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An integrated approach for profiling oxidative metabolites and glutathione adducts using liquid chromatography coupled with ultraviolet detection and triple quadrupole-linear ion trap mass spectrometry

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ABSTRACT

The use of liquid chromatography (LC) coupled with triple quadrupole linear ion trap (Qtrap) mass spectrometry (MS) for both quantitative and qualitative analysis in drug metabolism and pharmacokinetic studies is of great interest. Here, a new Qtrap-based analytical methodology for simultaneous detection, structural characterization and semi-quantitation of in vitro oxidative metabolites and glutathione trapped reactive metabolites was reported. In the current study, combined multiple ion monitoring and multiple reaction monitoring were served as surveying scans to trigger product ion spectral acquisition of oxidative metabolites and glutathione adduct, respectively. Then, detection of metabolites and recovery of their MS/MS spectra were accomplished using multiple data mining approaches. Additionally, online ultraviolet (UV) detection was employed to determine relative concentrations of major metabolites. Analyses of metabolites of clozapine and nomifensine in rat liver microsomes not only revealed multiple oxidative metabolites and glutathione adducts, but also identified their major oxidative metabolism and bioactivation pathways. The results demonstrated that the LC/UV/MS method enabled Qtrap to perform the comprehensive profiling of oxidative metabolites and glutathione adducts in vitro.

1. Introduction

Liquid chromatography/mass spectrometry (LC/MS) plays a critical role in both quantitative and qualitative analyses in drug metabolism and pharmacokinetic (DMPK) studies. More specifically, triple quadrupole and high resolution mass spectrometer (HRMS) instruments are frequently employed for bioanalysis and drug metabolite identification, respectively. In the pharmaceutical industry, there is an increased need for the use of a single LC/MS platform in various drug metabolism and disposition studies, especially in small DMPK groups at both academic institutes and biopharmaceutical companies. This is able to reduce cost and improve productivity. In addition, several types of drug metabolism studies require both quantitative and qualitative analysis. For example, metabolic stability assay that measures the disappearance of drugs and follow up metabolic soft-spot analysis that determines structures of major metabolites are often performed by the same scientists at the same groups [1]. Similarly, identification of metabolites and determination of their relative concentrations in plasma from the first in human study, which involve both qualitative and quantitative analyses, are carried out in the same studies [2,3]. In these cases, the use of a single LC/MS instrument can significantly improve analytical throughput.

In addition to HRMS, triple quadrupole-linear ion trap (Qtrap) mass spectrometer is an alternative platform that is well suited

Abbreviations: GSH, glutathione; LC/MS, liquid chromatography/mass spectrometry; MRM, multiple reaction monitoring; HRMS, high resolution mass spectrometry; Qtrap, triple quadrupole linear ion trap; EMS, enhanced MS; NL, neutral loss scan; PI, precursor ion scan; MIM, multiple ion monitoring; EPI, enhanced product ion scan; NAC, *N*-acetylcysteine; EIC, extracted ion chromatography; EMC, extracted MRM chromatography; NLF, neutral loss filter; PIF, product ion filter; NADPH, β-nicotinamide adenine dinucleotide 2-phosphate reduced tetrasodium salt; RLM, rat liver microsome; HLM, human liver microsome; SPE, solid phase extraction; UV, ultraviolet; DMPK, drug metabolism and pharmacokinetic.

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for both quantitative analysis and structural characterizations in DMPK studies. On top of the multiple reaction monitoring (MRM) scanning mode that is identical to that of triple quadruple instruments, Qtrap is capable of performing enhanced MS (EMS), neutral loss scan (NL), precursor ion scan (PI), and MRM as survey scans to trigger acquisition of enhanced product ion scan(EPI) of analytes [4,5]. These scan functions were utilized for fast and sensitive profiling and structural characterization of in vitro and in vivo drug metabolites, including screening of reactive metabolites [6–9]. For example, negative precursor ion scanning of *m/z* 272 to trigger acquisition of positive MS/MS spectra via polarity switching has shown a high through screening capability for glutathione (GSH) adducts [10]. On the other hand, MRM-EPI was capable of targeting up to 500 known analytes with sensitivity and selectivity much better than PI-EPI or NL-EPI. However, MRM-EPI was incapable of detecting drug metabolites if their fragmentations are unknown or unpredictable. A Qtrap-based multiple ion monitoring (MIM)-EPI scan method was developed to overcome the limitation of MRM-EPI and applied to the detection and identification of unknown drug metabolites regardless of their fragmentation patterns [11]. Additionally, various data mining approaches were applied for filtering out oxidative metabolites and reactive metabolites-trapped by GSH, NAC and cyanide, including extracted ion chromatography (EIC) based on predicted metabolite molecular weights, extracted MRM chromatography (EMC) based on predicted metabolite MRM transitions, and neutral loss filter (NLF) and product ion filter (PIF) based on predicted metabolite fragmentation [12,13]. The data mining approaches enabled high throughput analysis, in which data-acquisition can be continuously performed using a generic method, while detection of metabolites ions and recovery of their MS/MS spectra can be carried out via data mining.

