UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF CONVENIENT QUALITY CONTROL ANALYSIS METHOD AND IMPROVEMENT ON THE RADIOCHEMICAL YIELD USING F-18-LABELLED CHOLINE

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By

MUHAMMAD HISHAR BIN HASSAN

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Chair : Professor Dato' Abdul Jalil bin Nordin, PhD
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Prostate cancer continues to be the most prevalent cancer in men in Malaysia. It is anticipated that the number of prostate cancer sufferers' will increase in future. In the case of Positron Emission Tomography / Computed Tomography (PET/CT), clinicians cannot solely rely on F-18 Fluorodeoxyglucose (F-18 FDG) as there were few studies demonstrated the use of F-18 FDG was pointless in prostate cancer imaging. Thus, this current study presents development of convenient quality control analysis method and improvement performed on the azeotropic drying of no-carrier-added (n.c.a.) F-18 Fluorine to increase the F-18 Fluorocholine (F-18 FCH) radiochemical yield.

From these studies, a convenient, efficient and reliable method for quality control analysis of F-18 FCH was successfully developed and validated to comply all the release criteria even in a limited equipment set-up. As most of the equipment set-up in the quality control laboratory in the present study was meant for the routine quality control analysis of F-18 FDG, several parameters were changed in order to adapt to the F-18 FCH quality control method without affecting the routine quality control analysis of F-18 FDG. The synthesis of F-18 FCH was successfully performed on a commercial synthesis module, GE TracerLab Mx_{FDG} with fairly good radiochemical yields, between 5 to 15%, which decay is not corrected. Interestingly, the finding from this study shows the potential of improvement on the azeotropic drying condition of n.c.a F-18 Fluorine as a preferred technique to improve the radiochemical yield of F-18 FCH.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PEMBANGUNAN KAEDAH ANALISIS KAWALAN KUALITI YANG BERSESUAIAN DAN PENAMBAHBAIKAN KEPADA HASIL RADIOKIMIA MENGGUNAKAN F-18-LABEL CHOLINE

Oleh

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Kanser prostat terus menjadi kanser yang paling lazim di kalangan lelaki di Malaysia. Adalah dijangkakan bahawa bilangan pesakit kanser prostat akan meningkat pada masa akan datang. Bagi pengimejan modaliti Positron Emission Tomography / Computed Tomography, para doktor tidak boleh bergantung sepenuhnya pada F-18 Fluorodeoxyglucose (F-18 FDG) kerana terdapat beberapa kajian menunjukkan penggunaan F-18 FDG adalah terhad dalam pengimejan kanser prostat. Atas sebab ini, kajian semasa ini membentangkan pembangunan kaedah analisa kawalan kualiti yang mudah dan penambahbaikan yang telah dilakukan ke atas pengeringan azeotrop pada F-18 Fluorine yang tidak dilabel untuk meningkatkan hasil radiokimia F-18 Fluorocholine (F-18 FCH).

Dari kajian ini, satu kaedah yang mudah, cekap dan berkesan untuk analisis kawalan kualiti F-18 FCH telah berjaya dibangunkan dan disahkan mematuhi semua kriteria pelepasan walaupun dalam keadaan peralatan yang terhad. Oleh kerana kebanyakan peralatan di makmal kawalan kualiti ditetapkan untuk analisis kawalan kualiti F-18 FDG, beberapa parameter telah diubah bersesuaian dengan kaedah kawalan kualiti F-18 FCH tanpa menjejaskan rutin analisis kawalan kualiti F-18 FDG. Sintesis F-18 FCH berjaya dihasilkan dengan menggunakan modul sintesis komersial, GE TracerLab Mx_{FDG} dengan hasil radiokimia yanag agak baik iaitu, antara 5 hingga 15%, yang mana separuh hayat tidak diperbetulkan. Menariknya, hasil penemuan daripada kajian ini menunjukkan potensi penambah baikan pengeringan azeotrop kepada F-18 Fluorine yang belum dilabel sebagai kaedah untuk meningkatkan hasil radiokimia F-18 FCH.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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TABLE OF CONTENTS

