Hydrous 18F-fluoroethylation – leaving off the azeotropic drying

Kniess, T.; Laube, M.; Steinbach, J.;

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Smart [^{18}F]fluoroethylation of cyclooxygenase-2 (COX-2) inhibitors: a method without azeotropic drying.

Torsten Kniess[1]*, Markus Laube[1], Jörg Steinbach[1,2]

1) Helmholtz-Zentrum Dresden-Rossendorf, Institute of Radiopharmaceutical Cancer Research, Bautzner Landstrasse 400, 01328 Dresden, Germany, 2) Technische Universität Dresden, Department of Chemistry and Food Chemistry, Helmholtzstrasse 10, 01062 Dresden, Germany.

ABSTRACT: The study describes the development of a simple and effective method for [^{18}F]fluoroethylation, called as smart [^{18}F]fluoroethylation without azeotropic drying, by elution of a [^{18}F]fluoride loaded QMA column with a K_2CO_3/K_{222}/acetonitrile solution containing 2% (v/v) water directly to the 1,2-ethylene glycol-bis-tosylate precursor. The radiosynthesis of the labeling agent 2-[^{18}F]fluoroethyltosylate ([^{18}F]FETs) was performed in acetonitrile containing 2% water in high radiochemical yields. The method was exemplified on the formation of three COX-2 inhibitors with different core structures. In comparison to conventional [^{18}F]fluoroethylation, the reaction time was generally shortened and the radiochemical yield was improved in each case by factor 4-5 by the new approach.

1. INTRODUCTION

Positron emission tomography (PET) is a powerful *in vivo* modality and widely used in nuclear medicine for monitoring, diagnosis, and staging such as cancerous[1], neuropsychiatric[2], and cardiovascular[3] diseases. Drugs labeled with a positron emitting radionuclide, so called radiotracers allow the visualization and quantification of biological processes at a molecular level.

Fluorine-18 (half-life = 109.77 min, β^+ energy = 635 keV) is one of the most used PET radionuclides and among the variety of radiolabeling methods for introduction of ^{18}F the [^{18}F]fluoroethylation is a common approach. The [^{18}F]fluoroethyl group is an essential labeling unit since it can be considered, e.g., as surrogate for a [^{11}C]methyl moiety what may be beneficial for the transfer of a promising carbon-11 labeled radiotracer into its fluorine-18 labeled counterpart taking advantage of the longer half-life of fluorine-18. Amidst the [^{18}F]fluoroalkylating agents 2-[^{18}F]fluoroethyl tosylate ([^{18}F]FETs) is one of the mostly used due to its easy preparation, high reactivity and sufficient stability.[4] One major benefit of [^{18}F]fluoroethylation is the objective that no mesylated or tosylated labeling precursor of the target molecule has to be synthesized to perform a nucleophilic substitution with [^{18}F]fluoride; a circumstance that saves much effort in organic synthesis. Generally [^{18}F]fluoroethylation is rather simple performed by reacting the beforehand produced [^{18}F]FETs with the easily available precursors with either phenolic OH, amino, or thiol group. However on a closer look [^{18}F]fluoroethylation covers a multi-stage procedure comprising a) the azeotropic drying of [^{18}F]fluoride, b) the nucleophilic substitution with 1,2-ethylene glycol-bis-tosylate, c) the purification of [^{18}F]FETs, d) the reaction of [^{18}F]FETs with the precursor molecule and e) the final separation of the [^{18}F]fluoroethylated radiotracer with semi-preparative HPLC (Figure 1). In turn, the need for additional equipment or automated systems for such multistep radiolabeling procedures as well as in most cases the long synthesis time of 90-120 min and the resulting low 2-10 % overall radiochemical yields (RCY) of the final radiotracers often contradicts an [^{18}F]fluoroethylation as a routinely applied method. In particular PET laboratories with restrictions in equipment or in automated systems needed for such multistep radiolabeling will often not be able to integrate [^{18}F]fluoroethylation in the portfolio of routine ^{18}F-radiolabeling.

