

Preparation of Some Radiolabeled Pharmaceutical Compounds of Expected Biological Activity

Thesis Presented by Marwa Eid Sayyed Farrag

(Pharmacist)
Egyptian Atomic Energy Authority (EAEA)

For the partial fulfillment of master degree In Pharmaceutical Sciences (Pharmaceutics)

Under Supervision of

Prof. Dr. Gehanne Abd El-Samie Awad

Professor of pharmaceutics and Industrial Pharmacy,

Department of Pharmaceutics and Industrial Pharmacy,

Faculty of Pharmacy, Ain Shams University.

Prof. Dr. Mohamed Abd El-Motaleb Abd El-Rahman

Professor of Radiochemistry,

Labeled Compounds Department,

Hot Labs Center, Egyptian Atomic Energy Authority.

Dr. Ismail Taha Ibrahim

Assistant Professor of pharmacology,

Labeled Compounds Department,

Hot Labs Center, Egyptian Atomic Energy Authority.

Department of Pharmaceutics

Faculty of Pharmacy

Ain Shams University

بسم الله الرحمن الرحيم



صدق الله العظيم

Acknowledgement

First of all I would thank "Allah" for helping me to fulfill this work.

I would like to express my deep gratitude and appreciation to Professor Dr. Gehanne Abdel Samie Awad, Professor of Pharmaceutics and industrial pharmacy, Faculty of Pharmacy, Ain Shams University, for kindly supervising this work, continous support, available help and careful revision of this work.

I am sincerely grateful to Professor Dr. Mohamed Abd El-Motaleb, Professor of Radiochemistry, Labeled Compounds Department, and Hot Labs. Center, Egyptian Atomic Energy Authority for effective supervision, help and instructions through the whole work.

I am sincerely grateful to **Dr. Ismail Taha Ibrahim**, Assistant Professor at Department of Labeled Compounds Department, Hot Labs Center, Egyptian Atomic Energy Authority, for his help.

Also, I would thank mr. Rihab Osman for her support, help and support to make this work appear like this.

I am sincerely grateful to my mother, my husband, my brother, my daughters for their great help and continuous support and encouragement.

The Author Marwa Eid

Contents

LIST OF CONTENTS

Title	Page
List of Abbreviations	V
List of Tables	VIII
List of Figures	XI
List of Equations	XIV
Abstract	XVI
General Introduction	1
Radiopharmaceuticals (Radiodiagnostics/Radiotracers)	1
Therapeutic radiopharmaceuticals	2
Diagnostic radiopharmaceuticals	2
The Instrumentations used for radioisotopes production	5
Cyclotron for the production of radioisotopes	5
Nuclear reactor	6
Radioiodination	6
Iodine chemistry	6
Principles of iodination	7
Electrophilic radioiodination	8
Some of oxidizing agents commonly used for electrophilic substitution	9
Nucleophilic radioiodination	11
Nuclear properties of iodine	12
Isotopes of iodine	12
1. Iodine-123	13
2. Iodine-124	14
3. Iodine-125	14
3. Iodine-131	15
Labeling with technetium-99m (^{99m} Tc):	16
Pertechnetate (99m TcO ₄) Reduction	17
Quality control for radiopharmaceuticals	20

1. Paper Chromatography	20
2. Thin layer chromatography	20
3. Electrophoresis	21
4. Liquid liquid extraction (LLE)	23
5. HPLC	24
Use of radiopharmaceuticals in brain imaging	25
Brain imaging techniques	26
1. Computed Axial Tomography (CAT)	26
2. Magnetic resonance imaging (MRI)	27
3. Emission tomography (ET)	27
3.A.Single photon emission computed tomography (SPECT)	27
3.B.Positron emission tomography (PET)	28
4. Gamma camera	30
Radiopharmaceutical for brain imaging	31
Routes for brain imaging	33
Intravenous route (IV)	33
Intranasal route (IN)	34
Scope of work	36
CHAPTER I: Optimization of CNS active radiopharmaceuticals	38
Introduction	38
CNS active drugs	38
Experimental	41
Materials	41
Methodology	44
I. Labeling of the three ligands	44
I.1. labeling with iodine	44
I.2. Labeling with ^{99m} Tc	45
II. Analysis of the reaction mixture (Radiochemical yield/ purity) of	45
radiolabeled compounds	45

