

SOLID PHASE MEMBRANE TIP EXTRACTION - MICROEMULSION  
ELECTROKINETIC CHROMATOGRAPHY OF SELECTED  
NON-STREOIDAL ANTI-INFLAMMATORY DRUGS

IZDIANI MOHD YATIM

UNIVERSITI TEKNOLOGI MALAYSIA

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IZDIANI MOHD YATIM

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*In the name of Allah, the Most Merciful and the Most Beneficent. This thesis is dedicated to my beautiful mother Zalilah Naemat, my late father Mohd Yatim Sa'at, my beloved husband Muhammad Ilyas Mohd Ramdzan, my family and friends.*

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## ABSTRACT

Arylalkanoic acid drugs belong to the group of non-steroidal anti-inflammatory drugs (NSAIDs). These drugs are often used for the treatment of fever and minor pain due to its capability to inhibit prostaglandin productions which act as a messenger molecule in human body. There are many methods used in previous research to analyse arylalkanoic acids drugs but most of the methods require high organic solvent consumption, time consuming and involve complex sample derivatization. Solid phase extraction (SPE) is the common method used for sample preparation of NSAIDs but also involves high organic solvent consumption and is time consuming. To overcome the problems, solid phase membrane tip extraction (SPMTE) coupled with microemulsion electrokinetic chromatography (MEEKC) were used in this study and its performance was evaluated. Under the optimum MEEKC and SPMTE conditions, good linearity was obtained in the range of 0.25 to 4.00  $\mu\text{g/mL}$  with good coefficient of determination ( $r^2 > 0.9985$ ). Good repeatability was obtained with percentage relative standard deviation (% RSD) of 1.04 - 1.31% ( $n=3$ ). Limit of detection (LOD) ( $S/N = 3$ ) was satisfactory for all the selected drugs (0.14 - 0.18  $\mu\text{g/mL}$ ). The average relative recoveries of the selected drugs in spiked water sample were good (99-104%). Combination of SPMTE procedure and the MEEKC method was then applied to the determination of spiked sulindac, ketorolac and aceclofenac in human urine samples. The percentage recoveries of the three NSAIDs obtained from the SPMTE-MEEKC method were good, ranging from 79 to 96%. Percentage relative standard deviation (% RSD), ( $n=3$ ) for the extraction process was also good ( $< 3.7\%$ ). The result was then compared to SPE-MEEKC method. SPE-MEEKC method shows slightly higher percentage recovery (95 - 112%) and lower RSD % ( $n=3$ ) (1.33 - 2.06%) than SPMTE-MEEKC method. The SPMTE-MEEKC method was proven to be applicable to human urine analysis of sulindac, ketorolac and aceclofenac with faster analysis time and low amount of organic solvent used than in SPE-MEEKC method.

## ABSTRAK

Dadah asid arilalkanoik tergolong di dalam kumpulan dadah anti-radang bukan-steroid (NSAIDs). Dadah ini biasanya digunakan bagi merawat demam dan sakit kecil kerana kebolehnya untuk merencat penghasilan prostaglandin yang bertindak sebagai molekul penghantar di dalam badan manusia. Banyak kaedah yang telah digunakan dalam penyelidikan terdahulu untuk analisis dadah asid arilalkanoik tetapi kebanyakan daripada kaedah tersebut melibatkan penggunaan pelarut organik yang banyak, mengambil masa yang lama dan melibatkan penerbitan sampel yang kompleks. Pengekstrakan fasa pepejal (SPE) adalah kaedah yang biasa digunakan untuk penyediaan sampel NSAIDs tetapi juga melibatkan penggunaan pelarut organik yang banyak dan mengambil masa yang lama. Untuk mengatasi masalah ini, pengekstrakan muncung membran fasa pepejal (SPMTE) yang digabungkan dengan kromatografi elektrokinetik mikroemulsi (MEEKC) telah digunakan dan prestasinya dinilai. Di bawah keadaan optimum SPMTE dan MEEKC, kelinearan yang baik diperoleh dalam julat 0.25 hingga 4.00  $\mu\text{g/mL}$  dengan pekali penentuan yang baik ( $r^2 > 0.9985$ ). Kebolehulangan yang baik diperoleh dengan peratus sisihan piawai relatif (% RSD) ialah 1.04 - 1.31% ( $n=3$ ). Had pengesanan (LOD) ( $S/N = 3$ ) adalah memuaskan untuk semua dadah terpilih (0.14 - 0.18  $\mu\text{g/mL}$ ). Perolehan purata relatif dadah terpilih yang dipakukan dalam sampel air adalah baik (99 ke 104%). Gabungan prosedur SPMTE dan MEEKC kemudiannya telah digunakan untuk menentukan aseklofenak, ketorolak dan sulindak yang dipakukan dalam sampel air kencing. Peratus perolehan tiga NSAIDs yang diperoleh daripada kaedah SPMTE-MEEKC adalah baik dalam julat 79 ke 96%. Peratus sisihan piawai relatif (% RSD), ( $n=3$ ) untuk proses pengekstrakan juga adalah baik ( $< 3.7\%$ ). Keputusan yang diperoleh dibandingkan dengan kaedah SPE-MEEKC. Kaedah SPE-MEEKC menunjukkan peratus perolehan semula yang sedikit tinggi (95 - 112%) dan % RSD ( $n=3$ ) yang sedikit rendah (1.33 - 2.06%) daripada kaedah SPMTE-MEEKC. Kaedah SPMTE-MEEKC terbukti boleh digunakan untuk analisis aseklofenak, ketorolak dan sulindak dalam air kencing manusia dengan masa analisis yang lebih cepat dan penggunaan pelarut organik yang kurang berbanding dengan kaedah SPE-MEEKC.