		Page
ABSTRACT		i
ABSTRAK		ii
ACKNOWLEDGEMENTS		iii
APPROVAL		iv
DECLARATION		vi
LIST OF TABLES		x
LIST OF FIGURES		xi
LIST OF ABBREVIATIONS		xiii
CHAPTER		
1	**GENERAL INTRODUCTION**	1
1.1	Background of Study	1
1.2	Problem Statement and Justification	1
1.3	Aims	2
1.4	Organisation of Thesis	3
2	**LITERATURE REVIEW**	4
2.1	Fluorine-18 and Its Production	4
2.1.1	Properties of F-18 Fluorine	4
2.2.1	Improving the No-carrier-added (n.c.a) F-18 Fluorine Anion Reactivity	5
2.2	F-18 Radiopharmaceuticals	6
2.3	Quality Control Analysis and Synthesis of F-18 FCH	7
2.3.1	Development of Quality Control Analysis Method for F-18 FCH	7
2.4	Synthesis of F-18 FCH	10
2.5	Summary of Research Findings & Gap Analysis	12
3	**MATERIALS AND METHODS**	13
3.1	Materials	13
3.1.1	Reagents and Apparatus	13
3.2	Methods	14
3.2.1	Convenient and Efficient Method for Quality Control Analysis of F-18 FCH: For a Small Scale GMP-based Radiopharmaceuticals Laboratory Set-up	14
3.2.2	Synthesis of Fluorine-18-Labelled Choline (F-18 FCH): Towards an Early and Accurate Management of Prostate Cancer in Malaysia	17
3.2.3	Optimising the Azeotropic Drying of F-18 Fluorine Way to Improve the F-18 FCH Radiochemical Yield	17

4	**CONVENIENT AND EFFICIENT METHOD FOR QUALITY CONTROL ANALYSIS OF F-18 FCH: FOR A SMALL SCALE GMP-BASED RADIOPHARMACEUTICALS LABORATORY SET-UP**	19
	Article	19
	Acceptance Letter	31
5	**SYNTHESIS OF FLUORINE-18-LABELLED CHOLINE (F-18 FCH): TOWARDS AN EARLY AND ACCURATE MANAGEMENT OF PROSTATE CANCER IN MALAYSIA**	32
	Article	32
	Acceptance Letter	43
6	**OPTIMISING THE AZEOTROPIC DRYING OF F-18 FLUORINE WAY TO IMPROVE THE F-18 FCH RADIOCHEMICAL YIELD**	44
	Article	44
	Acceptance Letter	54
7	**CONCLUSION**	56
	7.1 Summary and Links to Articles	56
	7.2 Significant Findings / Contributions	57
	7.3 Recommendations	57
REFERENCES		58
BIODATA OF STUDENT		62
LIST OF PUBLICATIONS		63

LIST OF TABLES

Table		Page
1	Selected nuclear reactions to produce F-18 Fluorine	4
2	Overview of the reference solutions and HPLC results	15
3	Summary of the reference solutions prepared	16
4	Overview of the reference solutions and GC results	16
5	Overview of the azeotropic drying strategy in the synthesis of F-18 FCH	18
6	Overview of the reference solutions and HPLC results	22
7	Overview of the reference solutions prepared	23
8	Overview of the reference solutions and GC results	23
9	Quality control of F-18 FCH synthesis	30
10	Overview of the reference solutions and GC results	37
11	Overview of the azeotropic drying strategy in the synthesis of F-18 FCH	46
12	Overview of the F-18 FCH radiochemical yields %	48

LIST OF FIGURES

Figure		Page
1	Preparation of reactive F-18 Fluorine ion through the Formation of [F-18]F^-/Kryptand/K^+-complex	6
2	Synthesis of F-18 FCH	7
3	Schematic diagram of the automated synthesis of F-18 FCH on the GE TracerLab MX_{FDG} in Kryza *et al.*, method	10
4	Schematic diagram of the automated synthesis of F-18 FCH on the GE TracerLab MX_{FDG} in the present study	11
5	Radiochemical yield of F-18 FCH at the end of synthesis	24
6	Radio-HPLC and HPLC chromatogram of F-18 FCH	25
7	Retention time of F-18 FCH observed in the chromatogram of radio-HPLC	25
8	Reference solutions; fluoromethylcholine chloride and choline chloride observed in the chromatogram	26
9	F-18 FCH peak highlighted in red colour confirmed that the radiochemical purity was 100%.	27
10	The retention factor of F-18 FCH was observed in the Chromatogram of radio-TLC	27
11	Chromatogram of F-18 FCH	28
12	Levels of ethanol and acetonitrile in F-18 FCH	29
13	Synthesis of F-18 FCH	35
14	Overview of the F-18 FCH radiochemical yields (%) at the end of synthesis	37
15	Mean radiochemical yield of F-18 FCH at the end of synthesis	39
16	Radio-chromatogram showing purity of F-18 FCH	40
17	F-18 FCH peak highlighted in red colour confirmed that the radiochemical purity was 100%	41
18	Chromatogram of F-18 FCH	41
19	Synthesis of F-18 FCH	46