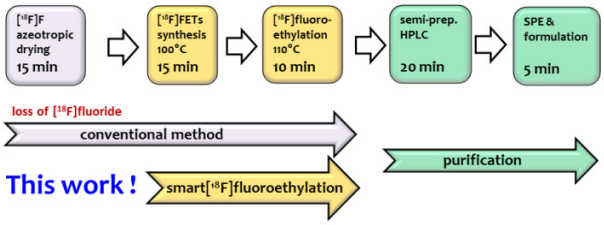


Figure 1. Course of conventional and smart [^{18}F]fluoroethylation

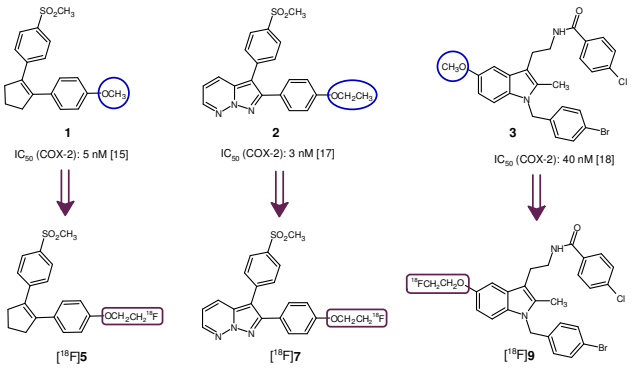
To overcome the drawbacks of conventional [^{18}F]fluoroethylations and to set the stage for a broader application of this important reaction, we aimed at the development of an smart and fast [^{18}F]fluoroethylation using [^{18}F]FETs. This is characterized by i) performing the whole reaction sequence in one vial, ii) circumventing the azeotropic drying of [^{18}F]fluoride and iii) avoiding the separation/purification of[^{18}F]FETs (Figure 1). The superi-

ority of smart [^{18}F]fluoroethylation over the conventional approach will be exemplified by comparing of the overall isolated RCYs and the total reaction time in case of [^{18}F]fluoroethylation of three different cyclooxygenase-2 inhibitors.

Since the discovery of the enzyme cyclooxygenase-2 (COX-2) in the early 1990s, it has become an intensively investigated target in biology, pharmacy, and medicine due to the fact that its overexpression is attributed to a number of diseases, most of them closely associated with inflammation. For some years, occurrence of COX-2 was demonstrated on several types of cancer [5,6] and consequently it moved in the focus of oncology as diagnostic marker and therapeutic target. [7,8,9]

The first report on a radiolabeled COX-2 inhibitor dates back to 1993 describing the radiosynthesis of carbon-11 labeled arachidonic acid[10] and since that time manifold radiotracers were designed for COX-2 imaging as it is reflected in a number of comprehensive review articles. [11,12,13,14] As a part of our ongoing research interest in imaging of COX-2 with PET[15,16], we identified from the literature three high affine COX-2 inhibitors with cyclopentene (**1**)[15], pyrazolo[1,5-*b*]pyridazine (**2**)[17], and indomethacin (**3**)[18] core structure, to transfer them into the corresponding fluorine-18 labelled radiotracer [^{18}F]**5**, [^{18}F]**7**, and [^{18}F]**8** by replacing the methyl or ethyl group by a [^{18}F]fluoroethyl moiety (Scheme 1). It is suggested that the low nanomolar affinity of the compounds **1-3** towards COX-2 will be only slightly decreased by the [^{18}F/^{19}F]fluoroethyl moiety as it was already demonstrated for other compounds in radiotracer design.[4] ^{18}F-radiolabeled COX-2 inhibitors hold more promise than their [^{11}C]methoxy labeled counterparts that suffer from the short half live of carbon-11 (20.4 min) and a notorious metabolic instability of the [^{11}C]methoxy group *in vivo*.