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**LIST OF ABBREVIATIONS**

ACN	- Acetonitrile
Ar	- Argon
BGE	- Background electrolyte
CAS	- Chemical abstracts service
CE	- Capillary electrophoresis
CNT	- Carbon nanotube
COX	- Cyclooxygenase
CZE	- Capillary zone electrophoresis
DAD	- Diode-array detectors
DCNP	- Dichloro nitrophenyl phosphate
EME	- Electromembrane extraction
EOF	- Electroosmotic flow
GC	- Gas chromatography
He	Helium
HPLC	- High performance liquid chromatography
IPA	- Isopropanol
IUPAC	- International Union of Pure and Applied Chemists
LC	- Liquid chromatography
LOD	- Limit of detection
LOQ	- Limit of quantitation
LPME	- Liquid phase microextraction
MEEKC	- Microemulsion electrokinetic chromatography
MEKC	- Micellar electrokinetic chromatography
MeOH	- Methanol
MWCNTs	- Multiwalled carbon nanotubes
N <sub>2</sub>	- Nitrogen gas

NSAIDs	- Non-steroidal anti-inflammatory drugs
PDAC	- Poly (diallyldimethylammonium chloride)
RSD	- Relative standard deviation
SDS	- Sodium dodecyl sulphate
SLM	- Supported liquid membrane
SPE	- Solid phase extraction
SPME	- Solid phase microextraction
SPMTE	- Solid phase membrane tip extraction

## CHAPTER 1

### SUMMARY OF THESIS

#### 1.1 Background of Study

Non-steroidal anti-inflammatory drugs (NSAIDs) belong to the class of acidic compounds which include a various number of different chemical types such as derivatives of arylacetic acid, arylalkanoic acid, arylpropionic acid, indolic acid and anthranilic acid. Commonly, NSAIDs exhibit  $pK_a$  values in the range of 3-6 and exist in anionic form in pH values greater than 7 (Jorgensen and Lukacs, 1981a). NSAIDs are usually taken at higher doses because of their anti-inflammatory effect and in small doses for their analgesic and antipyretic actions (Jorgensen and Lukacs, 1981b).

The most common anti-inflammatory mechanism for NSAIDs is the inhibition of cyclooxygenase enzyme (COX) which is necessary in the formation of prostaglandins. Prostaglandins can cause strong physiological effects like swelling and pain. The usage of NSAIDs however can give a few side-effects such as irritation of the stomach, vomiting and sometimes nausea. NSAIDs can be taken orally, systemically or as localized injection. NSAIDs are widely used and easily available. Thus, they are extensively used by patients (Hontela, 2006). However, because of their polar structures, high water solubility and poor degradability, NSAIDs usually cannot be completely eliminated through the sewage treatment plant and these facilitate their penetration through all natural filtration steps and enter the ground and drinking water (Jorgensen and Lukacs, 1981a; Jorgensen and Lukacs 1981b; Hollister, 1991; Sherma and Jain, 2000; Hontela, 2006). Arylalkanoic acid is one of the NSAIDs class and it usually contains one or more aryl groups in the

structure. Many important arylalkanoic acid drugs like sulindac and ketorolac play an important role in treating osteoarthritis, cancer, and smooth-muscle pain (O'Donnel, 1997; Schrier, 2007).

The growing demands for arylalkanoic acid class of NSAIDs drug and its bad effect towards the environment from their production and decomposition process ignite the needs to develop new analytical methods regarding their qualitative and quantitative analysis. There are many methods used in previous research on arylalkanoic class analysis such as high performance liquid chromatography (HPLC) (Sun *et al.*, 2003; Payan *et al.*, 2011), gas chromatography (GC) (Martinez-Algaba, 2004), liquid chromatography (LC) (Hoshina *et al.*, 2011), voltammetric (Ali, 1999), spectrophotometric and spectrofluorometric methods (Gouda *et al.*, 2011).

