



UNIVERSITI PUTRA MALAYSIA

**DEVELOPMENT AND APPLICATION OF HPLC-MS/MS METHOD
FOR DETERMINATION OF HUMAN PHARMACEUTICALS AND
SYNTHETIC HORMONES IN RIVER WATER AND SEWAGE
EFFLUENTS**

NAJAT AHMED AL-ODAINI

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By

NAJAT AHMED AL-ODAINI

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

October 2010



DEDICATION

In the name of Allah, the Beneficent, the Merciful

“My Lord! Increase me in knowledge”
(Qur'an 20:114)

To Prophet Mohammad

To the Memory of My Late Father

To My Beloved Mother and Brother Akram



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
fulfilment of the requirement for the degree of Doctor of Philosophy

**DEVELOPMENT AND APPLICATION OF HPLC-MS/MS METHOD FOR
DETERMINATION OF HUMAN PHARMACEUTICALS AND SYNTHETIC
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October 2010

Chair: Associate Prof. Mohamad Pauzi Zakaria, PhD

Faculty: Environmental Studies

Pollution of the aquatic environment by residues of human pharmaceuticals and synthetic hormones has become a cause for increasing concern in recent years and acknowledged as one of the most emerging environmental issues. The occurrence of human pharmaceuticals and synthetic hormones in the Malaysian aquatic environment has never been reported. This lack of data could be attributed to the absence of an internationally recognized method for the analysis of pharmaceutical residues in water matrices. The main objective of this study is to develop a sensitive and selective method for the simultaneous determination of 23 pharmaceuticals of interest in environmental matrices such as river water and sewage effluents. The 23 pharmaceuticals consist of different therapeutic classes which include both top prescribed and over the counter (OTC)



pharmaceuticals. The developed method was based on sample pre-treatment using Solid Phase Extraction (SPE) followed by analysis using High Performance Liquid Chromatography-Tandem Mass Spectrometry (HPLC-MS/MS). The method was validated and tested against environmental samples. The validation results indicated that the method is able to simultaneously detect and quantify the targeted pollutants with good selectivity and sensitivity. The method performed well for the majority of the pharmaceuticals evaluated, with recoveries greater than 70% for most pharmaceuticals. To the researcher's knowledge, 5 pharmaceuticals (i.e. amlodipine, chlorpheniramine, chlorothiazide, perindopril and gliclazide) out of the 23 pharmaceuticals had never been analyzed in environmental matrices. The other objectives of this study were to identify and quantify pharmaceuticals residues in environmental samples collected from the Langat River and in effluents of sewage treatment plants (STPs) using the developed method. Seventeen out of twenty-three targeted pharmaceuticals were detected in river water samples namely metformin, atenolol, acetaminophen, metoprolol, mefenamic acid, salicylic acid, salbutamol, perindopril, gliclazide, glibenclamide, loratadine, furosemide, levonorgestrel, cyproterone, diclofenac, chlorothiazide and nifedipine. The median concentration of detected pharmaceutical ranged between less than the Method Detection Limit (<MDL) for furosemide, loratadine and nifedipine to higher concentration of 112.7 ng/L for diclofenac. The highest concentration found was acetaminophen (346.3 ng/L). On the other hand, nineteen targeted



pollutants were detected in effluent samples from STPs namely metformin, atenolol, acetaminophen, metoprolol, mefenamic acid, salicylic acid, salbutamol, perindopril, glimepiride, diclofenac, furosemide, chlorothiazide, glibenclamide, lovastatin, loratadine, amlodipine, 17 α -ethinylestradiol, norethindrone and cyproterone. The median concentration of the detected pollutants were less than the Method Detection Limit (<MDL) for lovastatin, loratadine, norethindrone, cyproterone, amlodipine and 17 α -ethinylestradiol to a higher concentration of 3270.6 ng/L for metformin. The highest concentration found was metformin (34228.6 ng/L). This study has confirmed that the Malaysian aquatic environment is impacted by pharmaceutical residues at varying levels. Mefenamic acid and Salicylic acid were found to be present in all the river water and STPs effluent samples, suggesting their widespread use and high degree of persistency in the tropical aquatic environment. However, chlorpheniramine and simvastatin were not found in any sample for all matrices indicating their high degradability and low persistency in the tropical aquatic environment.

