Quantification of tracer mass in cardiac positron emission tomography using high-performance liquid chromatography

Quantification de la masse tracante en tomographie par émission de positrons cardiaques à l'aide de la chromatographie en phase liquide à haute performance

Jenna Abu-Dieh[1*], Benjamin Rotstein[1]

1. University of Ottawa, Ottawa, ON, Canada
*Corresponding author. Email: jabud024@uottawa.ca

Abstract | Résumé

Cardiac sympathetic nervous system dysfunction is associated with multiple cardiovascular diseases and may be assessed non-invasively using positron emission tomography (PET) imaging. Meta-fluorobenzylguanidine labeled with fluorine-18 ([18F]mFBG) is a radiotracer that targets the norepinephrine transporter (NET) and enables quantitative evaluation of sympathetic nerve function. However, tracer formulations contain both radioactive and non-radioactive forms, and excess non-radioactive mass may compete for transporter binding and affect imaging accuracy. This proposal aims to develop a high-performance liquid chromatography (HPLC)-based method to quantify total tracer mass and determine molar activity of [18F]mFBG. The protocol uses calibration standards of non-radioactive mFBG under formulation-matched solvent conditions and provides analysis using UV-detected HPLC to generate a calibration curve. The system analyzes formulated tracer samples to determine total mFBG concentration and allows for the calculation of molar activity as the ratio of radioactivity to total tracer mass. This approach is expected to provide a reproducible method for ensuring biologically negligible tracer mass in preclinical PET studies and may support future clinical translation.

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Le dysfonctionnement du système nerveux sympathique cardiaque est associé à de multiples maladies cardiovasculaires et peut être évalué de manière non invasive à l'aide d'imagerie par tomographie par émission de positrons (TEP). La méta-fluorobenzylguanidine marquée au fluor-18 ([18F]mFBG) est un radiotraceur qui cible le transporteur de noradrénaline (NET) et permet une évaluation quantitative de la fonction du nerf sympathique. Cependant, les formulations tracer contiennent à la fois des formes radioactives et non radioactives, et une masse non radioactive excessive peut rivaliser pour la liaison du transporteur et affecter la précision de l'imagerie. Cette proposition vise à développer une méthode basée sur la chromatographie en phase liquide à haute performance (CLHP) pour quantifier la masse traceuse totale et déterminer l'activité molaire de [18F]mFBG. Le protocole utilise des standards d'étalonnage pour mFBG non radioactif dans des conditions de solvant adaptées à la formulation et fournit une analyse utilisant du CLHP détecté par UV pour générer une courbe d'étalonnage. Le système analyse des échantillons traceurs formulés pour déterminer la concentration totale de mFBG et permet de calculer l'activité molaire comme le rapport radioactivité à la masse totale du traceur. Cette approche devrait fournir une méthode reproductible pour garantir une masse traceuse biologiquement négligeable dans les études précliniques TEP et pourrait soutenir la traduction clinique future.

Keywords: Cardiac PET imaging, fluorine-18 meta-fluorobenzylguanidine ([18F]mFBG), norepinephrine transporter, high-performance liquid chromatography, molar activity, radiotracer quantification, sympathetic nervous system imaging, radiopharmaceutical quality control.

Introduction

The cardiac sympathetic nervous system plays a critical role in regulating cardiac function, and its dysfunction is associated with conditions such as heart failure and arrhythmias. Positron emission tomography (PET) imaging enables non-invasive assessment of sympathetic innervation using radiotracers that target the norepinephrine transporter (NET).

Meta-fluorobenzylguanidine labeled with fluorine-18 ([18F]mFBG) is a PET radiotracer that enters sympathetic nerve terminals via NET and exhibits measurable myocardial uptake and washout kinetics (1, 2). These characteristics provide a quantitative assessment of cardiac sympathetic activity. However, each tracer formulation contains both radioactive and non-radioactive ("cold") mFBG, and excessive non-radioactive mass may compete with the radiotracer for NET binding, potentially confounding imaging results (1).

Molar activity, defined as the ratio of radioactivity to total tracer mass, is therefore a critical parameter. Ensuring high molar activity minimizes biological perturbation and improves the validity of PET measurements. Despite the growing use of [18F]mFBG for cardiac sympathetic nervous system imaging,

studies rarely standardize quantification of total tracer mass and molar activity in formulated samples. Existing studies primarily focus on radiochemical synthesis, biodistribution, and imaging performance, while they inconsistently describe accurate measurements of non-radioactive ("cold") mFBG content (1, 2). This represents an important limitation because excess non-radioactive mass may compete with radiotracer uptake through the NET, potentially altering tracer kinetics and reducing quantitative imaging accuracy. In addition, low analyte concentrations and solvent incompatibilities may complicate reproducible HPLC-based quantification. Therefore, the development of a formulation-matched and reproducible analytical workflow may improve quality control practices and support standardized assessments of biologically negligible tracer mass in preclinical PET imaging.