11. 1. Ascending paper chromatography analysis	40
II. 2. Paper electrophoresis	50
II. 3. HPLC analysis	51
III. Study of the factors affecting the percent labeling yield of ¹²⁵ I-	52
trazodone, ¹²⁵ I-haloperidol and ^{99m} Tc-phenobarbital	34
III. 1. Effect of oxidizing agent (Chloramine-T) content for ¹²⁵ I-	
trazodone and ¹²⁵ I-haloperidol	52
III. 2. Effect of reducing agent (SnCl ₂) content for ^{99m} Tc-phenobarbital	52
III. 3. Effect of substrate Amount	52
III. 4. Effect of pH of the reaction medium	52
III. 5. Effect of reaction time	53
III. 5. Effect of reaction temperature	53
III. 6. <i>In-vitro</i> stability of labeled compounds.	53
Statistical analysis	53
Results and discussion	54
I. Labeling yield of ¹²⁵ I and ^{99m} Tc labeled compounds	54
I.1. Ascending paper chromatography	54
I.2. Paper electrophoresis	55
I.3. HPLC analysis	57
II. Factors affecting the percent labeling yield of ¹²⁵ I-trazodone	58
HCl, ¹²⁵ I-haloperidol and ^{99m} Tc-phenobarbital	
II.1. Effect of oxidizing agent (CAT) amount for ¹²⁵ I-TZ and ¹²⁵ I-HP	58
II.2. Effect of reducing agent (SnCl ₂) amount for ^{99m} Tc-PB	60
II.3. Effect of substrate amount	61
II.4. Effect of pH of reaction medium	64
II.5. Effect of reaction time	68
II.6. Effect of reaction temperature	71
II.7. In-vitro stability study	74
CONCLUSION	77

Chapter II: Preparation of different IN formulations of radiopharmaceuticals and study of their biodistribution	79
Introduction	79
Experimental	82
Materials	82
Methodology	83
1. Labeled trazodone and haloperidol microemulsions (ME) preparation	83
2. Characterization of labeled drug microemulsion	83
3. Biodistribution study	84
4. Statistical analysis	85
RESULTS AND DISCUSSION	86
1. Physicochemical characterization of microemulsion (ME) formulation containing both TZ & HP	86
2. Biodistribution results	87
2.1. ¹²⁵ I-trazodone	87
2.2. ¹²⁵ I-hal operidol	90
2.3. 99m Tc-pheno bar bital	93
Conclusion	97
English Summary	98
References	103
Arabic Summary	1-6

LIST OF ABBREVIATIONS

AED	Antiepileptic drug
ANOVA	Analysis of variance
BBB	Blood brain barrier
ВСВ	Blood cerebrospinal fluid barrier
CAB	Chloramine-B (N-chloro-benzenesulphonic acid)
CAT	Chloramine-T (N-chloro-p-toluene sulfonamide sodium salt)
CBF	Cerebral blood flow
CNS	Central Nervous System
CPS	Count per second
CSF	Cerebro Spinal Fluid
CT/CAT	Computed axial tomography
Da	Dalton
D	Deutron
DMF	Dimethyl formamide
DTPA	Diethylene triamine pentaacetate
EC	Electron Capture
ECD	Ethylcystinate dimmer
fMRI	Functional magnetic resonance imaging
gm	Gram
g/mol	Gram per mole
HMPAO	Hexamethyl propylene amine oxime
HPLC	High Performance Liquid Chromatography
hr	Hour
IB ZM	Iodobe nzami de
I.D.	Injected dose
IMP	iso propyl iodoamphatamine
INDD	Intranasal drug delivery

I.V.	Intravenously
KBq	Kilo Becquerel
KeV	Kilo electron volt
LLE	Liquid liquid extraction
MBq	Mega Becquerel
mCi	Millicurie
MeV	Mega electron volt
mg	Milligram
min	Minute
ml	Millilitre
μl	Microlitre
MnO ₄	Permanganate ion
Мо	Molybdenum
MRI	Magnetic resonance imaging
NBS	N-bromosuccinimide
NCS	N-chlorosucci ni mi de
p	Level of significance
P	Proton
PC	Paper chromatography
PET	Positron emission tomography
PETR	positron emitting tracers
p.i.	Post injection
PTSM	Pyruvaldehyde bis (N ₄ ,N ₄ -dimethylthiosemicarbazon)
RIA	Radi oi mmuno assay
SPECT	Single photon emission computed tomography
TLC	Thin layer chromatography
α	Alpha particles
β	Beta particles
β^+	Positron

