

RAPID QUANTIFICATION OF QUININE BY MULTI-STACKING IN A  
PORTABLE MICROCHIP ELECTROPHORESIS SYSTEM

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## **DEDICATION**

*Specially dedicated to my beloved families for all support and  
encouragement in completing this study*

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## ABSTRACT

A new multi-stacking pre-concentration procedure based on field-enhanced sample injection (FESI), field-amplified sample stacking, and transient isotachopheresis was developed. The new procedure was implemented in a compact microchip electrophoresis (MCE) with a double T-junction glass chip, coupled with an on-chip capacitively coupled contactless conductivity detection ( $C^4D$ ) system. A mixture of the cationic target analyte and the terminating electrolyte (TE) from the two sample reservoirs was injected under FESI conditions within the two sample-loading channels. At the double-T junction, the stacked analyte zones were further concentrated under field-amplified stacking conditions and then subsequently focused by transient-isotachopheresis and separated along the separation channels. The proposed multi-stacking strategy was verified under an Universal Serial Bus (USB) fluorescence microscope employing Rhodamine 6G as the model analyte. This developed approach was subsequently used to monitor the target quinine present in human plasma samples. The total analysis time for quinine was approximately 200 s with a sensitivity enhancement factor of approximately 61 when compared to the typical gated injection. The detection and quantification limits of the developed approach for quinine were 3.0  $\mu\text{g/mL}$  and 10  $\mu\text{g/mL}$ , respectively, with intraday and interday repeatability (%RSDs,  $n=5$ ) of 3.6 % and 4.4 %. Recoveries in spiked human plasma were 98.1 % – 99.8 %.

## ABSTRAK

Satu prosedur pra-pemekatan pelbagai timbunan baharu yang berdasarkan suntikan sampel dipertingkatkan medan (FESI), timbunan sampel diperkuatkan medan, dan isotakoforesis sementara telah dibangunkan. Teknik baharu ini telah dilaksanakan dalam elektroforesis kapilari mikrochip (MCE) padat dengan cip kaca dua simpang-T, digandingkan dengan sistem pengesanan kekonduksian tanpa sentuh gandingan kapasitif atas cip. Campuran analit sasaran kationik dengan elektrolit penamat (TE) dari dua takungan sampel telah disuntik di bawah keadaan FESI di dalam dua saluran masukan-sampel. Di dua simpang-T, zon timbunan analit telah dipekatkan lagi di bawah keadaan timbunan diperkuatkan medan dan kemudian difokuskan oleh isotakoforesis sementara dan dipisahkan di saluran permisahan. Strategi pelbagai timbunan yang dicadangkan ini telah disahkan di bawah mikroskop pendarfluor Bas Bersiri Semesta (USB) dengan menggunakan Rhodamine 6G sebagai analit model. Pendekatan yang dibangunkan ini kemudian digunakan untuk memantau kehadiran kuinin sasaran di dalam sampel plasma manusia. Masa keseluruhan analisis bagi kuinin ialah 200 s dengan faktor peningkatan kepekaan lebih kurang 61 kali ganda berbanding dengan suntikan melalui kawalan biasa. Had pengesanan dan kuantifikasi kaedah ini bagi kuinin ialah masing-masing 3.0 µg/mL dan 10 µg/mL dengan kebolehulangan dalam-hari dan antara-hari (%RSD,  $n = 5$ ) masing-masing 3.6 % dan 4.4 %. Perolehan semula analit daripada sampel pakuan plasma manusia adalah dalam julat 98.1 % – 99.8%.

