

Synthesis of Borono Fluoro Deoxy D glucose as Boron carrier for Boron neutron capture therapy (BNCT)

Zafer Akan^{1*}, Taner Ozel², Gokhan Oto³, Hulya Ozdemir³

Abstract

Objective: The ¹⁸F-labeled Deoxy-D-Glucose exhibits high affinity to cancer tissue as a PET (Positron Emission Tomography) imaging agent for metastatic cancers. Complexation of ¹⁰B to ¹⁸F-DG complex may create a useful BNCT (Boron Neutron Capture Therapy) agent for cancer therapy. In previous studies, complexation and characterization of ¹⁰B with DG were evaluated and biodistribution analysis was completed successfully. Radio labeled ¹⁰B-DG will be a useful approach for uses for BNCT applications.

Methods: The ¹⁸F-DG synthesized by ion exchange and complexed with ¹⁰B(OH)₃ via pH reactions. ¹⁸F-DG-¹⁰B complexation was assayed with Agilent 1260 Infinity HPLC-DAD and Agilent 6420 Triple Quad LC/MS. Complexed molecule defragmented and fragmentation products assayed with Agilent 6420 Triple Quad LC/MS for confirmation.

Results: ¹⁰B-¹⁸F-DG complex was obtained with ion-exchange pH reaction successfully. Complexation of two ¹⁰B(OH)₃ to ¹⁸F-DG was determined with Triple Quad LC/MS. Purification of ¹⁰B-¹⁸F-DG is currently in progress.

Conclusions: Radio-labelled ¹⁰B-DG will supply new insight to research for BNCT studies. Cancer detection and therapy will be applicable in the same schedule with ¹⁰B-¹⁸F-DG complex.

Key words: Boron Neutron Capture Therapy, Borono 2-Deoxy-D-glucose, Boronophenylalanine, Glucose, 2-Fluoro-2-deoxy-D-glucose

Introduction

Boron Neutron Capture Therapy (BNCT) is a alternative radiotherapy method used in the treatment of head and neck cancers, especially for Glioblastoma (GBM) and Astrocytoma tumors (1).

Tumour tissue exposes to neutron after boron (¹⁰B) accumulation in the tumor cells, neutron exposed ¹⁰B's captures the neutrons and nuclear fusion reaction occurs (¹⁰B+¹n→¹¹B→⁷Li+⁴He+γ). Usually, epithermal energetic neutrons (0.4 keV-10 or 20 keV) are used for treatment. Today, clinical trials of BNCT are performed in a few nuclear reactors and BNCT aimed cyclotron include centers (1, 2).

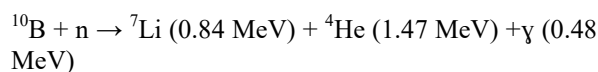
Produced cytotoxic 4He's from the fusion reaction, triggers the apoptosis and damages the tumor tissue specifically.

These charged particles can not move too far and release their all energy into the tumor cells, thus damages the DNA and directs to apoptosis the tumor cells. These products, which are the result of irradiation of ¹⁰B, have Linear energy transfer (LET)

characteristics (LET about 150 keV·m⁻¹ for He particle and about 175 keV·m⁻¹ for Li).

The length of these reaction products is between 4.5-10 nm, which is about the diameter of a cell.

The basic reaction of BNCT is as follows:



Basic problems at BNCT

- 1- Boron accumulation in to tumour tissue
- 2 - Neutron source.

For use as a neutron source for clinical trials, beside nuclear reactors, SUMITOMO has produced two 30 MEV energized cyclotrons (HM-30) (2, 3, 4, 5).

