

DOCTORAL THESIS

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

Fong, Mei Wah Bonnie

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

FONG Mei Wah, Bonnie

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

Principle supervisor: Dr. LEUNG Sze Yin, Kelvin

Hong Kong Baptist University

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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.

Table of Contents

Declaration.....	i
Abstract.....	ii
Acknowledgements.....	iv
Table of Contents.....	v
List of Tables.....	ix
List of Figures.....	xi
List of Abbreviations and Symbols	xv
Chapter 1 Introduction.....	1
1.1 Brief introduction to mass spectrometry.....	1
1.2 The use of LC-MS/MS in clinical laboratories.....	1
1.3 Aims of the study.....	7
1.4 Biological sample collection.....	9
1.5 References.....	11
Chapter 2 Instrumentation and interface of liquid chromatography and tandem mass spectrometry.....	15
2.1 Inlet.....	17
2.1.1 Reversed phase chromatography (RPC).....	17
2.1.2 Normal phase chromatography (NPC).....	18
2.2 Ionization.....	18
2.2.1 Electrospray Ionization (ESI)	19
2.2.2 Atmospheric Pressure Chemical Ionization (APCI).....	21
2.3 Mass analyzers.....	27
2.3.1 Quadrupole MS.....	29
2.3.2 Tandem mass spectrometry.....	31
2.4 Scanning techniques in tandem mass spectrometry.....	32
2.4.1 Product ion scanning.....	32
2.4.2 Precursor ion scanning.....	33
2.4.3 Selected reaction monitoring (SRM).....	33
2.4.4 Constant neutral loss scanning.....	34
2.5 QTRAP MS.....	36
2.6 Vacuum system.....	37
2.7 Detection.....	38
2.8 LC-MS/MS.....	39
2.9 References.....	40

Chapter 3 Improved liquid chromatography-tandem mass spectrometry method in clinical utility for the diagnosis of Cushing’s syndrome.....41

3.1	Introduction: Cushing’s syndrome.....	41
3.2	The structure and biosynthetic pathway of cortisol.....	42
3.2.1	Regulation of cortisol level in blood and its function in humans..	44
3.2.2	Diurnal variation of cortisol production.....	45
3.3	The diagnosis of Cushing’s syndrome.....	47
3.3.1	Urinary free cortisol.....	47
3.3.2	LDDST.....	48
3.3.3	Late night salivary cortisol.....	48
3.4	Methods for the determination of urinary free cortisol.....	49
3.5	Experimental.....	50
3.5.1	Materials and instrumentation.....	50
3.5.1.1	Standards and reagents.....	50
3.5.1.2	Chromatography.....	51
3.5.1.3	Mass spectrometry.....	51
3.5.2	Assay development.....	52
3.5.2.1	Quantitative and qualitative identification.....	57
3.5.3	Sample preparation.....	60
3.5.3.1	Patient samples.....	60
3.5.3.2	Sample preparation for immunoassay.....	60
3.5.3.3	Sample preparation for LC-MS/MS.....	62
3.5.4	Method validation.....	63
3.5.5	Clinical utility.....	64
3.6	Results.....	64
3.6.1	Linearity and limit of quantitation.....	64
3.6.2	Determination of extraction recovery.....	64
3.6.3	Inter- and intra-assay precision.....	66
3.6.4	Accuracy.....	67
3.6.5	Ion suppression or enhancement.....	68
3.6.6	Interferences.....	72
3.6.7	Sample stability.....	72
3.6.8	Method comparison.....	76
3.7	Discussion.....	79
3.8	Chapter summary.....	83
3.9	References.....	84

Chapter 4 Development and validation of a high-throughput double solid phase extraction–liquid chromatography–tandem mass spectrometry method for the determination of tetrodotoxin in human urine and plasma.....87

4.1	Introduction.....	87
4.2	TTX poisoning incidents and clinical grading system.....	88
4.3	Method choices for TTX determination	91
4.4	Experimental.....	94
4.4.1	Materials and reagents.....	94
4.4.2	Liquid chromatography.....	95
4.4.3	Mass spectrometry.....	95

4.5	Assay development.....	96
4.5.1	Quantitative and qualitative identification.....	98
4.6	Sample preparation.....	99
4.6.1	Patient samples.....	99
4.6.2	Sample preparation for LC-MS/MS.....	99
4.6.3	Determination of creatinine in urine samples.....	100
4.6.4	Method validation.....	100
4.7	Results and discussion.....	102
4.7.1	Effect of ion pair reagent content on column retention.....	102
4.7.2	Ion suppression or enhancement.....	106
4.7.3	Recovery.....	115
4.7.4	Quantitative study of matrix effect.....	116
4.7.5	Linearity, LLOD and LLOQ.....	117
4.7.6	Precision.....	118
4.7.7	Accuracy.....	118
4.7.8	Comparison with other methods.....	119
4.7.9	Patient samples for TTX determination.....	119
4.8	Chapter summary.....	123
4.9	References.....	124

Chapter 5 Quantification of acylglycines in human urine by HPLC electrospray ionization-tandem mass spectrometry and the establishment of pediatric reference interval in local Chinese.....129

5.1	Introduction.....	129
5.2	Experimental.....	133
5.2.1	Materials and reagents.....	133
5.2.2	Liquid chromatography.....	133
5.2.3	Acylglycine standards butylation procedure.....	134
5.2.4	Mass spectrometry.....	134
5.2.5	Biological samples.....	142
5.2.6	Determination of creatinine in urine samples.....	142
5.2.7	Solid phase extraction and preparation of acylglycine-butyl ester.....	142
5.2.8	Standard curves for acylglycines.....	143
5.2.9	Matrix effect study.....	143
5.3	Results and discussion.....	144
5.3.1	Ion suppression or enhancement.....	146
5.3.2	Linearity and recovery.....	148
5.3.3	LLOD, LLOQ, accuracy and imprecision assessment.....	151
5.3.4	Sample stability.....	154
5.3.5	Application to biological samples.....	154
5.4	Chapter summary.....	167
5.5	References.....	168

Chapter 6	Determination of plasma cholesterol sulfate by LC-APCI-MS/MS in the context of pediatric autism.....	171
6.1	Introduction.....	171
6.2	Experimental.....	175
6.2.1	Materials.	175
6.2.2	Standard preparation.....	176
6.2.3	Calibrator preparation.....	176
6.2.4	Biological samples.....	176
6.2.5	Sample preparation.....	177
6.2.6	LC-APCI-MS/MS.....	177
6.3	Method validation.....	180
6.3.1	Linearity, recovery and matrix effect.....	180
6.3.2	Lower limit of quantification and lower limit of detection.....	181
6.3.3	Accuracy and precision.....	182
6.3.4	Carryover.....	182
6.3.5	Stability.....	183
6.4	Results and discussion.....	183
6.4.1	Linearity, recovery, matrix effect, LLOQ and LLOD.....	187
6.4.2	Accuracy and precision.....	191
6.4.3	Carryover.....	191
6.4.4	Sample stability.....	192
6.4.5	Application to biological samples.....	193
6.5	Chapter summary.....	197
6.6	References.....	198
Chapter 7	Concluding remarks.....	201
	Outcome of Thesis Work.....	203
	Curriculum Vitae.....	206