Keywords: Human pharmaceuticals, synthetic hormones, water pollution, HPLC-MS/MS, Langat River



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PEMBANGUNAN DAN APLIKASI KAEDAH KCPT-JS/JS UNTUK
PENENTUAN FARMASEUTIKAL MANUSIA DAN HORMON SINTETIK DI
DALAM AIR SUNGAI DAN EFLUEN SISA KUMBAHAN**

Oleh

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Oktober 2010

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Pencemaran persekitaran akuatik oleh sisa farmaseutikal manusia dan hormon sintetik semakin mendapat perhatian sejak sedekad yang lalu dan ia dikenalpasti sebagai salah satu masalah persekitaran yang paling membimbangkan. Penghasilan farmaseutikal manusia dan hormon sintetik dalam persekitaran akuatik di Malaysia belum pernah dilaporkan. Kekurangan data mungkin disebabkan oleh ketiadaan kaedah yang diiktifaf di perigkat antarabangsa untuk menganalisa sisa-sisa farmaseutikal dalam matrik air. Objektif utama kajian ini adalah untuk membangunkan kaedah yang sensitif dan terpilih dalam penentuan 23 farmaseutikal dalam matriks persekitaran seperti air sungai dan efluen sisa kumbahan. Dua puluh tiga farmaseutikal ini terdiri daripada kelas terapi yang berbeza merangkumi prekripsi doktor dan belian farmaseutikal



melalui kaunter (MK). Kaedah yang dibangunkan ini adalah berdasarkan prarawat sampel dengan menggunakan Ekstrak Fasa Pepejal (EPP) diikuti dengan analisis menggunakan Kromatografi Cair Berprestasi Tinggi-Tandem Jisim Spektrometri (KCBT-JS/JS). Kaedah ini disahkan dan diuji dengansampel persekitaran. Keputusan pengesanan menunjukkan bahawa kaedah ini mampu mengesan secara serentak dan menentukan bahan pencemar yang disasarkan dengan pemilihan dan kepekaan yang baik. Kaedah ini digunapakai dengan baik untuk sebahagian besar farmaseutikal yang dinilai, dengan nilai pemulihan lebih besar dari 70% untuk semua farmaseutikal. Untuk pengetahuan penyelidik, ini buat pertama kalinya 5 farmaseutikal (i.e. amlodipin, klorpheniramin, klorothiazid, perindopril dan gliclazid) daripada jumlah 23 farmaseutikal telah dianalisis dari matrik alam sekitar. Objektif lain dalam kajian ini adalah untuk mengenalpasti dan menentukan sisa farmaseutikal dalam sampel persekitaran yang diambil dari Sungai Langat dan efluen loji rawatan kumbahan (LRK) dengan menggunakan kaedah yang telah dibangunkan. Tujuh belas daripada 23 farmaseutikal yang disasarkan dapat dikesan dalam sampel air sungai adalah metformin, atenolol, acetaminophen, metoprolol, asid mefenamat, asid salisilat salbutamol, perindopril, gliklazid, glibenklamid, loratadin, furosemid, levonorgestrel, siproteron, diklofenak, klorothiazide dan nifedipin. Kepekatan median farmaseutikal yang dikesan adalah kurang dari Kaedah Batas Pengesanan (<KBP) untuk furosemid, loratadin dan nifedipin kepada kepekatan yang lebih tinggi iaitu 112.7 ng/L untuk diklofenak. Kepekatan tertinggi yang

ditemui adalah asitaminophen (346.3 ng/L). Selain itu, 19 bahan pencemar yang disasarkan telah dikesan dalam sampel efluen loji rawatan kumbahan (LRK) iaitu metformin, atenolol, asitaminophen, metoprolol, asid mefenamat, asid salisilat, salbutamol, perindopril, gliklazid, diklofenak, furosemid, klorothiazid, glibenklamid, lovastatin, loratadin, amlodipin, 17 α -etinilestradiol, norethindron dan saiproteron. Kepekatan median dari pencemaran yang dikesan adalah kurang dari Kaedah Batas Pengesanan (<KBP) untuk lovastatin, loratadin, norethindron, amlodipin saiproteron, dan 17 α -etinilestradiol untuk kepekatan yang lebih tinggi ia itu 3.270.6 ng/L untuk metformin. Kepekatan tertinggi yang ditemui adalah metformin (34.228.6 ng/L). Kajian ini telah mengesahkan bahawa persekitaran akuatik Malaysia dipengaruhi oleh sisa-sisa farmasi dari pelbagai kepekatan. Asid mefenamat dan asid salisilat ditemui dalam semua sampel air sungai dan efluen loji rawatan kumbahan (LRK), menunjukkan penggunaan secara meluas dan berpersisten tinggi dalam perairan akuatik tropika. Manakala, klorphernamin dan simvastatin tidak dikesan dalam mana-mana sampel untuk semua matriks dan ini menunjukkan bahan-bahan ini menghancurkan dengan cepat serta mempunyai daya persisten yang rendah dalam persekitaran akuatik tropika di Malaysia.

Kata kunci: Farmaseutikal, hormon sintetik, pencemaran air, KCBT-JS/JS, Langat Basin

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I certify that a Thesis Examination Committee has met on 29 October 2010 to conduct the final examination of Najat Ahmed Al-Odaini on her Doctor of Philosophy thesis entitled “Development and application of an HPLC-MS/MS method for determination of human pharmaceuticals and synthetic hormones in river water and sewage effluents” in accordance with Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that student be awarded the Doctor of Philosophy.

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institutions.

NAJAT AHMED AL-ODAINI

Date: 29 October 2010



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