However, many of these methods are solvent and time consuming. LC, spectrophotometric method and spectrofluorometric method also exhibit low sensitivity properties (Sun *et al.*, 2003; Payan *et al.*, 2011; Martinez-Algaba, 2004; Hoshina *et al.*, 2011; Ali, 1999; Gouda *et al.*, 2011; Harvey, 2000). Therefore, one of the CE modes which is MEEKC was selected as the separation method to overcome the large solvent consumption problem faced in the previous method. Microemulsion electrokinetic chromatography (MEEKC), an electrodriven separation technique is one of the capillary electrophoresis modes in a nanoseparation method.

MEEKC offers a highly efficient separation of both charged and neutral solutes covering a various range of water solubility. This technique basically separates the solutes based on their hydrophobicities and electrophoretic mobilities using microemulsion buffers. This method was proved to be rigid, faster, low solvent consumption and cost effective compared to other rapidly used separation methods such as high performance liquid chromatography (HPLC) and micellar electrokinetic chromatography (MEKC) (Sun *et al.*, 2003; Payan *et al.*, 2011; Huang *et al.*, 2003; Hansen *et al.*, 2001). Therefore, MEEKC was selected as the separation method in this research in order to achieve a fast, environmental friendly and cost effective separation process of arylalkanoic acid drugs. In this research, an MEEKC method with DAD detector was used and the background electrolyte (BGE) composition was



investigated to provide better result for the analysis of the selected aryalkanoic acid drugs which were aceclofenac, ketorolac and sulindac.

In previous researches, various types of extraction methods have been used to extract NSAIDs such as solid phase extraction (SPE) (Hoshina *et al.*, 2011), electromembrane extraction (EME) (Payan *et al.*, 2011), liquid phase microextraction (LPME) (Es'haghi, 2009) and solid phase microextraction (SPME) (Fan *et al.*, 2005). However, SPE needs a lot of steps which is time consuming and uses a large amount of organic solvent that are potentially toxic and relatively expensive. SPME and LPME both have a similar disadvantage which is lack of selectivity for specific adsorption of certain analytes (Li *et al.*, 2011). EME in the other hand offers faster analysis time but the analyte's percentage of recovery in real samples is low compared to other extraction methods (Lee *et al.*, 2009).

Therefore, solid phase membrane tip extraction (SPMTE) was used in this research as it offers shorter extraction time, low solvent usage, cost effectiveness, high analyte percentage recovery and is easy to use. SPMTE involves the use of tiny-cone shaped membrane tip protected multiwall carbon nanotubes (MWCNTs). In a previous research done on pesticide analysis, SPMTE method was able to minimize the extraction time as well as reduce cost and solvent usage. This extraction method was proven to give comparable LODs as well as method reproducibility (See *et al.*, 2010).

## 1.2 Summary

This study was conducted to separate three different aryalkanoic acid drugs namely aceclofenac, ketorolac and sulindac in urine sample using MEEKC coupled with SPMTE method. There are four important objectives in this study which are firstly the optimization of MEEKC method followed by SPMTE method, thirdly is the validation of the SPMTE-MEEKC method and the final objective is application of the validated method in the analysis of human urine sample.

Chapter 2 combines the explanation of the selected drug properties, previous separation and extraction methods used in arylalkanoic acid drugs analysis and introduction to capillary electrophoresis and solid phase membrane tips extraction. Objectives of the study, significance of the study and the scope of the study are also covered in this chapter.

Chapter 3 reports the optimization of microemulsion electrokinetic chromatography method for the separation of the selected drugs. Throughout this chapter, the procedure and the effects of eleven parameters towards the separation process using MEEKC were investigated. The parameters investigated were sodium tetraborate buffer pH and concentration, SDS concentration, acetonitrile concentration, butan-1-ol concentration, ethyl acetate concentration, temperature, wavelength, applied voltage, injection time and solvent type.

Chapter 4 discussed the results obtained from the optimization of SPMTE techniques used in the extraction of the selected drugs in deionized water. Six parameters were optimized namely effect of organic solvent used for conditioning, sample pH, salt addition percentage, sample volume, extraction times and desorption times. The results were then compared with the results obtained from a published SPE method. The optimum conditions of SPMTE-MEEKC were then applied for the analysis of selected NSAIDs in urine samples.

The final chapter discussed the conclusions and future directions for further studies. The results obtained throughout this study such as the analytical performance and optimized parameters were concluded and compared. Future directions of the research are also highlighted in this chapter.

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