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## LIST OF ABBREVIATIONS

BGE	-	Background electrolyte
CE	-	Capillary electrophoresis
CGE	-	Capillary gel electrophoresis
CIEF	-	Capillary isoelectric focusing
CITP	-	Capillary isotachopheresis
CZE	-	Capillary zone electrophoresis
C <sup>4</sup> D	-	Capacitively coupled contactless conductivity detector
DI	-	Deionised
EC	-	Electrochemical
EOF	-	Electroosmotic flow
FASI	-	Field amplified sample injection
FESI	-	Field enhanced sample injection
HPLC	-	High performance liquid chromatography
ITP	-	Isotachopheresis
LE	-	Leading electrolyte
LIF	-	Laser-induced fluorescence
LOD	-	Limit of detection
LOQ	-	Limit of quantification
LVSS	-	Large volume sample stacking

MEKC	-	Micellar electrokinetic capillary chromatography
MeOH	-	Methanol
MGE	-	Microchip gel electrophoresis
MS	-	Mass spectrometry
MSS	-	Micelle to solvent stacking
NaDCHA	-	Sodium deoxycholate
NaOH	-	Sodium hydroxide
SDS-PAGE	-	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
S/N	-	Signal to noise
TDM	-	Therapeutic drug monitoring
TE	-	Terminating electrolyte
tITP	-	Transient isotachopheresis
USB	-	Universal Serial Bus

## LIST OF SYMBOLS

cm	-	Centimetre
I.D	-	Internal diameter
kV	-	Kilovolts
MΩ-cm	-	Megaohm-centimetre
mg/mL	-	Milligram per millilitre
min	-	Minute
mm	-	Millimetre
mM	-	Millimolar
ng/mL	-	Nanogram per millilitre
nm	-	Nanometre
pg/mL	-	Picogram per millilitre
s	-	Second
µg/mL	-	Microgram per millilitre
µL	-	Microlitre
µm	-	Micrometre
µS/cm	-	MicroSiemens per centimetre
V·s	-	Volt·second

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# CHAPTER 1

## INTRODUCTION

### 1.1 Background of Study

Electrophoresis is the movement of electrically charged particles in a conductive medium according to their charge-to-size ratio under the influence of an electric field. Electrophoresis of cation is called cataphoresis, while electrophoresis of anion is called anaphoresis. In 1930, Arne Tiselius introduced the use of electrophoresis as an analytical technique to separate proteins in free solution [1]. Capillary electrophoresis (CE) is now a mature and powerful technique for analytical separation which provides several advantages such as high efficiency and the consumption of small amount of sample [2]. Nowadays, simple, fast and sensitive analytical methods and instrumentation are common in separation science. Microchip capillary electrophoresis (MCE) was invented by Manz and co-workers in the early 1990s. Miniaturized MCE, also referred to as lab-on-a-chip, integrates all functions of conventional CE into a single device. Unlike conventional CE which consists of essentially a single capillary, multiple fluidic channels or different designs of the microchip offer the potential for high-throughput and innovative and versatile analytical methods [3]. Hence, MCE has brought a lot of benefits such as low consumption of materials (including the sample), rapid analysis and portability.

Despite their advantages, the detection limit of MCE systems is one of their often-cited limitations, particularly in comparison to other instrumentation like conventional CE and liquid chromatography (LC) [4]. In order to overcome the sensitivity limitation, researchers have proposed several on-line pre-concentration techniques such as field-amplified stacking injection [5-7], large-volume sample stacking (LVSS) [8], isotachopheresis (ITP) [9-11], sweeping [12], dynamic pH junction [13], multi-stacking strategy [14-15] and electrokinetic supercharging [16-19].

Field-amplified sample stacking (FASS) is the simplest method to increase the sensitivity in CE. Haglund and Tiselius were the first group to introduce the stacking method using low-conductivity background electrolyte (BGE) for the sample [20]. In order to perform FASS, lower conductivity of sample solution than BGE solution is mandatory. Electrokinetic supercharging (EKS) was first introduced by Hirokawa and co-workers in 2003 [16]. EKS is a powerful online pre-concentration technique in electrophoresis which combines field enhanced sample injection (FESI) and transient isotachopheresis (tITP). In EKS, analytes are introduced electrokinetically between the leading electrolyte (LE) and terminating electrolyte (TE). Although EKS is considered as a most powerful on-line pre-concentration technique in CE, it is still not widely implemented in MCE. Until now, there have only been a few publications that have discussed integrating EKS into microchip gel electrophoresis to perform DNA fragment analysis using single channel or cross-column geometry chips [17-19].