20	Mean radiochemical yield of F-18 FCH at the end of synthesis for 2 groups	49
21	Radio-chromatogram showing purity of F-18 FCH after using the newly improved procedure	51
22	F-18 FCH peak highlighted in red colour confirmed that the radiochemical purity was 100%	51
23	Chromatogram of F-18 FCH after the newly improved procedure	52

LIST OF ABBREVIATIONS

CDA	Compressed Dry Air
CT	Computed Tomography
DBM	Dibromomethane
DMEA	*N*-*N*-dimethyethanolamine
EOS	End of Synthesis
F-18 $BrCH_2F$	F-18 Fluorobromomethane
F-18 FDG	F-18 Fluorodeoxyglucose
F-18 FCH	F-18 Fluorocholine or F-18 Fluoromethylcholine
FID	Flame Ionization Detector
GMP	Good Manufacturing Practice
H_2SO_4	Sulphuric Acid
HCl	Hydrochloric Acid
HPLC	High Performance Liquid Chromatography
K_2CO_3	Potassium Carbonate
LOD	Limit of Detection
MRI	Magnetic Resonance Imaging
mRNA	micro Ribonucleic Acid
NaH_2PO_4	Sodium dihydrogen phosphate
n.c.a	No-Carrier-Added
NCI	National Cancer Institute
PEEK	Polyether Ether Ketone
PET	Positron Emission Tomography
Radio-HPLC	Radio-High Performance Liquid Chromatography
Radio-TLC	Radio-Thin Layer Chromatography
RID	Refractive Index Detector
Rf	Retention Factor

Rt	Retention Time
SUV	Standardized uptake value
SPE	Solid Phase Extraction
$TBA.HCO_3$	tetrabutylammonium bicarbonate
$^{18}O\text{-}H_2O$	Enriched water

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background of Study

Prostate cancer, a typical adenocarcinoma, occupies fourth position in the chart of the most frequent cancer modalities in men in Malaysia. In developed countries like United States of America and United Kingdom, prostate cancer is in the top three most prevalent cancers in men. In Malaysia, according to Malaysian Oncological Society on 2013, the Chinese ethnic population was recorded to be the highest incidence of prostate cancer compared to other local ethnicities (Malaysian Oncological Society, 2013). The cancer commences when normal semen-secreting gland cells in the prostate start to proliferate without control. The primary mutating cells are able to metastasise into other parts of the body and affect other normal tissue.

The risk factors believed to be associated with prostate cancer are age, family background and diet. The incidence of prostate cancer rises rapidly as men age of 50. The problem will then get worse as the man ages (Kelloff *et al.*, 2009). The cancer risk also runs in family. With regard to diet, it is observed that men with a diet high in saturated animal fat and low in fruits and vegetables are at an increased risk of getting the prostate cancer. Unfortunately, there are no specific symptoms during the early stage, as the cancer bears a small size in its early manifestation. However, some men diagnosed with prostate cancer, responded that they experienced painful or burning during urination, blood in the urine, pain in the lower back, shoulders and prostatism.

With the emergence of new clinical diagnostic techniques, the stage of the prostate cancer can be localised and classified with greater accuracy. Among the non-invasive imaging techniques such as ultrasound, computed tomography (CT), positron emission tomography (PET) and magnetic resonance imaging (MRI), the prospect of hybrid imaging technique of positron emission tomography and computed tomography, known as PET/CT, is promising. PET/CT imaging technique measures the metabolic activity or response of cells in the human body. The relative tissue uptake or response to a radioisotope labelled with specific marker is measured as standardised uptake value (SUV) (Hricak *et al.*, 2007). The benefit offered by PET/CT imaging technique, eliminates the necessity for the patient to go through the painful procedure such as biopsy technique, which could be missed if in the case of low-grade prostate cancer (Mercola, 2013).