In addition to neutron source problem, Boron carriers that are alternative to Boron Phenyl Alanine (BPA) are still being worked on. In previous study ¹⁰B-DG

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1 Lycia Press Inc. Ltd, 3rd Floor, 86 - 90 Paul Street, EC2A 4NE London, UK

2 Celal Bayar University, Faculty of Medicine, Dept. of physiology, Manisa, TR

3 Yuzuncu Yil University, Faculty of Medicine, Dept. of Pharmacology, Van, TR

* Corresponding Author: Zafer AKAN E-mail: zafer_akan@hotmail.com Phone: +44 203 289 92 94



has been synthesized and bio-distribution analysis were completed. In this project, we proposed a reaction for the synthesis of the radioactive labelled boron carrier, Borono-Floro-Deoksi-D-Glucose (^{10}B - ^{18}F -DG).

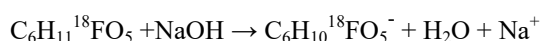
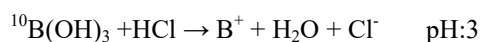
^{10}B - ^{18}F -DG complex was obtained with ion-exchange pH reaction successfully. Complexation of two $^{10}\text{B}(\text{OH})_3$ to ^{18}F -DG was determined with Triple Quad LC/MS and shown to be an alternative boron carrier to BPA by in-vitro comparison studies (6, 7, 8).

Material and Methods

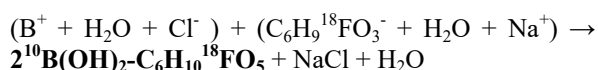
To the compromise, yield of complexation reactions, two different pH reactions prepared.

Reaction 1 (R1): pH:3 Reaction: Boric Acid ($^{10}\text{B}(\text{HO})_3$: Sigma-Aldrich, Saint Louis, MO, 63103, USA; MW: 61,03 g/mol) solutions has been prepared in 0.08, 0.16 ve 0.32 mM pH:3 concentrations and ; 0.08 mM ^{18}F -DG ($\text{C}_6\text{H}_{11}^{18}\text{FO}_5$, MW: 181,1495 g/mol) pH:3. Solute were mixed and pH elevated to physiological pH:7,4 with NaOH.

Reaction 2 (R2): pH:3, pH:11 Reaction: Boric Acid ($^{10}\text{B}(\text{HO})_3$: Sigma-Aldrich, Saint Louis, MO, 63103, USA; MW: 61,03 g/mol) solutions has been prepared in 0.08, 0.16 ve 0.32 mM pH:3 concentrations and ; 0.08 mM ^{18}F -DG ($\text{C}_6\text{H}_{11}^{18}\text{FO}_5$, MW: 181,1495 g/mol) pH:11. Solute were mixed and pH fixed at physiological pH:7,4.



pH:11



pH:7

The ^{18}F -DG complexed with $^{10}\text{B}(\text{OH})_3$ via low-high pH reaction. ^{18}F -DG- ^{10}B complexation was assayed with Agilent 1260 Infinity HPLC-DAD and Agilent 6420 Triple Quad LC/MS. Complexed molecule defragmented and fragmentation products assayed with Agilent 6420 Triple Quad LC/MS for confirmation.

For ^{10}B uptake compromise analysis: 0, 20, 40, 80 $\mu\text{g}/\text{ml}$ ^{10}B - ^{18}F FDG and ^{10}B PBA were prepared; Mat LyLu: ATCC JHU-92 cell line (5×10^6 cell) maintained in CO_2 incubator at 37 $^\circ\text{C}$, 0.5 CO_2 , RPMI1640, 10% FCS, 0.5% penicillin/streptomycin (Sigma Aldrich).

For ^{10}B uptake compromise analysis at different concentrations ^{10}B - ^{18}F FDG (0, 20, 40, 80 $\mu\text{g}/\text{ml}$) and ^{10}B PBA were add to Mat LyLu cell suspension and

incubated for 4 hours. Then cells were washed with phosphate buffer saline (PBS; Sigma-Aldrich, Sigma-P4417) solutions for three times and lysed with ultrasonography.

Cell debris centrifuged and clean intracellular solution used for ^{10}B analysis in Agilent 7700 ICP-MS analysis.

