

## DOCTORAL THESIS

### Development and applications of liquid chromatography-tandem mass spectrometry in clinical areas

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**Development and applications of liquid chromatography-  
tandem mass spectrometry in clinical areas**

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**A thesis submitted in partial fulfillment of the requirements  
for the degree of  
Doctor of Philosophy**

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## Abstract

Over the last 20 years, there has been tremendous growth in the area of mass spectrometry (MS) and it has gained a prominent position among analytical methods due to its versatility, high sensitivity and the commercial availability of instruments that are relatively affordable, robust, and increasingly user friendly. The ability to separate complex mixtures and identify the components provides an indispensable tool for scientists for monitoring environmental pollution, investigating crime, and recently, in clinical field, MS has expanded from research to clinical routine applications.

All the developed analytical methods in my research works are based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) which uses LC as a separation platform and detection being made by MS. Focus of the study is on applications of this technique on clinical areas which include endocrinology, toxicology and inborn errors of metabolism.

For endocrinology, an improved LC-MS/MS method was developed for the determination of urinary free cortisol for the diagnosis of Cushing's syndrome and is described in **Chapter 3**. The improvement includes sample preparation procedure and analytical method. Data comparison between this novel approach and conventional immunoassay showed markedly improved assay selectivity and sensitivity. The present method also showed improvement in the positive predictive value for the diagnosis of Cushing's syndrome.

In the area of toxicology, determination of tetrodotoxin in human urine and plasma in real patient samples are recorded in **Chapter 4**. The new method uses C18 and hydrophilic interaction liquid chromatography solid phase extraction (C18-HILIC SPE) to reduce matrix interference and overcome severe ion suppression problem which is a major challenge in detection of TTX in urine.

Applications of LC-MS/MS in the study of inborn errors of metabolism (IEM) have drawn much attention in recent years over the world. Among various metabolites, we focused on urinary acylglycines which helps in the diagnosis of some IEM. A new method was developed and validated; pediatric reference intervals in local Chinese was established and recorded in **Chapter 5**. Furthermore, a novel liquid chromatography-atmospheric pressure chemical ionization-tandem mass spectrometry (LC-APCI-MS/MS) method for the determination of cholesterol sulfate (CS) in human plasma was introduced in **Chapter 6**. Previously the measurement of plasma CS mainly help in the differential diagnosis of ichthyosis, until recently, a hypothesis suggested that CS deficiency might be related to autism. Although the relationship between CS deficiency and autism is only a hypothesis, it is worth exploring. Setting up a novel analytical method for plasma CS, with simple sample preparation and high analytical sensitivity, and using it to determine plasma CS in autistic and non-

autistic children are the specific purposes of this chapter. In the broader context, our main goal is to offer some help for those suffering from autistic conditions.

The above studies are just the tip of the iceberg of LC-MS/MS applications in clinical areas, reliance on this technique for biochemical diagnosis is ever increasing as evidence by the increasing number of publications. The future of LC-MS/MS in the clinical laboratory is promising and exciting.

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