Scheme 1. Design of [^{18}F]fluoroethylated COX-2 inhibitors from methoxy/ethoxy-substituted lead compounds

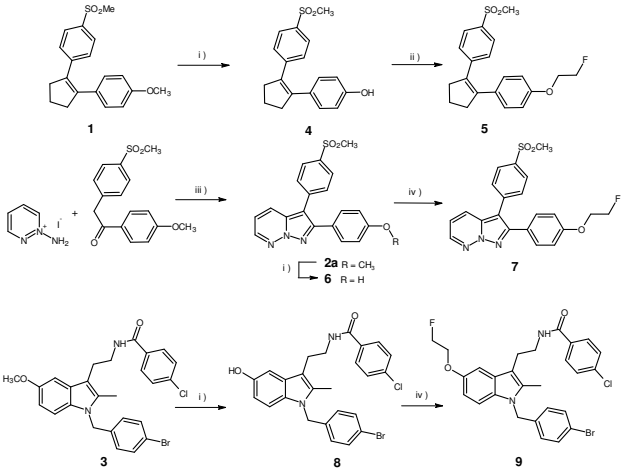


2. RESULTS AND DISCUSSION

2.1. Synthesis of labeling precursors and reference compounds. As starting materials for our studies, the respective [^{19}F]fluoroethylated reference compounds and precursors for radiolabeling were synthesized from their methoxy-substituted analogs (Scheme 2). In detail, the phenols **4**, **6**, and **8** served as educts for the synthesis of the corresponding non-radioactive reference compounds **5**, **7**, and **9** as well as precursors for [^{18}F]fluoroethylation. The potent COX-2 inhibitor 1-methoxy-4-{2-[4-(methanesulfonyl)phenyl]cyclopent-1-enyl}-benzene **1**, whose carbon-11 radiolabeled derivative has shown a distinct accumulation in mouse tumor xenografts[15], was demethylated using boron tribromide to yield the phenol **4**. The respective fluoroethylated reference **5** was formed by reacting **4** with 1-bromo-2-fluoroethane in presence of sodium hydride in DMF. For the pyrazolo[1,5-*b*]pyridazine based derivatives, the methoxy-substituted pyrazolo[1,5-*b*]pyridazine **2a** was prepared as a key substance according to the literature by aza-mannich type reaction of *N*-aminopyridazinium iodide with 1-(4-methoxyphenyl)-2-[4-(methylsulfonyl)phenyl]ethanone using $TiCl_4$ and Et_3N followed by dehydrogenation with Pd/C. (Scheme 2) Demethylation of **2a** with BBr_3 provided the pyrazolo[1,5-*b*]pyridazin-2-yl substituted phenol **6** as a labeling precursor**.** Finally, the reaction of **6** with 2-fluoroethyl-4-nitrobenzene sulfonate (FENs) formed the fluoroethylated pyrazolo[1,5-*b*]pyridazine **7**. The indomethacin based COX-2 inhibitor **3** was prepared according to the literature[18] and subsequent demethylation with BBr_3 provided the phenol **8**. The fluoroethylated indomethacin **9** was formed in analogy to **7** by reaction of **8** with FENs.

After having in hand the appropriate phenol precursors and [^{19}F]fluoroethylated reference compounds we focused on the optimization of [^{18}F]fluoroethylation with [^{18}F]FETs.

Scheme 2. Synthesis of phenol precursors and [^{19}F]fluoroethylated COX-2 inhibitors



Reaction conditions; i) BBr_3, CH_2Cl_2; ii) $BrCH_2CH_2F$, NaH, DMF; iii) $TiCl_4$, Et_3N, CH_2Cl_2, Pd/C, CH_2Cl_2; iv) FENs, K_2CO_3, CH_3COCH_3

2.2. Previous methods of [^{18}F]radiofluorination without azeotropic drying. As mentioned earlier the overall isolated yield of most protocols via common [^{18}F]fluoroethylation lies not higher than 2-10% within 90 -120 min total syntheses time.[4] Two main reasons can be identified as drawbacks of the hitherto applied methods:

first the azeotropic drying is time consuming and a distinct quantum of [^{18}F]fluoride stays unreactive in this process and second some losses of [^{18}F]FETs by its purification by HPLC or SPE are unavoidable. Hence as a first step to improve the overall RCY of [^{18}F]fluoroethylation we aimed for the development of a synthesis of [^{18}F]FETs where the azeotropic drying is abdicable.