Quinine is one of the most powerful antimalarial agents and is widely used to treat *Plasmodium falciparum*, a parasite that causes malaria, in areas where multiple drug resistance. The treatment of malaria with quinine was the first successful use of a chemical compound in the treatment of an infectious disease. Until 1820, the *Cinchona* bark was dried, ground into powder, mixed with a liquid and administered

orally to a sick person. Since 1820, quinine has been extracted from the *Cinchona* bark [21]. Over the years, quinine was considered as a safe drug but adverse allergic reactions can still occur to some of patients [22]. Currently, quinine is still the drug of choice for severe and complicated malaria disease caused by the *Plasmodium falciparum*. Moreover, the Food and Drug Administration (FDA) also warned against the usage of quinine in beverages for the treatment of leg cramps. In order to overcome this problem, therapeutic drug monitoring (TDM) was conducted to ultimate the dosage of the drug in the human body. A quinine level of 10 – 15  $\mu\text{g/mL}$  in patient plasma was suggested for effective parasite clearance and tolerable side effects, but these levels were described as toxic in non-malaria subjects [23].

## 1.2 Problem Statement

Various techniques have been described for the determination of quinine in biological fluids and beverages, which include spectrophotometry [24], liquid chromatography [25], fluorometry [26], polarography [27], and CE [28] techniques with a variety of detection systems. Although the established methods are beneficial in monitoring quinine content in biological fluid and beverages samples, they are also existed some drawbacks like time-consuming and require expensive instrumentation. Moreover, bulky size of the instrument also become a critical issue in portability when requires to access TDM for patients living in rural and remote areas. Furthermore, the determination of quinine using the MCE platform has not yet been reported. Although MCE able to provide a rapid monitoring for target analyte within few minute but poor detection sensitivity always be its limitation in practical analysis of real sample. With this, a rapid, portable and more sensitive on-line pre-

concentration technique is developed for the determination of quinine with a portable MCE platform.

### **1.3 Objectives of the Study**

The objective of this study are:

- To study the new multi-stacking strategy which involves the integration of EKS and FASS into MCE and visualize the process involved using Rhodamine 6G as a model analyte detected by a fluorescence microscope.
- To optimize the operational parameters and subsequently validate the newly established multi-stacking strategy with MCE coupled with C<sup>4</sup>D detector for monitoring of quinine in human biological fluid.

