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Enhanced Detection and Characterization of Glutathione-Trapped Reactive Metabolites by Pseudo-MS^3 Transition Using a Linear Ion Trap Mass Spectrometer

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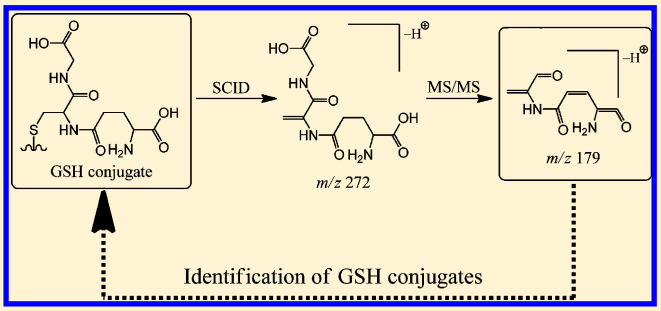
Supporting Information

ABSTRACT: We present a simple and label-free approach to characterize glutathione (GSH)-trapped reactive metabolites from a single LC-MS analysis employing a linear ion trap mass spectrometer. The GSH specific fragment anion m/z 272 was first generated from the nonselective in-source fragmentation of intact conjugates. GSH conjugates were then detected by selected reaction monitoring (SRM) of the anion pair m/z 272 → 179 or 210. The resultant SRM peaks represented putative GSH conjugates which were then further characterized from their MS and MS^2 data acquired in both positive and negative ion modes. The method is demonstrated with test compounds that are all known to form GSH conjugates.



Metabolic bioactivation of a drug can generate reactive intermediates that are capable of covalently modifying cellular biomolecules and are implicated in drug induced toxicity.[1] Reactive metabolites are usually detected by trapping them with L-glutathione (GSH) followed by characterization of the resultant conjugate(s) with liquid chromatography (LC)-mass spectrometry (MS). Many approaches have been developed based on attributes of different MS platforms. Methods implemented on a triple quadrupole or Q-Trap mass spectrometer include constant neutral loss scan,[2] precursor ion scan,[3−5] and MRM,[6] all based on monitoring characteristic GSH conjugate fragmentation. Methods utilizing high resolution mass spectrometry (HRMS) implemented on TOF and Orbitrap platforms typically include data processing and filtering techniques. These techniques include comparison with control samples,[7−10] background subtraction,[7,11] mass defect filtering,[7−10,12] and mass extraction[13] to detect and characterize GSH conjugates. Ion trap mass spectrometers have been employed for the detection of GSH conjugates, and published methods[14−16] have the disadvantage of requiring the use of a mixture of natural and stable isotope labeled GSH as the trapping agent.

We recently reported a sensitive and selective method called XoPI[13] to screen for and characterize GSH conjugates using a hybrid Orbitrap spectrometer. The method relied on fragments produced from nonselective in-source collision-induced dissociation (SCID; also called in-source fragmentation) and detection of accurate mass of GSH conjugate product ion at m/z 272.0888, representing the deprotonated gamma-glutamyl-dehydroalanyl-glycine (γ-EdAG). The presence of γ-EdAG was identified from an extracted product ion chromatogram of m/z 272.0888 (referred to as $XPIC_{272.0888}$).[13] Its precursor GSH conjugate was then confirmed and characterized from the corresponding full scan MS and MS^2 spectra collected in alternate scans of the same chromatographic peak. The XoPI method requires a HRMS instrument such as a hybrid Orbitrap or TOF MS. In this rapid report, we demonstrate an effective and isotope-label-free approach for the detection of GSH conjugates using a unit resolution linear ion trap mass spectrometer.

γ-EdAG can be identified from its characteristic product ion. Figure 1 shows the negative ion mode HRMS tandem mass spectrum obtained by wideband activation of the γ-EdAG ion generated from an amodiaquine GSH conjugate A5 (Figure 2) under SCID. It is essentially the MS^3 spectrum of the GSH conjugate; however, since this spectrum was not an MS^3

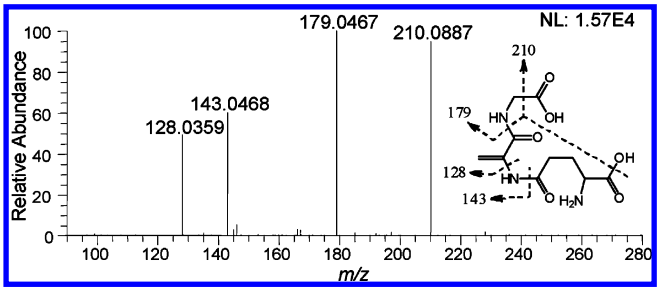


Figure 1. γ-EdAG HRMS tandem mass spectrum (MS^2; pseudo-MS^3 spectrum of the GSH conjugate A5) obtained by wideband activation of m/z 272 ion in the negative ion mode. The proposed assignment of the observed fragments is shown on the inset γ-EdAG structure.