β-	Beta particles
У	Gamma emission

LIST OF TABLES

Table No	Title	Page
(I)	Examples of radiopharmaceuticals used as therapeutic agents	2
(II)	Examples of radiopharmaceuticals used as diagnostic agents	3
(III)	Production methods and radioactive properties of ¹²³ I, ¹²⁴ I, ¹²⁵ I and ¹³¹ I	13
(IV)	Comparison between PET and SPECT	30
(1)	Radiochromatographic behavior of different radiochemical Species and their R_f values for 125 I-haloperidol, 125 I-trazodone in paper Chromatography	54
(2)	Electrophoretic behavior for ¹²⁵ I-trazodone, ¹²⁵ I-haloperidol and ^{99m} Tc-phenobarbital	55
(3)	Effect of CAT amount on the percent labeling yield of 125 I-TZ	59
(4)	Effect of CAT amount on the percent labeling yield of 125 I-HP	60
(5)	Effect of Sn(II) amount on the percent labeling yield of ^{99m} Tc-PB complex	61
(6)	Effect of TZ amount on the percent labeling yield of 125 I-TZ	62
(7)	Effect of HP amount on the percent labeling yield of ¹²⁵ I- HP	63
(8)	Effect of PB amount on the percent labeling yield of 99mTc-PB	64
(9)	Effect of pH value on the percent labeling yield of ¹²⁵ I-TZ	66

(10)	Effect of pH value on the percent labeling yield of 125 I-HP	67
(11)	Effect of pH value on the percent labeling yield of 99mTc-PB complex	68
(12)	Effect of reaction time on the percent labeling yield of ¹²⁵ I-TZ	69
(13)	Effect of reaction time on the percent labeling yield of 125I-HP	70
(14)	Effect of reaction time on the percent labeling yield of 99mTc-PB complex	71
(15)	Effect of reaction temperature on the percent labeling yield of ¹²⁵ I-TZ	72
(16)	Effect of reaction temperature on the percent labeling yield of ¹²⁵ I-HP	73
(17)	Effect of reaction temperature on the percent labeling yield of ^{99m} Tc-PB complex	74
(18)	<i>In-vitro</i> stability of ¹²⁵ I-TZ	75
(19)	<i>In-vitro</i> stability of ¹²⁵ I-HP	75
(20)	<i>In-vitro</i> stability test for ^{99m} Tc-PB	76
(21)	Physicochemical properties of ME formulation	86
(22)	Timed biodistribution in different organs of normal Swiss albino mice post IV and IN administration of the three formulations of 125 I-trazodone HCl (% ID/g \pm SD, $n=3$)	88
(23)	Brain/Blood uptake ratio of ¹²⁵ I-TZ	89
(24)	Timed biodistribution in different organs of normal Swiss albino mice post IV and IN administration of the three formulations of 125 I-haloperidol (% ID/g \pm SD, n $= 3$)	91
(25)	Brain/Blood ratio of ¹²⁵ I-HP	92
I		

(26)	Timed biodistribution in different organs of normal Swiss albino mice post IV and IN administration of the three formulations of 99m Tc-phenobarbital (% ID/g ± SD, n = 3)	94
(27)	Brain/Blood ratio of ^{99m} Tc-PB	95
(28)	Comparison of biodistribution of ¹²⁵ I-trazodone, ¹²⁵ I-haloperidol and ^{99m} Tc-phenobarbital of the different formulations at 15 minutes post injection	95

LIST OF FIGURES

Figure No	Title	Page
(I)	Decay scheme of technetium-99m	19
(II)	Thin Layer Chromatographic Technique	21
(III)	Electrophoresis apparatus schema	22
(IV)	Electrophoresis Apparatus	23
(V)	Liquid liquid extraction using separatory funnel	24
(VI)	HPLC Apparatus	25
(VII)	Schema of a PET acquisition process	30
(VIII)	Scintillation and gamma camera	31
(IX)	Trazodone HCL (TZ)	39
(X)	Haloperidol (HP)	40
(XI)	Phenobarbital sodium (PB)	40
(XII)	Paper chromatographic development of ¹²⁵ I-HP and ¹²⁵ I-TZ	48
(XIII)	Radiochemical analysis pattern of 99m Tc-PB	49
(XIV)	Paper electrophoresis development of ¹²⁵ I-TZ and ¹²⁵ I- HP	50
(XV)	Paper electrophoresis development of ^{99m} Tc-PB	51
(1)	Profile of free iodide and ¹²⁵ I-TZ using paper electrophoresis	56
(2)	Profile of free iodide and ¹²⁵ I-HP using paper electrophoresis	56
(3)	Profile of free pertechnetate and ^{99m} Tc-PB using paper electrophoresis	56
(4)	HPLC chromatogram of TZ and ¹²⁵ I-TZ	57
(5)	HPLC chromatogram of HP and ¹²⁵ I-HP	57
(6)	HPLC chromatogram of PB and ^{99m} Tc-PB	58