Results

Concentration depending elevation of ^{18}F -DG shift has been shown in figure 1 by HPLC analysis (^{18}F -DG 0.08, 0.16 ve 0.32 mM). (Agilent 1260 Infinity HPLC-DAD) (Figure 1).

For complexation reaction 0.08 mM ^{18}F -DG complexed with $\text{B}(\text{OH})_3$ in three different concentrations (0.08, 0.16 ve 0.32 mM) with two different pH approaches (R1, R2) in figure 2.

Due to peak shifts, the best yield of complexation reaction observed in $^{10}\text{B}(\text{OH})_3$ (0.32 mM) pH:3 + ^{18}F -DG (0.08 mM) pH:11 (R2 Reaction) (Figure 2).

The LC/MS analysis has been used to complexation confirmation for R1 and R2 Reactions. Complexation observed with both R1 and R2 (Figure 3A, 4A).

Complexation peak of ^{10}B - ^{18}F -DG elevated at 268.8 MW clearly. For confirmation ^{10}B - ^{18}F -DG complex collided with ions in different collision energy (5, 10, 15) and complex defragmented. Defragmentation and Defragmentation products of complexed ^{10}B - ^{18}F -DG has been observed (Figure 3B, 3C, 3D, 4B, 4C, 4D).

The peak at 268.8 MW is compatible with our ^{10}B - ^{18}F -DG MW calculations. Due to our calculations $2^{10}\text{B}(\text{OH})_2\text{-C}_6\text{H}_{10}^{18}\text{FO}_5$ MW is about 268.

According to literature, BoronoPhenylAlanine (BPA) and radioactive tagged ^{18}F -BPA is most usefull Boron carrier for BNCT clinical applications. To compare ^{10}B PBA and ^{10}B - ^{18}F -DG uptake yield, invitro comparison analysis was settled.

For ^{10}B uptake compromise analysis: 0, 20, 40, 80 $\mu\text{g}/\text{ml}$ ^{10}B - ^{18}F FDG and ^{10}B PBA were prepared; Mat LyLu: ATCC JHU-92 cell line (5×10^6 cell) maintained in CO_2 incubator at 37 $^\circ\text{C}$, 0.5 CO_2 , RPMI1640, 10% FCS, 0.5% penicillin/streptomycin (Sigma Aldrich).

For ^{10}B uptake compromise analysis at different concentrations ^{10}B - ^{18}F FDG (0, 20, 40, 80 $\mu\text{g}/\text{ml}$) and ^{10}B PBA were add to Mat LyLu cell suspension and incubated for 4 hours. Then cells were washed with phosphate buffer saline (PBS; Sigma-Aldrich, Sigma-P4417) solutions for three times and lysed with ultrasonography. Cell debris centrifuged and clean intracellular solution used for ^{10}B analysis in Agilent 7700 ICP-MS analysis (Figure 4).

Due to statistical analysis, ^{10}B - ^{18}F FDG uptake yield better than ^{10}B PBA ($P < 0.001$)

