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The separation and detection of PET tracers via capillary electrophoresis for chemical identity and purity analysis

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ABSTRACT

CE coupled with UV detection was assessed as a possible platform for the chemical identity and purity analysis of positron emission tomography (PET) tracers using [^{18}F]FAC and [^{18}F]FLT as examples. Representative samples containing mixtures of the tracers plus well-known impurities, as well as real radioactive samples (formulated for injection), were analyzed. Using MEKC with SDS in a neutral phosphate buffer, the separation of all compounds in the samples was achieved with baseline resolutions in less than 4.5 min and 3 min for FLT and FAC samples, respectively. In comparison to the gold-standard for chemical analysis (i.e. HPLC/UV), we have demonstrated improvements in analysis times, and comparable LOD. Although the reproducibility in migration time is slightly lower than that of the HPLC, identification of the compounds was still possible due to good peak separation. In addition, we show that CE can be used to identify and quantify Krytofix2.2.2 (a toxic and commonly used phase transfer catalyst) in less than 2 min and with a LOD of 45 μg/mL (non-optimized). These results demonstrate adequate performance for chemical identity and purity analysis. Combined with the potential for miniaturization into a microchip format, these results suggest the potential of CE as an integral part of a miniaturized quality control system for PET tracers.

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1. Introduction

Positron emission tomography (PET) is a 3D medical imaging tool that uses radiolabeled molecular tracers to measure specific *in vivo* biochemical and biological processes non-invasively [1]. Though the ^{18}F-labeled glucose analog 2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG) has been a tremendously successful tracer, especially in oncology applications, it does not meet all research or clinical needs. Many other tracers are in clinical trials [2], and, in fact, over 3000 PET tracers for different biochemical pathways have been synthesized for clinical and research use [3]. However, very few PET tracers are widely available due to the high cost of tracer synthesis and the need for a centralized production model to leverage economies of scale that make tracers affordable. To alleviate the PET tracer production bottleneck, several groups are developing microscale batch radiosynthesis platforms to perform synthesis (and sometimes purification) of PET tracers [4–6] at substantially reduced cost of equipment and radiation shielding [7]. While effort has been devoted in developing these microscale synthesis technologies, there has been little development to address the downstream quality control (QC) process to ensure the safety of each batch for injection.

General requirements for QC testing of the final formulated PET tracer can be found in USP General Chapter <823> [8], FDA 21 CFR Part 212 [9] and recent reviews [10,11]. The testing of chemical purity depends on the particular tracer and synthesis method. In a typical PET tracer production, chemical identification is performed with high performance liquid chromatography (HPLC) by matching the retention time to a reference standard and/or by co-injection of the reference standard to validate the radioactive product peak. Chemical purity analysis of PET tracers is typically performed using separation techniques involving HPLC, thin layer chromatography (TLC) and/or gas chromatography (GC) coupled with detection techniques such as UV, pulsed amperometric detection (PAD), (refractive index) RI detection, flame ionization detection (FID) and/or beta or gamma radiation detection. While residual organic solvents are typically analyzed by GC [12], most other impurities are analyzed via HPLC. For example, in the synthesis of 1-(2′-deoxy-2′-[^{18}F]fluoro-β-D-arabinofuranosyl)cytosine ([^{18}F]FAC) one important side product is the α-anomer [13] as its presence can lead to ambiguous

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PET images. In the synthesis of 3′-deoxy-3′-[^{18}F]fluorothymidine ([^{18}F]FLT) from the precursor 3-N-Boc-5′-*O*-dimethoxytrityl-3′-*O*-nosyl-thymidine (Boc-FLT), Pascali et al., [14] reported the by-products thymidine, thymine, furfuryl alcohol, stavudine (an anti-HIV agent), and chlorothymidine, some of which have pharmacological effects.

Another potential impurity common in PET tracer synthesis is Kryptofix2.2.2 (K222), which must be tested due to its toxic nature (intravenous LD_{50} of 35 mg/kg in rats) [15]. Currently, there are various tests for K222. One accepted method of analysis is the colorimetric comparison of a test solution and a reference standard (typically involves developing the TLC plate in an iodine vapor chamber or a substrate pretreated with iodoplatinate reagent) [16,17]. Other methods include the use of GC with a nitrogen-selective detector [18], HPLC–UV [19], HPLC–ELSD [20], and LC/MS/MS [21]. Among these tests, LC/MS/MS has the lowest limit of detection (LOD) of 1.0 ng/mL. However each of these test have various drawbacks. For example, the colorimetric spot tests typically take more than 15 min to conduct. Shorter test times of 5 min can be achieved using the iodoplatinate indicator strip, but this method is prone to false positives [16].

The current QC practices involve many separate, expensive and bulky analytical systems and use complicated labor-intensive methodologies. Integrated and automated QC systems are in development, which utilize miniaturized versions of conventional techniques, e.g. QC1 (Münster, Germany) [22], to reduce the complexity and decrease the laboratory space needed. However by replacing conventional techniques with microscale analytical approaches, there is much further potential to significantly reduce the size, cost, and complexity of the overall system.

One such microscale analytical approach that may offer improved analytical performance is capillary electrophoresis (CE). Similar to HPLC, it can be used to separate, identify and quantify various components in a sample, but unlike HPLC, it can be readily miniaturized into microfluidic chips [23,24] and instrumentally does not require high pressure valves, pumps and connectors that typically add to the bulk size, complexity and cost of the system. As CE can be performed on-chip and fully actuated using digital electronics, it allows for ease of automation and the potential for a cheap and disposable chemical analysis platform.