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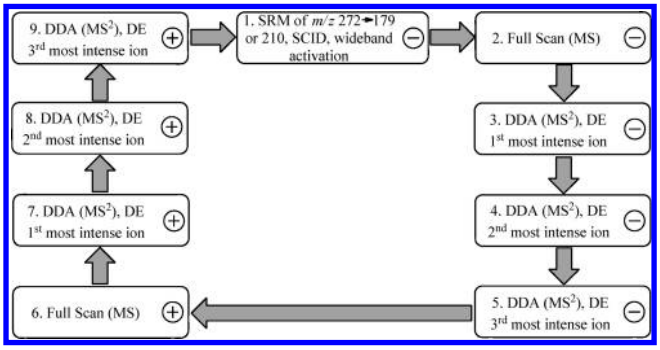
fragmentation of a selected precursor ion but rather from an MS^2 fragmentation of a nonselective SCID produced ion, it is considered to be a pseudo-MS^3 spectrum. The two most abundant product ions are at m/z 179 and 210 (Figure 1). Using a unit resolution mass spectrometer (e.g., linear ion trap MS), a product ion detected at m/z 272 is insufficiently selective to be deemed from a GSH conjugate because it may not necessarily be deprotonated γ-EdAG. However, an ion at m/z 272 with a product ion at m/z 179 or 210 is highly likely to be the deprotonated γ-EdAG. Thus, selected reaction monitoring (SRM) of anion pair m/z 272 → 179 or 210 is selective to identify γ-EdAG and therefore its precursor GSH conjugate.

Without wideband activation, the m/z 254 ion formed by a neutral loss of water (18 Da) was the most intense ion in the γ-EdAG MS^2 spectrum as shown in Figure S1 (Supporting Information). The loss of water is a nonspecific neutral loss because it readily occurs with a variety of functional groups containing an oxygen atom; therefore, anions m/z 272 → 254 would be a less selective SRM ion pair to specifically detect γ-EdAG than m/z 272 → 179 or 210 using wideband activation.

The analytical strategy of the method proposed in this rapid report is to detect putative GSH conjugates on a SRM chromatogram by monitoring the m/z 272 → 179 or 210 ion pair in the negative ion mode under SCID and wideband activation. A nine-event data acquisition cycle (Scheme 1) was

Scheme 1. Flow Chart of Data Acquisition Cycle Implemented on an LTQ Velos Linear Ion Trap Mass Spectrometer



implemented on a linear ion trap MS (LTQ Velos, Thermo Fisher Scientific Inc., San Jose, CA). In the first event, all components passing through the ion source are fragmented under SCID. A GSH conjugate is identified by SRM of m/z 272 → 179 or 210 under wideband activation. Once a peak is observed in the SRM chromatogram, the putative precursor GSH conjugate is then confirmed and its structure is elucidated from the MS and MS^2 data acquired in the alternate scans of the same chromatographic peak. The first event could instead be an MS^2 scan of m/z 272 under SCID. GSH conjugates could then be detected from extracted ion chromatograms of both m/z 179 and 210 from the MS^2 data. However, extraction complicates the method compared to the SRM approach, which does not require additional data processing. The next two sets of four-event scans (events 2 to 9 in Scheme 1) are to acquire full scan and data-dependent acquisition with dynamic exclusion of MS^2 spectra of the three most intense ions in the negative and positive ion modes, respectively. The total acquisition time for this nine-event cycle in our method was 1.5 to 1.6 s with each event ranging from 100 to 200 ms.

The effectiveness of the method was evaluated with four test compounds, amodiaquine, clozapine, diclofenac, and fipexide, following incubation in fresh rat hepatocytes. Data employing the HRMS XoPI method was also acquired from the same samples. The total ion chromatograms (TICs) from SRM events acquired using the current method and the $XPIC_{272.0888}$ from the XoPI method are displayed in Figure 2. Each SRM