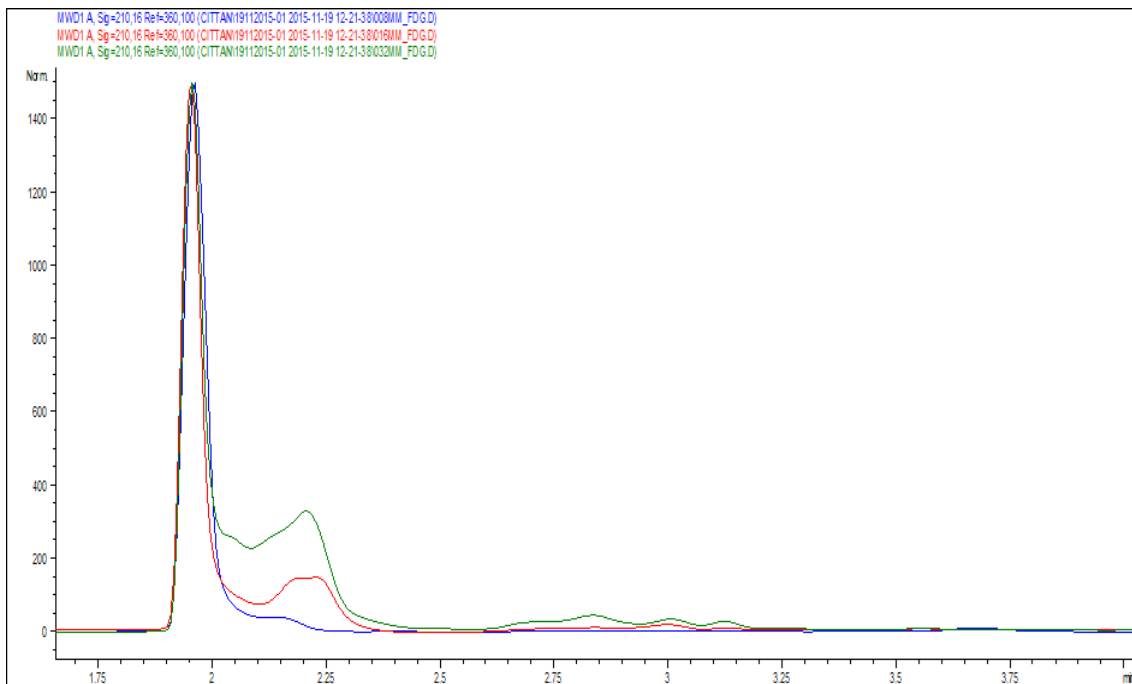


Figure 1: HPLC analysis of different concentration of ^{18}F -DG (0.08, 0.16 ve 0.32 mM). (Agilent 1260 Infinity HPLC-DAD)