### **1.4 Scope of the Study**

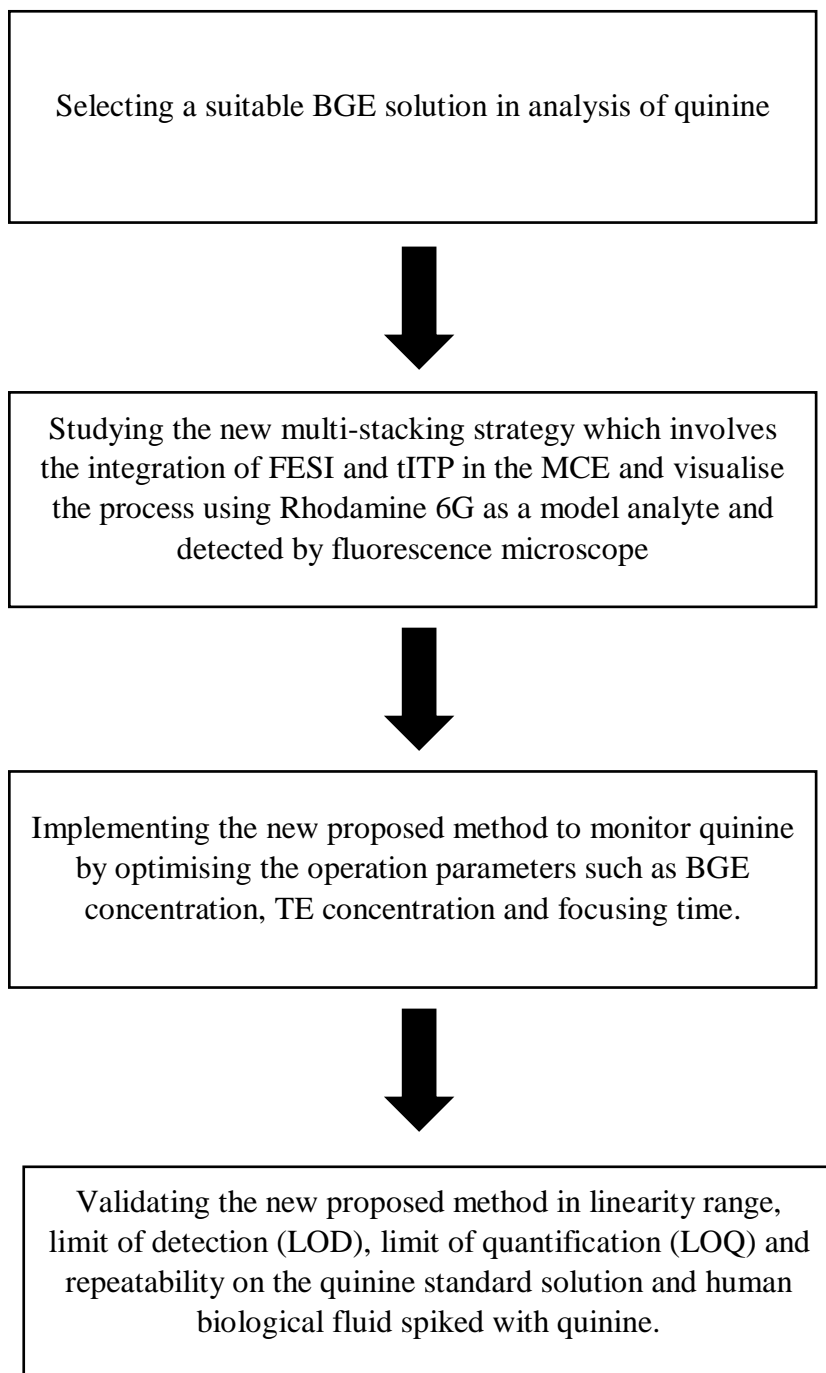
Non aqueous based MCE was demonstrated to analyse quinine in real human plasma. A new on-line pre-concentration technique that involves EKS and FASS was

implemented in an established battery-powered portable MCE system coupled with C<sup>4</sup>D detector. The multi-stacking strategy is visualized using Rhodamine 6G as a model analyte under a fluorescence microscope. The verified method is subsequently applied to the monitoring of quinine. Several operational parameters were comprehensively optimized in the MCE system such as the concentration of the background electrolyte, concentration of the leading and terminating electrolyte, concentration of acid in sample solution and focusing time. The developed method is then validated and subsequently applied for the determination of quinine in human biological fluid.

## **1.5 Significance of the Study**

In this study, a significant contribution in developing a new multi-stacking strategy in an established MCE system for the determination of quinine in biological fluid. Fundamental concept of the EKS technique in conventional CE system was transferred into a miniaturized MCE system. A monitoring assay for quinine sulfate is crucial for guiding treatment decisions, therapeutic monitoring, pharmacokinetic and bioavailability studies, as well as the quality control of the dosage forms. The portable MCE system allows the analysis to be performed on-site and this is important in improving the quality of health of people in remote locations and rural areas. Moreover, the development of new highly efficient, fast and simple methods to perform separation could improve the efficiency of analytical performance in quinine monitoring. It is believed that the new protocol can be applied in a wide range of applications.