The analysis of radiochemical purity can be performed through the incorporation of a radioactivity detector on to the capillary [25,26] and the use of CE for the radiochemical purity of PET tracers in particular has been suggested in a patent application by Hansteen et al. [27]. However, to date, we believe there have been no investigations in the use of CE for PET tracer chemical identity and purity analysis to explore its analytical advantages and disadvantages and suitability for this application. In particular, analysis time, automation and instrument size are especially important due to the short half-life and radioactive nature of the chemical species typically used for PET.

This paper explores the feasibility and suitability of using CE for the chemical identification and purity analysis of two ^{18}F-labeled PET tracers, namely [^{18}F]FLT and [^{18}F]FAC. The separation methods were developed using known reference standards (i.e. representative samples) and further tested using real, radioactive tracer samples that have been purified and reformulated for injection.

2. Materials and methods

2.1. Materials

Sodium phosphate monobasic (NaH_2PO_4), sodium phosphate dibasic dihydrate (Na_2HPO_4), boric acid, sodium dodecyl sulfate (SDS), ammonium acetate, ethanol, sodium chloride (NaCl), sodium deoxycholate (SDC), dichloromethane (DCM), acetonitrile (MeCN) and sodium hydroxide (NaOH), thymine, thymidine, furfuryl alcohol (FA), 2′,3′-didehydro-3′-deoxythymidine (stavudine), and 3′ deoxy-3′-fluorothymidine (FLT) were purchased from Sigma–Aldrich (Milwaukee, WI, USA). Zidovudine impurity B (chlorothymidine, CLT) was purchased from LGC Standards (Wesel, Germany). Kryptofix2.2.2 (K222), 1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)cytosine (β-FAC), 1-(2′-deoxy-2′-fluoro-α-D-arabinofuranosyl)cytosine, and (α-FAC), 3-N-Boc-5′-*O*-dimethoxytrityl-3′-*O*-nosyl-thymidine (Boc-FLT) were purchased from ABX (Radeberg, Germany). Hydrochloric acid 0.1 N was used as supplied (Beckman Coulter, CA, USA).

As the synthesis, purification (cartridge and/or semi-preparative HPLC) and QC methods of both tracers are still in development, we have chosen our list of compounds to be analyzed from well-known impurities and byproducts [14,28]. Two representative samples were prepared for experiments. One sample consisted of FLT, K222, thymine, thymidine, FA, stavudine, CLT, and Boc-FLT which was representative of a purified [^{18}F]FLT sample containing synthesis by-products [14]. As Boc-FLT was insoluble in aqueous solvents, it was prepared separately. A 100× concentrated stock solution was prepared by dissolving Boc-FLT in MeCN with 10% DCM (v/v) and then diluted with saline. The second sample consisted of a mixture of β-FAC, α-FAC and K222, was representative of the possible contaminants in a purified [^{18}F]β-FAC sample [28]. The lists of compounds may be incomplete but were deemed sufficient to demonstrate some of the analytical advantages of CE over HPLC. All samples were prepared in saline solution (9 g/L or 154 mM of NaCl) using 0.22 μm membrane filtered 18 MΩ deionized water or phosphate buffered saline to simulate a formulated injectable tracer dose. 30 mM phosphate buffer (PB) was prepared via titration with NaH_2PO_4 and Na_2HPO_4 and monitored with a pH meter (Mettler, Toledo, Easy five, Columbus, OH, USA). 75 mM borate buffer was prepared via titration of boric acid with NaOH. 100 mM SDS in 30 mM phosphate buffer (SDS-PB) was prepared by dissolving SDS in 30 mM PB. All buffers were degassed prior to use.

Radiolabeled [^{18}F]β-FAC was synthesized, purified and formulated as in published methods [28] using the ELIXYS radiosynthesizer (Sofie Biosciences, Inc., Culver City, CA, USA). [^{18}F]FLT was synthesized using a protocol by Lee et al. [29] adapted to the ELIXYS synthesizer [30].

2.2. Methods

2.2.1. Analysis of samples via CE

CE measurements were performed on a PA800-CE (Beckman Coulter, CA, USA) system using a 75 μm I.D., 375 μm O.D. uncoated fused-silica capillary with a total length of 31.2 cm (21 cm to detector window) from Beckman Coulter (Brea, CA, USA). Capillary preconditioning was performed by sequentially flushing at 10 psi with the following solutions, 0.1 M HCl for 3 min, 1 M NaOH for 10 min, 0.1 M NaOH for 30 min, and the separating buffer for 30 min. In between separations, the capillary was flushed with the separating buffer at 5 psi for 3 min. During the experiment, the capillary and samples were maintained at room temperature (22 °C). Samples were injected electrokinetically at +10 kV for 2.5 s and separation was performed at +12 kV. Detection was performed using a photodiode array (PDA) detector (Beckman Coulter, CA, USA) monitoring at various absorption maxima (205, 218, 254 and 268 nm). Data analysis was performed using the instrument's built-in 32 Karat software (Beckman Coulter, CA, USA).

2.2.2. Analysis of samples via HPLC

HPLC separations were performed on a Knauer Smartline HPLC system using a C18 Phenomenex Luna reverse phase (4.6 mm × 250 mm, 5 μm) column (Phenomenex, Torrance, CA,

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