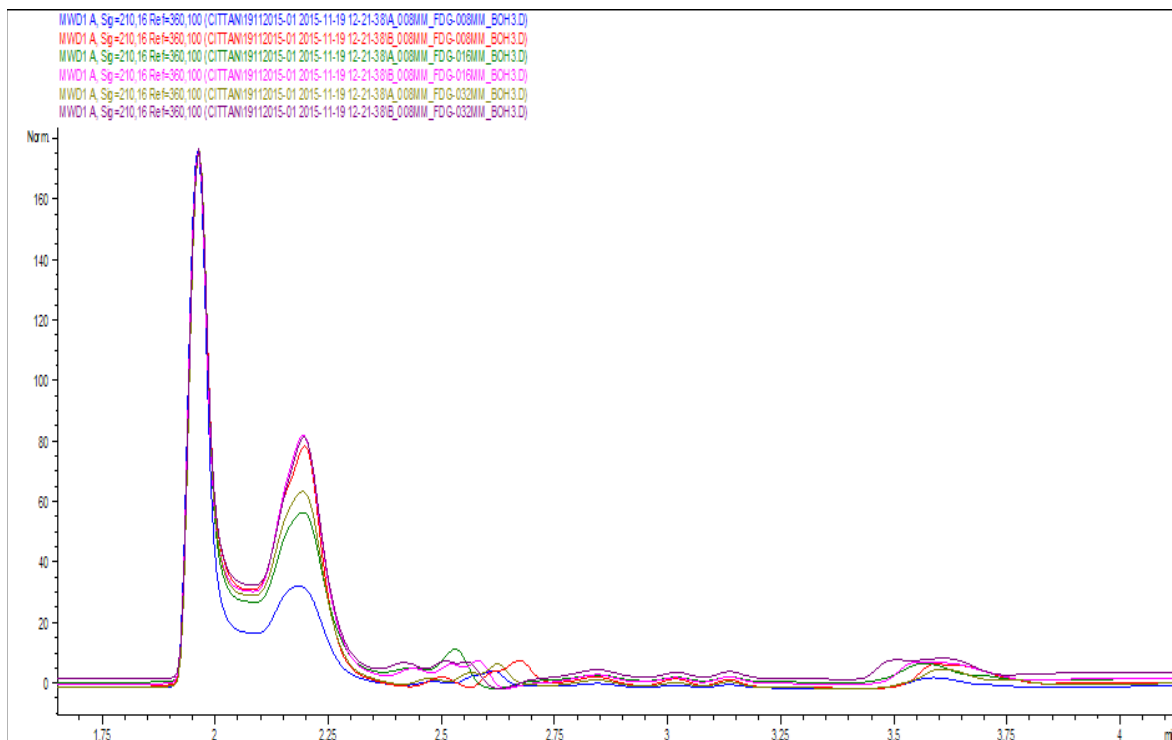


Figure 2: Complexation reaction analysis of FDG (0.08 mM) with 0.08, 0.16 ve 0.32 mM $^{10}\text{B}(\text{OH})_3$ with 2 different reaction conditions: $\text{FDG pH:3} + \text{B}(\text{OH})_3 \text{ pH:3} \rightarrow \text{pH:7}$ and $^{18}\text{F}\text{-DG pH:11} + ^{10}\text{B}(\text{OH})_3 \text{ pH:3} \rightarrow \text{pH:7}$. $\text{FDG pH:11} + \text{B}(\text{OH})_3 \text{ pH:3} \rightarrow \text{pH:7}$ reaction yield is more than first reaction (Agilent 1260 Infinity HPLC-DAD)