1.2 Problem Statement and Justification

In Malaysia, the prospect of PET/CT imaging technique in prostate imaging has increasingly prospered, with on-going improvement on novel radioisotope labelled markers and examination procedure. However, with regard to radioisotope labelled marker in prostate cancer imaging, it is limited only by the availability of radioisotope

Fluorine-18 Fluorodeoxyglucose (F-18 FDG). After almost a decade of first cyclotron installed in Malaysia, F-18 FDG is only the available marker in PET/CT imaging technique. Since then, it has become a common marker that is being used for most cancer cases in PET/CT imaging technique. As the number of patients diagnosed with prostate cancer has significantly increased over the year, it creates a strong urgency to provide an accurate diagnosis in management of prostate cancer patient. Clinicians cannot solely rely on F-18 FDG in PET/CT imaging technique as not all cancers behave in the same way. Evidently, specific cancer cases such as brain, neuroendocrine or prostate demonstrated that the use of F-18 FDG was pointless in PET/CT technique due to cancer cell behaviour. In the case of prostate cancer, previous studies performed using F-18 FDG in PET/CT imaging technique observed a low F-18 FDG avidity on PET/CT image. Researchers believed that the incident of low F-18 FDG avidity is associated to a very weakly expressed glucose transporter mRNA and protein in human prostate tissue (Candler *et al.,* 2003).

In order to accommodate strong demand from clinicians to provide an accurate diagnosis of prostate cancer, hence, there is a need to synthesise a specific marker labelled with radioisotope Fluorine-18. Therefore, this study attempts to synthesise the Fluorine-18 labelled choline or known as F-18 Fluorocholine or also known as F-18 Fluoromethylcholine (F-18 FCH) on a commercial synthesis module, GE TracerLab $MX_{FDG.}$ Since this was the first time F-18 FCH was synthesised locally, there were no available data or guideline for the acceptance criteria of F-18 FCH documented.

1.3 Aims

The overall aim of this present study is to improve the radiochemical yield of F-18 FCH at the end of the synthesis without affecting the quality control analysis result of F-18 FCH. In addition, this study also keen to develop a convenient and efficient method of quality control analysis for F-18 FCH in a Good Manufacturing Practice (GMP)-based radiopharmaceutical laboratory. As the equipment set-up in the GMP-based radiopharmaceuticals laboratory in the current study was meant for F-18 FDG routine production, this study tried to adapt the equipment set-up for F-18 FCH by minimising changes in the parameters to obtain good result for F-18 FCH.

In summary, the research aims are as follows:

- To develop a convenient and efficient method for quality control analysis of F-18 FCH in a GMP-based radiopharmaceutical laboratory that routinely produced F-18 FDG.
- To perform synthesis of F-18 FCH on a commercial synthesis module designed for F-18 FDG synthesis, (GE TracerLab MX_{FDG}) using Kryza method with minor modification (Kryza *et al.,* 2008).
- To investigate the potential of improvement on the azeotropic drying condition of n.c.a F-18 Fluorine in order to improve the radiochemical yield of F-18 FCH.

1.4 Organisation of the Thesis

The organisation of this thesis is as follows. In the next chapter, we review previous works in synthesis and quality control analysis of F-18 FCH. We discuss different parameters that are used in our quality control analysis method. The significance of improvement on the azeotropic drying of n.c.a F-18 Fluorine in order to improve the radiochemical yield of F-18 FCH is highlighted in the last subsection in Chapter 2.

Chapter 3 provides a brief description on how we develop and validate the quality control analysis method of F-18 FCH in a small scale GMP-based radiopharmaceutical laboratory. The synthesis of F-18 FCH and improvement made on the azeotropic drying condition of n.c.a F-18 Fluorine is briefly describe in the last subsection in this chapter. In Chapter 4, we present a convenient and efficient method for quality control analysis of F-18 FCH that is suitable for a small scale GMP-based radiopharmaceuticals laboratory set-up. The related work has been accepted for publication in Current Radiopharmaceuticals journal on 2015 and also presented at European Association of Nuclear Medicine Congress of 2015 in Hamburg, Germany.

Chapter 5 highlights the very first synthesis of F-18 FCH in Malaysia. The present work has been accepted for publication in Current Medical Imaging Reviews journal on 2015. Meanwhile, in Chapter 6, we discuss the potential of improving the azeotropic drying condition of n.c.a. F-18 Fluorine in order to improve the radiochemical yield of F-18 FCH. The related work has been accepted for publication as a full research manuscript in Current Radiopharmaceuticals journal and as a technical note in Journal of Labelled Compounds and Radiopharmaceuticals on 2015. The related work also has been presented at European Association of Nuclear Medicine Congress of 2015 in Hamburg, Germany.

Chapter 7 gives a summary of this thesis as well as discusses significant findings from the study and directions for future work. Last but not least, the last section of this thesis briefly describes the biographical data of the student and publication of manuscripts related to this study.

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