

## DOCTORAL THESIS

# Development of chemical derivatization methods for cis-diol-containing metabolite detection by using liquid chromatography-mass spectrometry

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## ABSTRACT

*Cis*-diol-containing metabolites have attracted increasing attention in recent years. These metabolites widely exist in the body fluids and tissues. They play important roles in the structure, function and metabolic activity of cells. Some of them are related to cell proliferation and metabolic processes. And they have been used to denote a state of disease as potential biomarkers. Several methods have been developed for the analysis of *cis*-diol-containing metabolites. However, these methods faced a challenge to separate and detect isomers of these compounds, particularly for compounds with low abundance and high polarity. Therefore, novel methods were necessary to improve the separation and detection sensitivity of this kind of metabolites.

With this aim, chemical derivatization methods were developed for *cis*-diol-containing metabolite detection by using liquid chromatography-mass spectrometry in this project. These methods were optimized and validated to achieve the optimal reaction conditions. And they were applied to study real-world biological systems, including the changes of modified nucleosides in hepatocellular carcinoma (HCC) nude mice and toxic effects of bisphenol A (BPA) exposure.

Firstly, the derivatization reaction of *cis*-diol compounds with acetone were optimized. Factors that affected reaction efficiency were investigated by reacting guanosine (G) with acetone. The optimal reaction conditions were validated by detecting four acetonides of urinary nucleosides by using LC-MS/MS. The results

showed that the approach had good linearity, accuracy and precision. The recoveries were ranged from 92.9% to 103.5%. It indicated that the assay was reproducible. The robust method should be potentially useful for the analysis of modified nucleosides and other *cis*-diol-containing metabolites in biological samples. The validated derivatization method was applied to determine urinary nucleosides by LC-MS. This method not only improved the retention of nucleosides on reversed-phase column, but also reduced the matrix effect from urine samples and enhanced detection sensitivity of mass spectrometry. Isotope labeling method with acetone-d<sub>6</sub> and multivariate statistical analysis enabled the positive identification of 56 nucleosides, including 52 modified nucleosides. The obtained results indicated that the derivatization method was practical, fast and effective for the identification of urinary nucleosides. It was successfully applied to study the changes of urinary nucleosides in nude mice bearing HCC. Some significantly changed nucleosides were identified as potential biomarkers.

Subsequently, this approach was modified by employing parallel reaction monitoring (PRM) method which was based on high resolution MS to detect urinary nucleosides in rats exposed to BPA. Comparing to the data acquired by triple quadrupole MS with neutral loss scanning, higher specificity and sensitivity were achieved by using PRM scanning mode. Therefore, more nucleosides were identified by using the method in urine samples (from 56 up to 66). The changes of the detected nucleosides were studied in the rats exposed to BPA. Various trends of

modified nucleosides were observed with different dose BPA exposure. Specifically, the high-dose exposure group was the most strongly affected. The biomarker of RNA oxidation, 8-hydroxyguanosine (8-oxoG), showed significant change in this group. It proved that BPA exposure could induce RNA damage when the dose of BPA was beyond a certain amount.

Except for nucleosides, other *cis*-diol-containing metabolites, such as carbohydrates, were also studied by using the derivatization method. Acetone and acetone- $d_6$  were applied to label the *cis*-diol metabolites. Based on the chemical isotope labeling, *cis*-diol metabolites were easily recognized from urine samples. Influence of BPA exposure on these metabolites was investigated by comparing different doses of BPA administration on rats. Analytes showed noticeable difference were highlighted. Pathway analysis indicated that galactose metabolism, nucleoside and its analogues metabolism were disturbed.

The derivatization method was extended to quantify nucleotides in plasma samples. According to the specific physical-chemical properties of nucleotides, the method was improved to fit the requirement of analysis by using 1,1-Dimethoxycyclohexane (DMCH) as derivatization agent and formic acid (FA) as catalyst. Tip micro-columns packed with  $TiO_2$  were used for selective adsorption of nucleotides in the plasma. Then in-situ derivatization were carried out to change the polarity of targeted compounds. LC-MS analysis of the derivatization products were employed without using ion-pairing reagents. This method exhibited a high

selectivity for the extraction of nucleotides. After derivatization, retention of nucleotides on reversed-phase C<sub>18</sub> column was improved. Complete separation of nucleotides with the same base was achieved. The peak shape was symmetrical and the tailing was eliminated by using high pH mobile phase. The method settled the problems of nucleotide detection, which were poor retention, trailing, in-source fragmentation and contamination of ion-pairing reagents. The quantitative method was successfully applied to determine the content of nucleotides in plasma samples of rats exposed to BPA. It was simple and fast, as well as good selectivity and stability. It could be extended to detection of other phosphorylated metabolites with similar structure.

To our best knowledge, it was the first time to employ derivatization methods to detect *cis*-diol-containing metabolites. The methods decreased the matrix effects of complex biological samples, and also decreased the polarity of *cis*-diol-containing metabolites. The changes of properties not only improved the chromatographic separation, but also enhanced the MS intensities. The methods overcame the problems of *cis*-diol-containing metabolite detection on reversed-phase column. They were successfully applied to study the changes of *cis*-diol-containing metabolites of HCC and toxic effects of BPA exposure. The method might be extended to determine other *cis*-diol-containing metabolites in urine samples as well as in cells, tissues and plasma samples. It might be valuable for the understanding of the roles of *cis*-diol-containing metabolites in in cell metabolism.

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