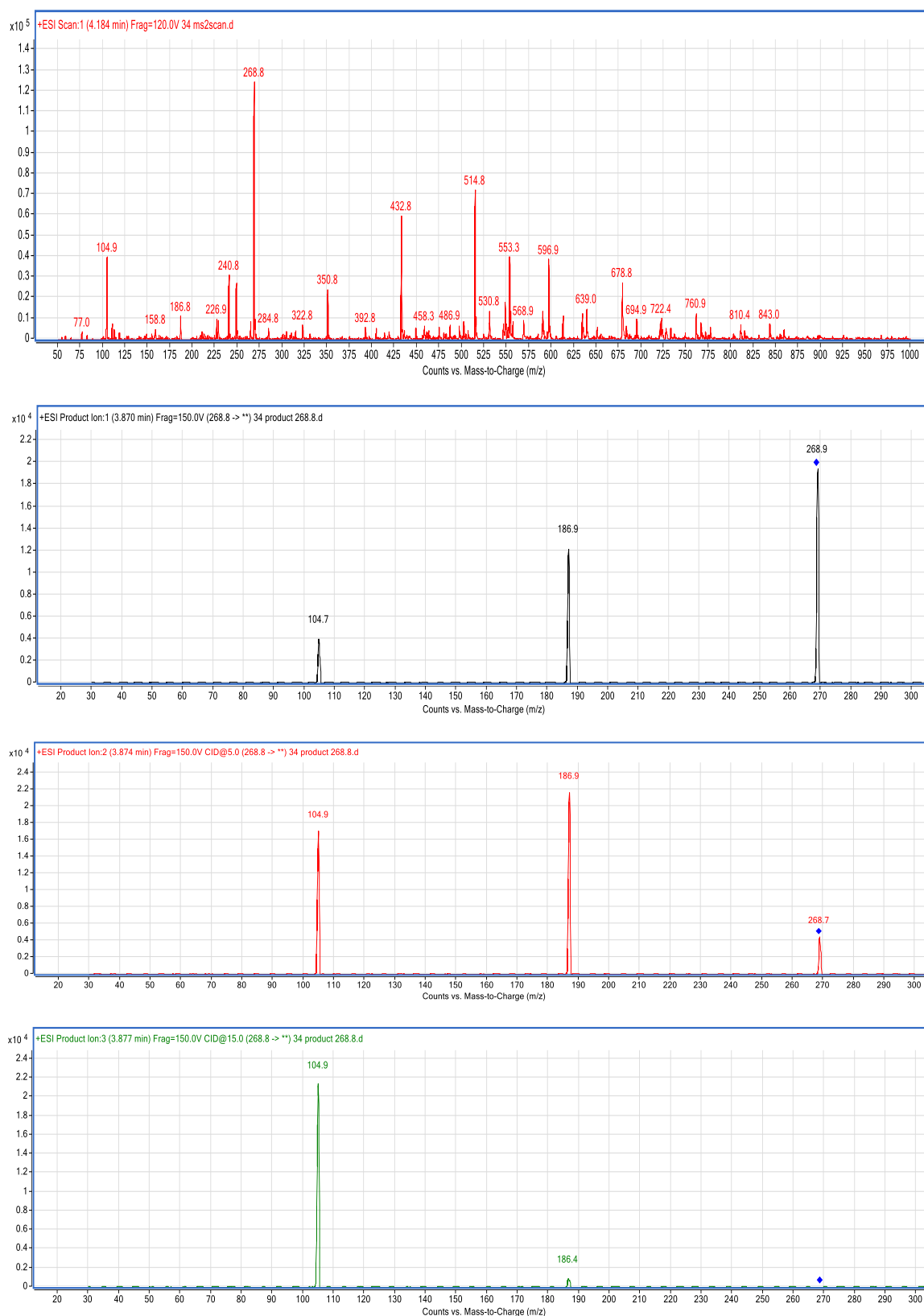


Figure 3: The results of ^{18}F -DG pH:3 + $^{10}\text{B}(\text{OH})_3$ pH:3 \rightarrow pH:7 complexation reaction, Agilent 6420 Triple Quad LC/MS. **A)** All $^{10}\text{B}(\text{OH})_3 - ^{18}\text{F}$ -DG complexation reaction products **B)** Complexation product (BFDG) pike at 268.9 MW (Collision Energy =5) **C)** Degradation product of complex at 268.9 MW (Collision Energy =10). **D)** Degradation product of complex at 268.9 MW (Collision Energy =15).

