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"Mixed" anionic and non-ionic micellar liquid chromatography for high-speed radiometabolite analysis of positron emission tomography radioligands

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ABSTRACT

A mixed micellar liquid chromatographic (LC) method, the mobile phase consisting of anionic and non-ionic surfactants, has been developed for the high-speed direct radiometabolite analysis of positron emission tomography (PET) radioligands in plasma. The addition of Triton X-100 on an anionic surfactant sodium dodecyl sulphate (SDS) mobile phase improved elution strength and peak efficiency for many PET radioligands. Several radioligands could be easily separated from their radioactive metabolites with short run time of only 4 min using a "pure" (without organic solvent) mixed micellar mobile phase and semi-preparative monolithic C_{18}-bonded silica column by simple isocratic elution without any treatment of plasma. Moreover, the use of "hybrid" mixed micellar mobile phase containing anionic, non-ionic surfactants and organic solvent was effective to further enhance peak efficiency and elute highly retained hydrophobic PET radioligands. These characteristics enabled significant shorting the radiometabolite analysis procedure of PET radioligands and simplifying the experimental setup.

1. Introduction

Positron emission tomography (PET) is a powerful scientific and clinical tool probing physiological and biochemical processes in human subjects and animals *in vivo*. This non-invasive imaging technique is valuable for clinical molecular diagnoses and also for drug development. The biochemical scope of PET is determined by the available array of positron-emitting radioligands, which are generally labelled with ^{11}C or ^{18}F [1–3]. Radiometabolite analysis in plasma during a PET study is one of the most important components for accurate pharmacokinetic modelling of PET radioligands. Liquid chromatography coupled with radioactivity detection (radio-LC) is the most widely accepted method to determine the relative composition of PET radioligands in plasma for quantitative PET measurements [4–6]. Semi-preparative reversed phase LC columns are commonly employed for this purpose owing to relatively large sample injection (*e.g.* 1 mL plasma); however it usually requires a long time for sample treatment and chromatographic separation, making the procedure laborious, error-prone and causing less sensitive analysis due to radioactivity decay (*e.g.* ^{11}C: $t_{1/2}$ = 20.4 min, ^{18}F: $t_{1/2}$ = 109.8 min).

Micellar liquid chromatography (MLC) is one of the reversed-phase liquid chromatographic modes with a mobile phase consisting of an aqueous solution of surfactant above its critical micellar concentration (CMC) [7–9]. In this condition, the variety of interactions between solutes, surfactant monomers modified stationary phase, aqueous phase and micelles yields unique selectivity for many compounds. One of the advantages in MLC is the possibility of direct sample injection of biological material (*e.g.* plasma, serum, urine) into the column without deproteination [10,11]. We previously introduced MLC to determine PET radioligands in plasma [12,13]. These methods permit the effective and repetitive analysis of diverse compounds in untreated plasma samples by solubilizing of plasma proteins based on the formation of micellar complex between plasma proteins and anionic surfactant sodium dodecyl sulphate (SDS). In addition to simplifying the processes the MLC analysis provides a more accurate metabolite analysis. One of the drawbacks of these methods include the limitation of organic solvents that can be injected into the column and thus the use of an organic solvent gradient elution is required to decrease the retention of analytes and obtain high peak efficiency. Recently, Ebrahimi and Hadjmohammadi [14,15] and Sun et al. [16] have studied the use of mixed micellar eluents with SDS and non-ionic surfactant Brij 35. The mixed MLC is superior to the single micellar system by producing various molecular forces to allow discrimination in the retention of the analytes without the need for gradient elution.

In this study, we investigate the use of mixed micellar mobile phase consisting of anionic SDS and non-ionic Triton X-100 surfactants with isocratic elution for simplifying experimental design and speeding up radiometabolite analysis procedure of PET radioligands.

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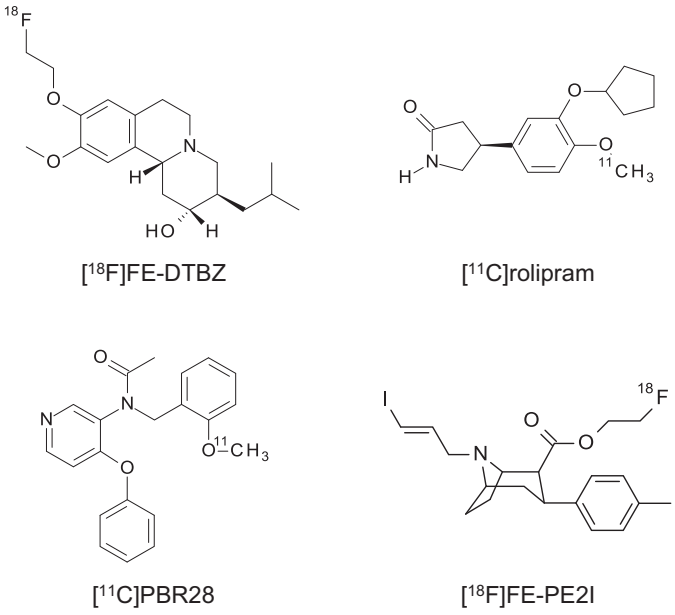

Fig. 1. PET radioligands investigated in this study.

