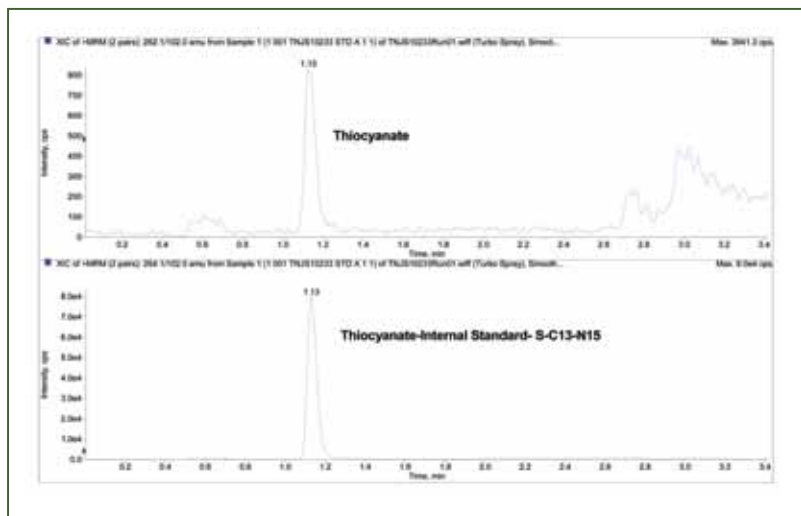
- Mass 262 m/z – 102 m/z has good specificity (See extracted blank - Figure 2)
- Analyte has retention in reversed phase (See extracted LLOQ- Figure 3 )

FIGURE 2: Chromatogram -Example of Blank with Internal Standard Added



Note: This chromatogram is an extracted water blank as human plasma samples exhibit some level of incurred thiocyanate.

FIGURE 3: Chromatogram - Example of Analyte Retention- at LLOQ- 2.00 µg/mL



- Thiocyanate resonance creates complex of 2 TEA cations per each thiocyanate anion, M+1 is now 262 m/z
- Fragment ion achievable - MS/MS mode
- Retention in reversed phase



## Results (continued)

### INCURRED THIOCYANATE IN HUMAN PLASMA

Human plasma (25 lots) was screened for incurred thiocyanate levels.

All screened plasma lots had some level of incurred thiocyanate ranging from ~ 0.200 ng/ml to 30.0ng/mL. See figure 4

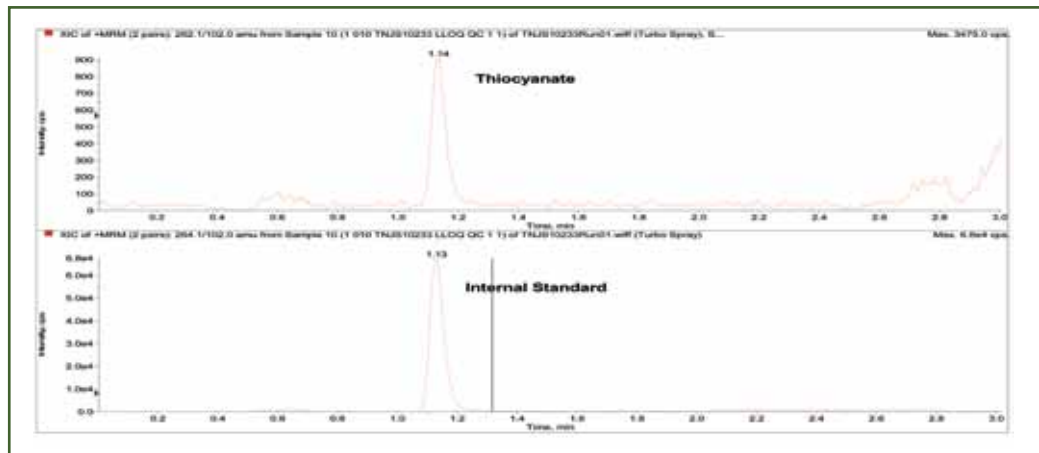
The analytical range of the assay was 2.00 - 200 ng/mL.

A strategy was needed for quantitation.

A surrogate matrix of water was chosen for spiking the calibration curve.

This has the advantage of having no analyte present so no low end bias is measured, but has the disadvantage that the analyte may behave differently in another matrix. For evaluation of the assay, quality control samples were prepared in human plasma and the incurred level was determined.