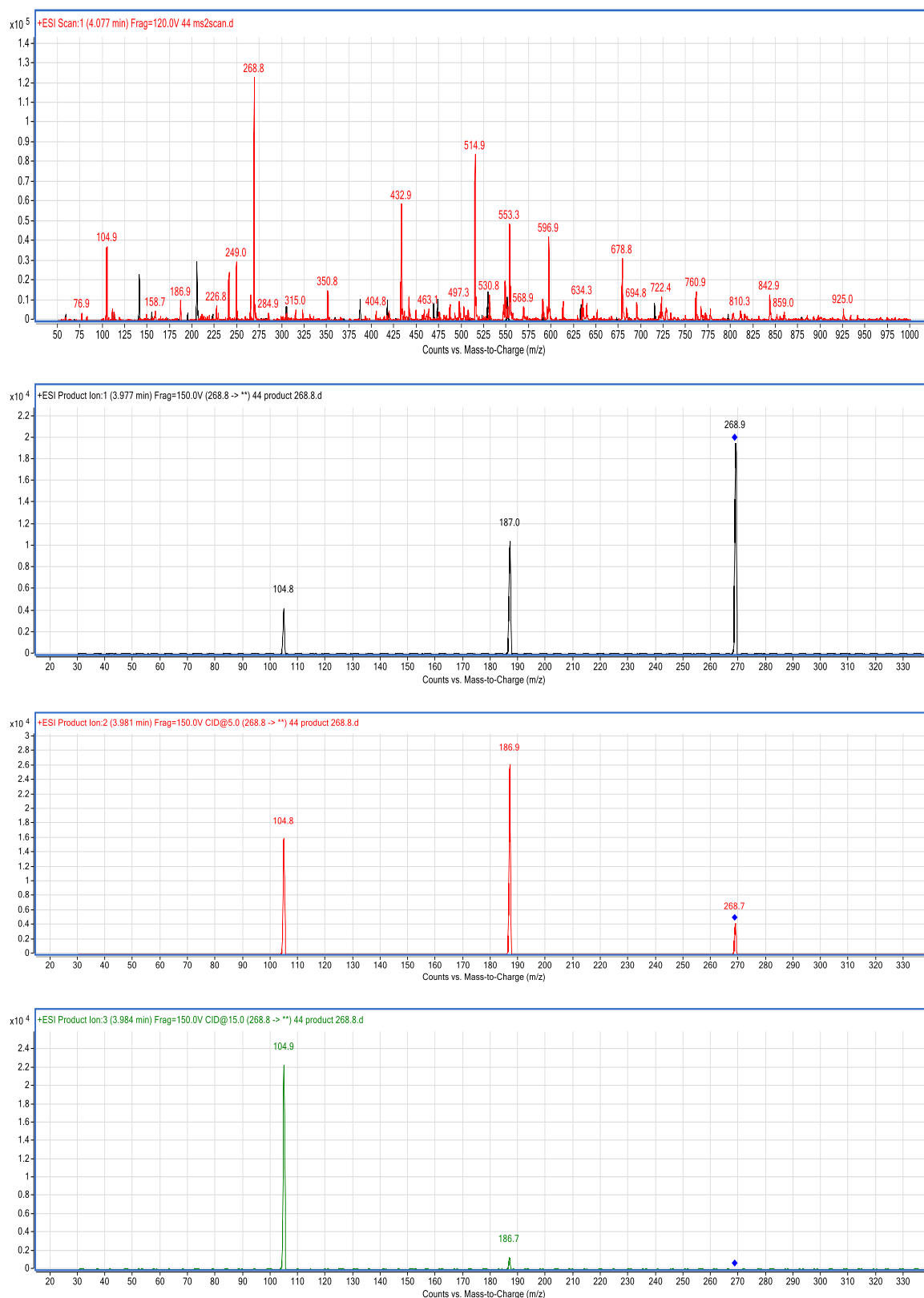


Figure 4: the results of ^{18}F -DG pH:11 + $^{10}\text{B}(\text{OH})_3$ pH:3 \rightarrow pH:7 complexation reaction, Agilent 6420 Triple Quad LC/MS. **A)** All $^{10}\text{B}(\text{OH})_3$ - ^{18}F -DG complexation reaction products **B)** Complexation product (BFDG) pike at 268.9 MW (Collision Energy =5) **C)** Degradation product of complex at 268.9 MW (Collision Energy =10). **D)** Degradation product of complex at 268.9 MW (Collision Energy =15).

