QPS IS YOUR LINK.

Effect on GSH and GSSG Levels in Rats Treated with Acetaminophen and Tamoxifen Using LC-MS/MS

Helen L. Shen, Lata Venkatarangan, Bruce Aungst, and Zamas Lam, QPS, LLC, Newark, DE 19711

OBJECTIVES

Many drug candidates form glutathione conjugates in treated animals. Glutathione levels and glutathione depletion may be associated with hepatotoxicity for some compounds. During lead optimization a common approach is to minimize reactive metabolite formation through appropriate structural modification. In this study, we developed LC-MS/MS methods to monitor glutathione (GSH) and glutathione disulfide (GSSG) levels in liver, bile, and plasma of rats dosed with compounds known to form glutathione conjugate.

EXPERIMENTAL

Analytical Methods Development

LC-MS/MS methods were developed for the analysis of GSH and GSSG in rat plasma, bile, and liver specimens. Because of their high endogenous levels surrogate matrices were used for the preparation of calibration standards. These were 4% bovine serum albumin (BSA) for plasma, and sodium taurocholate in saline at pH ~3.5 for bile and liver. GSH assay for plasma was carried out by derivatization with Ellman's reagent and monitoring of the derivatized product. The extent of derivatization in the surrogate matrix and matrix effect were also investigated. As shown below for plasma, LC-MS/MS responses for the standards were shown to be similar between the sample matrices and surrogate matrices for both the derivatized GSH and GSSG in plasma.

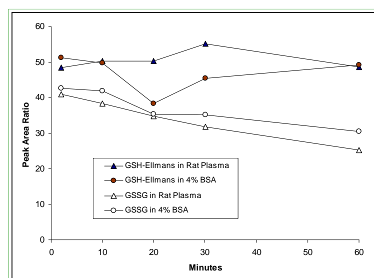
Conc (ug/mL)	Peak Area Ratio GSH-Ellman in plasma	GSH-Ellman in 4% BSA	GSSG in plasma	GSSG in 4% BSA
1.5	0.081*	0.041	0.058	0.054
5	0.180*	0.156	0.154	0.174
15	0.476	0.455	0.450	0.527
50	1.868	1.558	1.528	1.638
150	4.798	4.641	4.290	4.733
500	15.986	15.499	14.716	16.210
1000	33.784	33.840	ND	ND

*- peak area ratio biased high due to contribution from the endogenous levels in plasma

Ellman's reagent was added to whole blood and acid to bile as stabilizing agents during sample collection (Reference for bile stabilization -- J. Biol. Chem, 256(5), 2115-2117, 1981). Liver homogenization was carried out using acidified water (pH ~1.0) to maintain GSH and GSSG stability post homogenization. Stability tests were carried out for each matrix with stabilizing agent to ensure proper storage condition for the samples.

EXPERIMENTAL

This graph shows that GSH-Ellman forms immediately in rat plasma and is stable in rat plasma and the surrogate matrix, but GSSG was not stable in rat plasma requiring immediate sample processing.



The sample processing for plasma and bile samples were performed immediately after sample collection using a protein precipitation method, on wet-ice, initially with sulfosalicylic acid and then with acetonitrile. A similar method was employed for the processing of the homogenized liver samples. Stability of GSH and GSSG were shown in acidified bile and homogenized liver specimens stored on wet-ice and at -70 °C respectively. A 2-in-1 LC-MS/MS assay was used for the quantitation of GSH-Ellman and GSSG in rat plasma samples. Two discrete LC-MS/MS assays were used for the quantitation of GSH and GSSG in rat bile and liver samples. Stable labeled GSH and GSSG were used as internal standards for the LC-MS/MS assays.

In Vivo Evaluation

Bile duct-cannulated rats (n = 4) were treated by oral administration with vehicle, 300 mg/kg acetaminophen, or 45 mg/kg tamoxifen once daily for 4 days. Plasma samples were collected daily at 4 h post-dose, and bile was collected 0-4 h post-dose on Days 1-4. A terminal liver specimen was harvested on Day 4 after the collection of plasma and bile samples. Plasma and bile were stabilized as discussed above. GSH and GSSG concentrations in the plasma, bile, and liver tissue from the treated animals were compared to those from the vehicle control animals. The effect was also compared among the three matrices from the treated animals.

By Day 4, tamoxifen-treated rats showed adverse effects, and 1 of 4 animals died. The Day 4 dose was not administered and terminal specimens were collected.

RESULTS

Mean Day 4 plasma, bile, and liver GSH and GSSG concentrations are shown in the table below (Concentrations in µg/mL or µg/g)

	Plasma GSH	Bile GSH	Liver GSH	Plasma GSSG	Bile GSSG	Liver GSSG
Control	22.1±9.3	1.6±0.2	1023.2±97.7	3.7±2.4	165.2±78.1	105.5±15.8
Acetaminophen	12.9±4.6	BQL	899.8±358.4	2.7±1.0	239.2±56.7	86.3±26.9
Tamoxifen	14.3±4.7	BQL	464.9±233.7	1.5±0.9	53.3±35.7	87.2±34.8

GSH concentrations in plasma and liver were reduced in tamoxifen and acetaminophen rats.

GSH/GSSG ratios are shown below. In plasma and liver, the predominant form is GSH, but in bile GSSG concentrations were greater than GSH. The effects of drug treatment on GSH/GSSG ratios were not clear for this set of compounds.

	Plasma GSH/GSSG	Bile GSH/GSSG	Liver GSH/GSSG
Control	7.2±2.6	0.011±0.003	9.9±1.7
Acetaminophen	5.3±2.2	BQL	10.4±2.1
Tamoxifen	10.3±2.4	BQL	5.2±0.8

CONCLUSIONS

LC-MS/MS methods for determination of GSH and GSSH in plasma, bile, and liver specimens were established, using surrogate matrices because of the endogenous levels in these matrices. Stability studies were performed to understand the requirements of *in vivo* sample collection and processing for each specimen type. Finally, *in vivo* studies were performed in rats comparing GSH and GSSG levels in control rats with those treated with acetaminophen or tamoxifen for 4 days. These methods may be useful for assessing the effects of test compounds on glutathione levels *in vivo*, and the use of plasma and/or bile specimens would not require terminal sampling. Additional studies with other known compounds that affect GSH levele are planned