FIGURE 4: Chromatogram – Example of “Blank” Sample Showing Incurred Thiocyanate



### 2ND PROBLEM – Quantitating Incurred Thiocyanate in Human Plasma

- Surrogate matrix that is free of thiocyanate is required
- Need to determine “Target” values for QC’s (spiked conc. + incurred conc.)
- Accuracy (once determined) and precision must be in criteria of  $\pm 15\%$



## Results (continued)

### 2ND SOLUTION – Use water as Matrix for curve

- Lab grade water was thiocyanate free
- Results in no low end bias in accuracy
- Use of labeled internal standard compensates for matrix effects

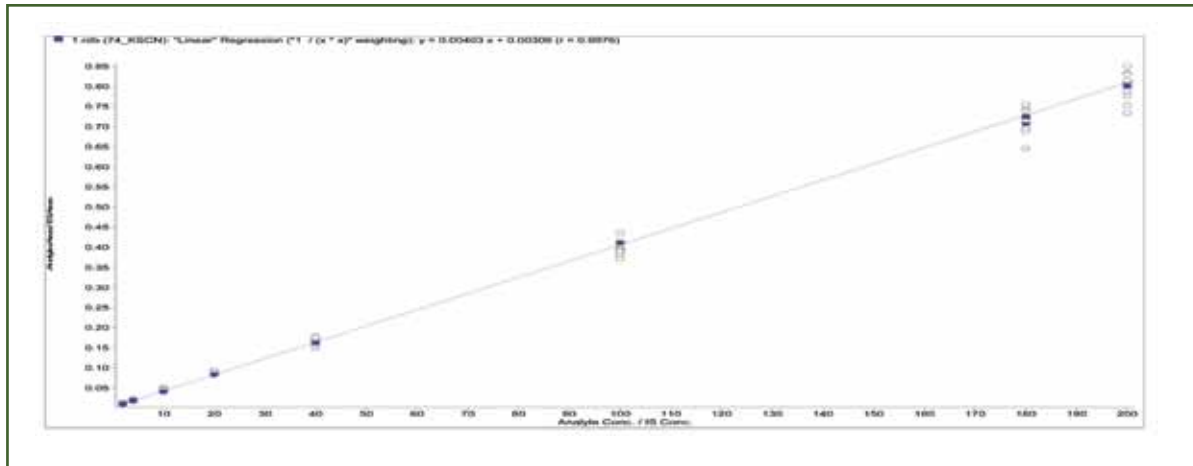
Method:

- 1- Select 6 lots at low incurred levels and spike as calibration curves to determine slopes and endogenous concentrations from water matrix curve.
- 2- Determine QC concentrations, "Targets", resulting from incurred and spiked thiocyanate levels.

### RESULTS

Slopes of matrix curves < 5% different from water spiked calibration curve -see Figure 5 and Table 1

FIGURE 5: Calibration Curve for 6 Different Lots of Human Plasma vs. Water curve



Note: Solid blue points are water curve calibrators

TABLE 1- Slopes for 6 lots of Human Plasma vs. Water Matrix							
	WATER MATRIX	Plasma Lot 1	Plasma Lot 2	Plasma Lot 3	Plasma Lot 4	Plasma Lot 5	Plasma Lot 6
Slope (m)	0.00406	0.00396	0.00406	0.00410	0.00413	0.00411	0.00415
% Difference in slope		-2.4	0.0	0.9	1.6	1.2	2.2
r <sup>2</sup>	0.9981	0.9962	0.9946	0.9923	0.9928	0.9979	0.9977
Absolute value of x intercept µg/mL	0.617	1.53	0.865	0.653	0.903	1.20	0.986
Incurred concentration µg/mL (Plasma conc. less Water conc.)		0.914	0.248	0.0358	0.286	0.587	0.369



## Results (continued)

### 2-QC CONCENTRATIONS

To determine QC concentrations, quality control samples were spiked and quantitated over 3 batches at n=6 using a water matrix curve to establish "Target" values.

TABLE 2						
Batch #		Quality Control Samples in Plasma				
		LLOQ QC	QC 1	QC 2	QC 3	QC 4
		µg/ml				
Batch 1	Spiked. conc.	2.00	6.00	15.0	80.0	160
	Mean found conc. (n=6)	2.45	6.42	15.9	81.9	160
Batch 2	Mean found conc. (n=6)	2.60	6.79	16.5	84.0	163
Batch 3	Mean found conc. (n=6)	2.60	6.83	16.1	81.8	158
TARGET	Mean found conc. (n=18)	2.55	6.68	16.2	82.5	160
	Incurred level	0.549	0.679	1.17	na	na

## Conclusion

The addition of TEA to the samples created a "masked" anion that was non-polar enough to retain using reverse phase chromatography. The resulting complex also was more massive and had an m/z of 262 for the M+1 ion which was easily cleaved in the collision cell to TEA product ions. Detecting at the higher mass and the ability to easily get a fragment ion increased specificity and reduced noise. Quantitative bias due to Incurred thiocyanate was overcome by use of water as a surrogate matrix. QCs in plasma were evaluated over 18 samples to establish thiocyanate concentrations.