Although each of above Qtrap-based methods has its own advantages in analyzing reactive metabolites trapped by various trapping agents, all of them share a major limitation that they were incapable of analyzing oxidative metabolites during the reactive metabolites screening. To obtain oxidative metabolite information from the same samples, the use of a different LC/MS method for determination of oxidative metabolites was required. In many cases, oxidative metabolites are precursors or downstream products of reactive intermediates. The identification of these oxidative metabolites can facilitate the elucidation of structures of reactive intermediates and chemical mechanisms of bioactivation [14]. More importantly, the quantitative information on bioactivation products related to a total of metabolites has been utilized to determine human body burden of reactive metabolites, which is one of key parameters in risk assessment of drug bioactivation in humans [15,16]. A common approach for quantitative determination of reactive metabolites and stable oxidative metabolites is to use radiolabeled test compounds. However, the synthesis of radiolabeled test compounds at the lead optimization stage is not practical.

The major objective of this study was to develop a practical Qtrap-based approach for qualitative and quantitative screening of both GSH-trapped reactive metabolites and stable oxidative metabolites in the same injection (Fig. 1). In the approach, combined MIM-MRM-EPI was employed to collect MS/MS data of the parent drug, oxidative metabolites and GSH adducts. Metabolite detection and recovery of their MS/MS spectra were carried out via multiple data mining processes. Additionally, on-line UV detection was employed to determine relative concentrations of oxidative metabolites and GSH adducts. Clozapine and nomifensine were used as model compounds to examine the effectiveness of this method, which are well known for undergoing extensive biotransformation to form multiple oxidative metabolites and GSH adducts in liver microsomes. The current approach was capable of performing LC/UV/MS data acquisition in a high throughput manner, while data mining and structural elucidation of metabolites of individual samples can be performed in parallel without interruption of the data acquisition.

2. Material and methods

2.1. Chemicals and materials

Nomifensine and clozapine were purchased from National Institutes for Food and Drug Control (Beijing, China). Trichloroacetic acid was acquired from Sinopharm Chemical Reagent Co., LTD (Shanghai, China). Reduced GSH and NADPH were purchased from Beijing Dingguochangsheng Biotechnology Co., LTD (Beijing,

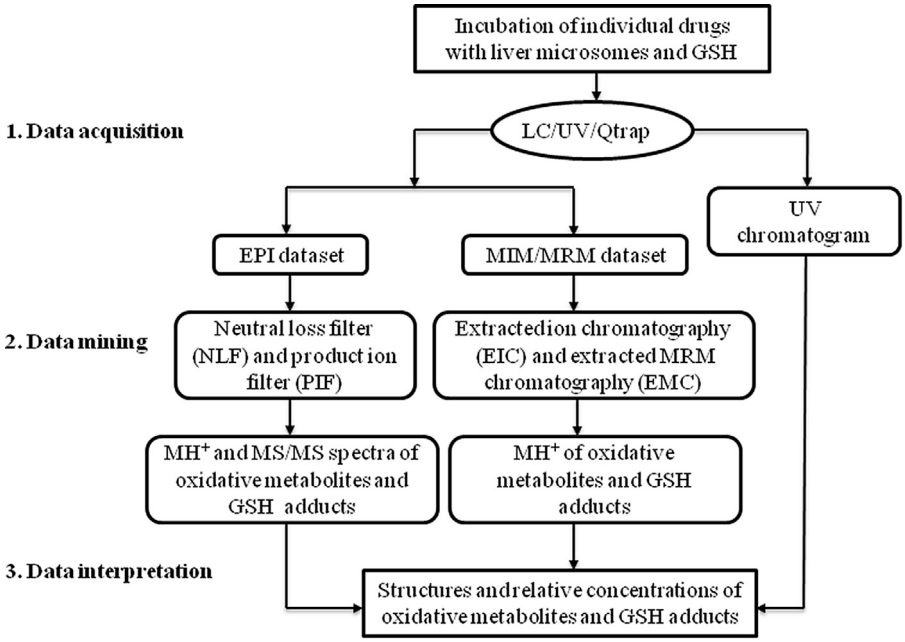

Fig. 1. Workflow of detection, structural characterization and quantitative estimation of oxidative metabolites and GSH adducts using LC coupled with UV detection and Qtrap.