## 1.6 Flowchart/Scheme of Planned Work



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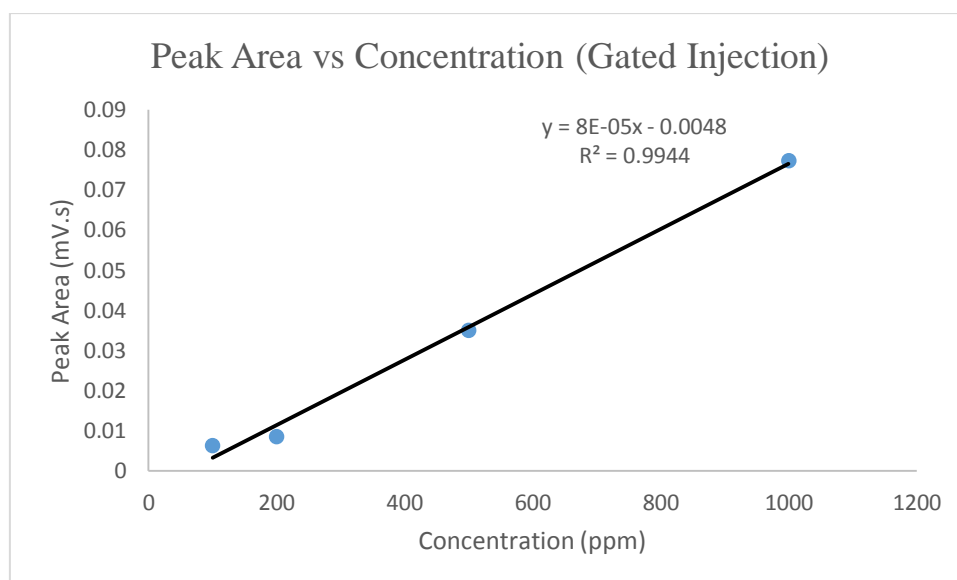
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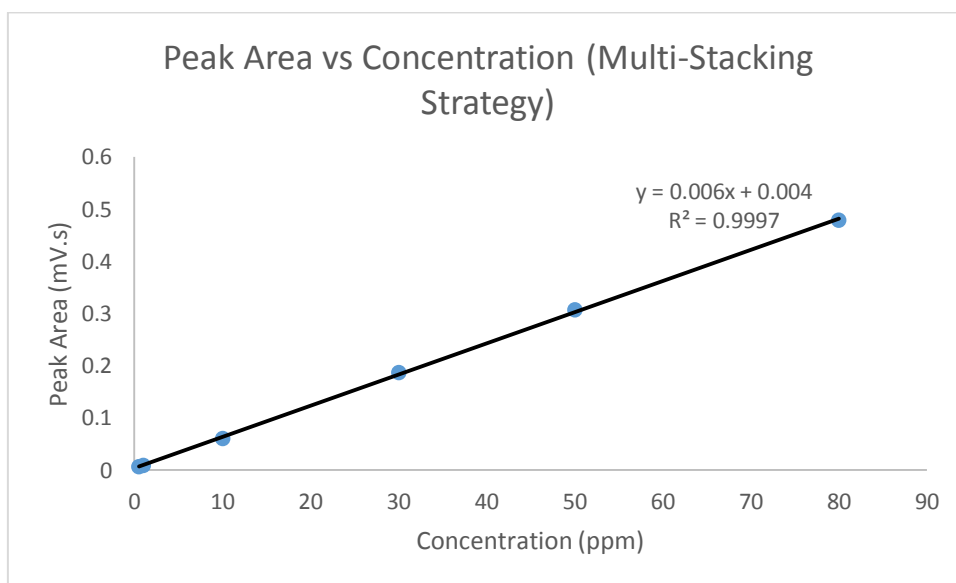
## APPENDICES A

### The calibration Graph for Typical Gate Injection Method



## APPENDICES B

### The calibration Graph for Proposed Multi-Stacking Strategy (Standard Solution)



## LIST OF PUBLICATION

1. Chee Tung Tai and Hong Heng See. Rapid Quantification of Quinine by Multi Stacking Strategy in a Portable Microchip Electrophoresis System. *Electrophoresis*, 2018. 00: 1-7.

## LIST OF PRESENTATION RELATED TO THIS STUDY

1. Chee Tung Tai & Hong Heng See. “Rapid Quantification of Quinine by Multi Stacking in a Portable Microchip Electrophoresis System”, Oral presentation at The 1<sup>st</sup> ACS Asia-Pacific International Chapters Conference 6<sup>th</sup> November, 2017, Jeju, Korea organized by ACS APICC.