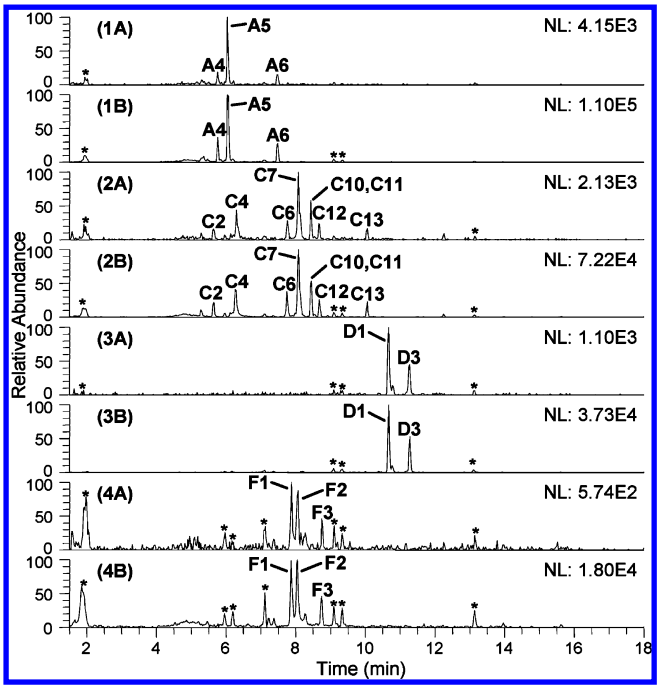


Figure 2. LC-MS profiles of rat hepatocyte incubations with test compounds acquired with SRM of the m/z 272 → 179 ion pair using a linear ion trap mass spectrometer or selected ion monitoring at m/z 272 ± 2.5 Da using a hybrid Orbitrap mass spectrometer all under SCID in the negative ion mode. Labels 1–4 refer to amodiaquine, clozapine, diclofenac, and fipexide, respectively. Panels labeled A display the total ion chromatogram from the SRM of the m/z 272 → 179 ion pair. Panels labeled B display the $XPIC_{272.0888}$, the extracted product ion chromatogram of m/z 272.0888 ± 5 ppm obtained using the XoPI method.[13] The chromatograms were constructed from data generated in the 1st event of the acquisition cycle from each method. The asterisk denotes the endogenous GSH conjugate peak, which was also observed in the nondosed rat hepatocyte samples.

TIC for the test compounds (panels 1A–4A) has a profile almost identical to the corresponding $XPIC_{272.0888}$ (panels 1B–4B). Analogous TIC profiles from SRM of the m/z 272 → 210 ion pair are shown in Figure S2 (Supporting Information).

The selectivity and sensitivity of the proposed SRM based method was comparable to the XoPI method. A total of 26 GSH conjugates from the four test compounds were identified and characterized using the SRM method from a single LC-MS run for each compound. Their MS and MS^2 properties are listed in Table S1 (Supporting Information). Identical GSH conjugates were detected using the XoPI method. The signal-to-noise ratio for each peak appeared to be similar in both SRM TICs and $XPIC_{272.0888}$ chromatograms. When the intensity of γ-EdAG ions from test compound GSH conjugates was low (Figure 2, panels 4A and 4B), a greater proportion of γ-EdAG ions from endogenous conjugates could be discerned.

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The fast scan rate of a modern linear ion trap mass spectrometer (e.g., LTQ Velos) provided the short cycle time and an adequate level of sensitivity to accommodate narrow ultra high performance liquid chromatographic peaks. Rapid scanning is necessary for the acquisition of the MS and three data dependent MS^2 scans in each polarity mode. Acquisition of data in both negative and positive ion modes helped to ensure the detection of all GSH conjugates. For example, the clozapine GSH conjugate C14 (Table S1, Supporting Information) was only detected in the negative ion mode. GSH conjugates are readily confirmed by product ions such as m/z 272 and 254 in the negative ion mode[17] as well as neutral losses such as 75, 129, 273, or 275 Da in both positive and negative ion modes.[18] To alleviate the effect of the low mass cutoff rule on the ion trap fragmentation, the activation Q value for CID was set to 0.18 (1/5 low mass cutoff) in the negative ion mode so that m/z 272 and 254 could be readily detected in the MS^2 spectrum of any precursor ion with an m/z of up to 1000. A specific example of LC-MS analysis is illustrated with the clozapine GSH conjugate C6 data presented in Figure S3 (Supporting Information).

The method described here is similar in selectivity and sensitivity to the HRMS-based XoPI method but does not require a high resolution instrument. Previously published approaches using a linear ion trap mass spectrometer required isotopically labeled GSH as the trapping agent[14−16] to facilitate detection of GSH conjugates. The current method does not require any isotope labeling and may offer a more efficient approach to bioactivation screening. In addition, the isotope labeling strategy has its own limitations: it can be used with *in vitro* incubations amenable to GSH supplementation such as microsomal incubations but not in hepatocytes or *in vivo* biomatrices. Also, for the method utilizing the data-dependent MS^2 scan initiated by the doublet peak pattern (from a mixture of the natural and stable isotope labeled GSH),[15,16] conjugates with only multiple charges or with multiply adducted GSH will not be triggered and thus not detected. Like all other fragment-structure-based approaches, the proposed method relies on formation of the deprotonated γ-EdAG ion at m/z 272 from a GSH conjugate. Although this product ion is formed from many different classes of GSH conjugates,[3−5,17] the method will not discern conjugates that do not produce the m/z 272 ion.

In conclusion, we have demonstrated a simple, selective, and label-free method for the rapid detection and characterization of GSH conjugates employing commonly available unit resolution linear ion trap mass spectrometers.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details, Figures S1−S3, and Table S1. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

γ-EdAG, gamma-glutamyl-dehydroalanyl-glycine; GSH, L-glutathione; HRMS, high resolution mass spectrometry; SCID, in-source collision-induced dissociation or in-source fragmentation; SRM, selected reaction monitoring; TIC, total ion chromatogram

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