This project proposes the development of a high-performance liquid chromatography (HPLC)-based method to quantify total mFBG concentration and determine molar activity in [18F]mFBG formulations used for cardiac PET imaging.

No published literature outlines a standardized HPLC-based workflow specifically for the reproducible quantification of total mFBG mass in formulated [18F]mFBG PET tracer preparations under formulation-matched solvent conditions. The proposed study therefore aims not only to quantify tracer mass, but also to establish a reproducible analytical strategy that may improve standardization of molar activity measurements in NET-targeted PET imaging studies.

Methods

Preparation of calibration standards
A series of non-radioactive mFBG calibration standards was prepared over a biologically relevant concentration range using a solvent system matching the tracer formulation. To improve solubility and analytical reproducibility, calibration standards were prepared using a formulation-matched solvent system containing 10% dimethyl sulfoxide in water. This approach was selected to minimize variability associated with solvent incompatibilities and improve consistency between calibration standards and formulated tracer samples. Serial dilutions were performed to generate standards spanning the expected concentration range of formulated samples.

HPLC analysis
All samples were analyzed using high-performance liquid chromatography equipped with UV detection. A fixed injection volume was used for all standards and samples to ensure consistency. Chromatographic separation was performed under standardized conditions, and the retention time corresponding to mFBG was identified based on reference standards. Peak areas were integrated using chromatography software.

Calibration curve generation
Calibration curves were generated by plotting integrated peak area as a function of known mFBG concentration. Linear regression analysis was performed to determine the relationship between concentration and detector response. Only calibration ranges that demonstrated linearity were used for quantification.

Quantification of tracer samples
Aliquots of formulated [18F]mFBG batches were analyzed using the same HPLC method. The integrated peak area corresponding to mFBG was used to determine total mFBG concentration by interpolation from the calibration curve. Total mFBG amount was calculated based on formulation volume.

Molar activity calculation
Total radioactivity of each tracer batch was measured independently using a dose calibrator. Molar activity was calculated as the ratio of radioactivity to total mFBG amount. All variables were defined prior to calculation to ensure consistency across samples.

Expected Outcomes
It is anticipated that calibration standards will produce a linear relationship between mFBG concentration and integrated HPLC peak area within a defined concentration range. The retention time for mFBG is expected to remain consistent across runs, allowing reliable identification of the compound.

Analysis of formulated [18F]mFBG samples is expected to yield measurable peaks corresponding to mFBG, enabling quantification of total tracer mass. Interpolation from the calibration curve should allow accurate estimation of mFBG concentration.

Molar activity values are expected to fall within a range consistent with biologically negligible tracer mass, supporting the validity of PET imaging measurements. The proposed method is anticipated to provide reproducible and consistent results across multiple samples.

Discussion

The proposed study aims to establish a practical and reproducible HPLC-based method for quantifying total mFBG concentration and determining molar activity in [18F]mFBG tracer formulations. Accurate molar activity measurement is essential to ensure that injected tracer mass does not interfere with biological processes, particularly in transporter-mediated imaging (1). A major strength of the proposed method is the incorporation of formulation-matched calibration standards designed to improve analytical reproducibility under conditions closely resembling formulated tracer samples. This may reduce variability associated with analyte solubility and solvent mismatch, which can affect chromatographic peak shape and detector response at low concentrations. By improving consistency of molar activity measurements, the proposed workflow enhances confidence that injected tracer masses remain biologically negligible during NET imaging studies. This is particularly important for transporter-mediated PET tracers, where competition between radioactive and non-

radioactive ligand may influence quantitative interpretation of uptake kinetics.

The use of a formulation-matched solvent system is expected to improve consistency and reduce variability in calibration. However, potential limitations include variability in injection volume, detector sensitivity, and chromatographic conditions. These factors introduce error and will require careful standardization.

This work contributes toward the development of more standardized quality control protocols for PET radiotracers where tracer mass effects are a concern. Beyond [18F]mFBG, the proposed workflow applies to other transporter-targeted PET tracers requiring accurate molar activity determination. Improved standardization of tracer mass quantification may ultimately support greater reproducibility across preclinical imaging studies and facilitate future clinical translation.

Conclusion

This proposal outlines the development of an HPLC-based method to quantify total mFBG concentration and calculate molar activity in [18F]mFBG tracer formulations. The expected outcomes include a reproducible calibration method and reliable estimation of tracer mass. This approach has the potential to improve the accuracy and biological validity of cardiac PET imaging studies and may be extended to other radiotracers in both preclinical and clinical settings.

References

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