2. Experimental

2.1. Chemicals and reagents

SDS, Triton X-100 (polyethylene glycol tert-octylphenyl ether), Tween 20 (polyoxyethylene-(20)-sorbitan monolaurate), Tween 80 (polyoxyethylene-sorbitan-(20)-monooleate), Brij 35 (polyoxyethylene-(23)-lauryl ether), ammonium phosphate monobasic, ammonium phosphate dibasic and 1-BuOH (1-butanol) were obtained from Sigma–Aldrich. Authentic samples of PET radioligands (Fig. 1) were obtained commercially or as gifts and used without further purification. ^{11}C and ^{18}F-labelled radiopharmaceuticals were prepared according to the published procedure [17–20].

2.2. Radio-LC system and radiometabolite analysis conditions

Radio-LC analysis was carried out using a quaternary LC pump (G1311A; Agilent), coupled to a manual injection valve (7725i; Rheodyne) with a 1.0 mL loop. Chromatographic separation was performed on a monolithic alkyl-bonded silica column (Onyx Monolithic Semi-PREP C18, Phenomenex, 100 mm × 10 mm I.D.) using a 1–2% (v/v) Triton X-100, 100 mM SDS, 0–5% (v/v) 1-BuOH in 200 mM ammonium-phosphate at pH 7 as the mobile phase at a flow rate of 8.0 mL/min. All experiments were conducted at room temperature. The effluent from the column was monitored by an UV absorption detector (G1314D; Agilent) in series with a dual bismuth germanium oxide coincidence radiation detector (S-2493Z; Oyokoken) housed in a shield of 50 mm thick lead. The accumulation time of radiation detector was 3.3 s and the flow cell volume was 400 μL. Data collection and control of the LC system were performed using chromatographic software (EZChrom Elite; Agilent).

2.3. Radiometabolite analysis of PET radioligands in human and monkey plasma

The human and monkey PET studies were approved by the Regional Ethics Committee and by the Animal research Ethical Committee in Stockholm. During PET measurements, whole blood samples were taken from human or monkey and collected in heparin-treated syringes at pre-specified time points after intravenous administration of radioligands. The blood samples were centrifuged at 2000 g for 2–4 min at room temperature to separate plasma. The plasma specimen was then collected and mixed with same volume of a 100 mM SDS. The resulting mixture was directly injected onto the radio-LC system.

3. Results and discussion

3.1. Retention behaviour on the mixed micellar LC

It is well known that anionic surfactants, such as SDS (Mw: 288, CMC: 8.4 mM [21]), can efficiently dissolve plasma proteins and displace the protein bound compounds [10,11]. This was not accomplished with a cationic and non-ionic surfactant. We checked that the cationic surfactant (cetyltrimethylammonium bromide; CTAB) precipitated plasma proteins and non-ionic surfactants (Brij 35 (Mw: 1200, CMC: 0.055 mM), Triton X-100 (Mw: 625, CMC: 0.19 mM) and Tween 80 (Mw: 1309, CMC: 0.027 mM) [22]) did not disrupt protein bound radioligands completely and generated an increase in column back-pressure when large sample was injected (1 mL plasma). Therefore, mobile phase should contain an anionic surfactant to allow for direct injection of the plasma samples into the column. However, pure aqueous anionic mobile phases (without organic modifier) caused long retention time and low peak efficiency for most PET radioligands. Although the weak solvent strength of anionic micellar eluents can be increased by the addition of an organic solvent which can also improve the efficiency of the chromatographic peak [8,9], high concentration of organic solvent in micellar mobile phases lead to protein precipitations, disturbing direct plasma injection with large sample volume.

It was found that the use of non-ionic surfactant in SDS mobile phases could dramatically decrease the elution of some radioligands (Fig. 2). The retention of the two radioligands ([^{11}C]rolipram and [^{18}F]FE-DTBZ) decreased markedly with the addition of non-ionic surfactants to SDS mobile phase. At the almost same concentration (8–9 mM), Triton X-100 showed strongest elution strength among four non-ionic surfactants (Triton X-100, Tween 20, Tween 80 and Brij 35, the most widely used non-ionic surfactants in biochemical and chemical processes and also in MLC [10,23–25]) investigated in this study. With the mixed micellar mobile phase containing 0.5% Triton X-100 and 100 mM SDS, the retention times of [^{11}C]rolipram and [^{18}F]FE-DTBZ were 70–79% shorter than those by a single anionic surfactant eluent (100 mM SDS).

The presence of Triton X-100 in an anionic surfactant may modify the anionic surfactant-coated stationary phase and decrease the surface charge density of their monomers, in addition to the increased hydrophobicity of the bulk solvent in the mobile phase, which would result in a decrease in retention time. The addition of non-ionic surfactant to an anionic surfactant may significantly reduce the CMC and increase the size of micelle [22]. Non-ionic surfactant molecules insert into the micelle of anionic surfactant and decrease the repulsion force among the ionic heads, together with the hydrophobic interaction of alkyl chain (SDS) and aromatic hydrocarbon (Triton X-100) of surfactants, facilitating the formation of mixed micelle. The mixed CMC of SDS/Triton X-100 at 100 mM/0.5% is approximately 1 (CMC_{SDS}) and 0.1 mM ($CMC_{Triton\ X\text{-}100}$) in which the molar ratio of SDS/Triton X-100 is *ca.* 10/1 [26]. However, further detailed studies are required to interpret this behaviour.

An increase in the SDS concentration also decreased the retention of analytes due to the saturation of surfactant monomers on stationary phase and the increased anionic micelles in aqueous mobile phase, however the mixed SDS/Triton X-100 (100 mM/0.5%) system was more effective; 54–62% smaller retention factors (k) were obtained for [^{11}C]rolipram and [^{18}F]FE-DTBZ as compare to