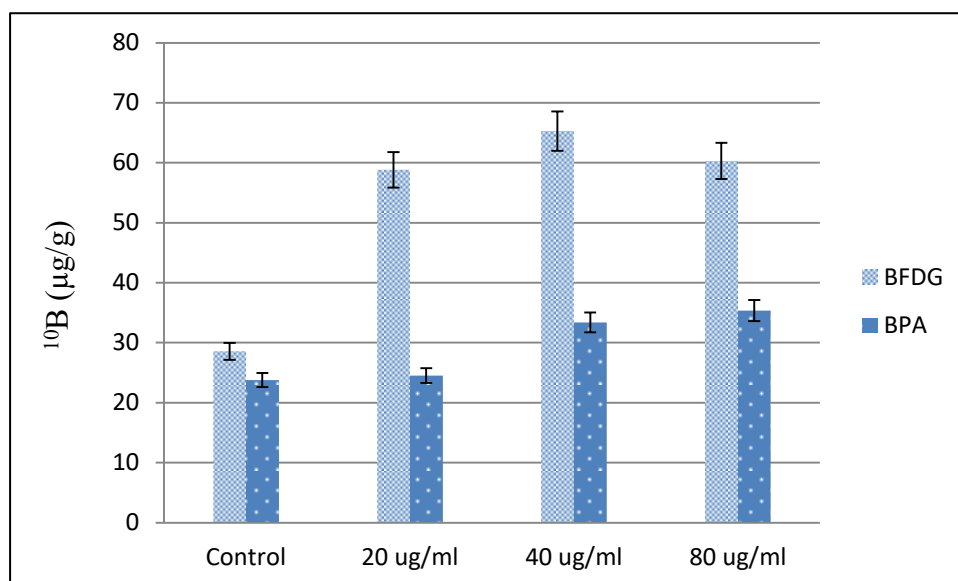


Figure 5: Invitro uptake compromise analysis of ¹⁰B: 0, 20, 40, 80 µg/ml ¹⁰B-¹⁸FDG and ¹⁰BPA were prepared; Mat LyLu: ATCC JHU-92 cell line (5x10⁶ cell) maintained in CO₂ incubator for 4 hours at 37 °C, 5% CO₂, RPMI1640, 10% FCS, 0.5% penicillin/streptomycin (Sigma Aldrich).

Discussion

Head and neck cancers such as Glio Blastoma Multiform (GBM), and astrocytomas treatment are among the most difficult type of cancers due to aspects of treatment modality. Chemotherapy and radiotherapy response is inadequate and surgery is quite risky cause of the very low surgical intervention. Due to lack of effective treatment, the life span of patients very low. Due to some survival reports for recurrent cancers the average life time of patient changes between 6-9 months after diagnoses of cancer (5).

Because of the success of treatment of head and neck tumors, many groups are working on alternative methods such as Boron Neutron Capture Therapy (BNCT). Private companies such as SUMITOMO carry out clinical trials with their own specific accelerators to prepare this treatment method as a routine treatment protocol (1, 2, 3, 4, 5, 6).

To date, approximately 200 BNCT clinical trials have completed and some of them reported worldwide. Beside complete recovery of some patients, average patient survival reported more than 24 months (9).

The biggest problem of BNCT as a routine treatment is the cost problem. Beside, thermal, epithermal neutron source, the boron carrier agent cost is the biggest obstacle to the routine implementation of BNCT.

As indicated in literature complexation of boron with sugar compounds based on pH reaction.

Lorand and Edwards published work quantifying the affinity of boric and phenylboronic acids for simple diols (e.g.-ethylene glycol, catechol) and common monosaccharides (i.e.-glucose, fructose, mannose, galactose) (10, 11).

The covalent product between a boronic acid and a diol is termed a boronate ester, analogous to a carboxylate ester. These interactions are favoured at basic pH ranges where the tetrahedral boronate ester is formed (Figure 6). The interchange between boronic acids and divalent ligands in aqueous solution can be complex and varied depending on pH.

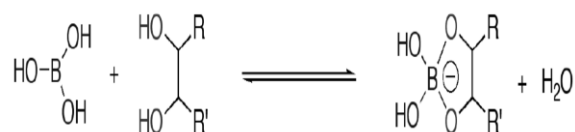


Figure 6: Boric Acid interactions with sugar (10)

In our previous studies, success of boron targeting of deoxy-d-glucose to tumor tissues (borono deoxy-d-glucose, BDG) has been shown which deoxy-d-glucose routinely used for ¹⁸F targeting to tumour tissue in positron emission tomography (PET) for tumor detection and staging.

In this study, we proposed a simple pH reaction method for the synthesis of ¹⁸F-¹⁰B-DG, (¹⁸F-BDG) which could be used beside to ¹⁸F-¹⁰B-PA, (¹⁸F-BPA)

Due to invitro uptake analysis, synthesized ^{18}F -BDG has shown more efficient carrier properties than BPA to the respect of boron accumulation test on cancer cells.

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Conflict of Interest: The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author's Contributions: ZA, GO, HO: Design of study, ZA, TO: Complexation studies, Invitro analysis Collecting of patients data, Patient examination and operation, writing and revision of article, HC: Statistical analysis of findings.

Ethical issues: All Authors declare that Originality of research/article etc... and ethical approval of research, and responsibilities of research against local ethics commission are under the Authors responsibilities. The study was conducted due to defined rules by the Local Ethics Commission guidelines and audits.

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