Generally, the [^{18}F]fluoride is provided in aqueous solution from the cyclotron target and has to be transferred into water free aprotic solvents to generate a highly reactive [^{18}F]fluoride. This transfer is most often accomplished by trapping [^{18}F]fluoride on an anion exchange column followed by elution of the [^{18}F]fluoride with an acetonitrile/water mixture containing a phase transfer reagent (Kryptofix, K_{222}) and a base (KOH, K_2CO_3, $K_2C_2O_4$, or NBu_4OH). In the early years of development of the column supported [^{18}F]fluoride activation the eluent was 100% aqueous[19]; later the content of water has been decreased to 5% (v/v) to ensure further complete recovery of fluorine-18 and high yields of [^{18}F]fluorination. Ideally the content of water should be minimized to generate a non-aqueous highly reactive [^{18}F]fluoride.[20] Nowadays the residual water is routinely removed by azeotropic drying, the repeated addition and removal of acetonitrile in a nitrogen stream, a time consuming and sensitive process because excessive temperature and incomplete drying strongly affect the reactivity of the [^{18}F]fluoride. To circumvent the azeotropic drying step a number of innovations has been made, e.g. ad-/desorption of [^{18}F]F^- on electrochemical cells[21,22,23,24], the utilization of ionic liquids[25] or macro porous copolymers[26], or the application of strong organic bases as additives [27].

Just recently a new, so-called 'minimalist' approach for ^{18}F-labeling of 'onium'-precursors avoiding the azeotropic drying as well as kryptands, bases and other additives was developed. [28] In another, so-called 'Munich-method' an anhydrous solution of Kryptofix K_{222}/KOH in acetonitrile, was successfully applied for aliphatic nucleophilic [^{18}F]fluorination resulting in high ^{18}F-incorporation yields, however this approach has the drawback of using strong alkaline conditions what is not suitable for all precursor compounds.[29]

The fact that the nucleophilic aliphatic substitution with [^{18}F]fluoride tolerates a minimum content of water was published by several groups in the past, e.g. 5% (v/v) aqueous acetonitrile[30], 3% (v/v) aqueous DMF[31], the use of [^{18}F]Et_4NF in different solvents containing up to 5% (v/v) water[32,33], or by minimizing the content of water of the [^{18}F]KF/K_{222}/K_2CO_3 eluent solution by predrying with a Na_2SO_4 SPE column[34]. More strikingly, direct [^{18}F]fluorination was reported to proceed even in organic solvents containing 25% (v/v) water with titanium nanoparticles[35], or with diaryliodonium precursors in DMF containing 0.25-28% (v/v) water[36].

2.3. Recovery and reactivity of [^{18}F]fluoride in aqueous acetonitrile. We found that ^{18}F-radiolabeling in aqueous acetonitrile was most promising and focused on the development of a solvent system containing acetonitrile, water (2-3 % v/v) and a kryptofix/potassium carbonate, oxalate, or hydroxide complex having a maximum [^{18}F]fluoride recovery from the anion exchange column. Coincidently the reactivity of [^{18}F]fluoride should be maintained in the subsequent reaction to form [^{18}F]FETs with 1,2-ethylene glycol-bis-tosylate precursor in high RCY.

For determination of the best [^{18}F]fluoride recovery from the anion exchange column (QMA-light®, 130 mg), eight solutions were prepared by varying the amount of Kryptofix K_{222}, the base (K_2CO_3, $K_2C_2O_4$, or KOH) and its concentration, and the content of water, 2 or 3 % (v/v) in acetonitrile. Table 1 shows the exact composition of the solutions ***1-8*** and the resulting [^{18}F]fluoride recovery after elution with 1 mL of ***1-8*** (see column "[^{18}F]fluoride recovery"). As a comparison, the respective data of previously published methods avoiding the azeotropic drying step are displayed. As visible for solution ***1*** containing 3% of water, about 97% of [^{18}F]fluoride recovery was achieved. A reduction of the water content to 2% (v/v) (solution ***2***) did not affect the high recovery. By lowering the quantities of K_2CO_3 (15 µmol) and K_{222} (30 µmol) as performed in solution ***3***, still an excellent recovery of [^{18}F]fluoride (85-98%) was observed. However, by further reduction of the amount of base (7 µmol) and K_{222} (15 µmol) the [^{18}F]fluoride recovery decreased to 71 % (solution ***4***). Similar to the results obtained with K_2CO_3, the use of 30 or 15 µmol $K_2C_2O_4$ as the base in acetonitrile containing 2% (v/v) water resulted in excellent [^{18}F]fluoride recovery (solution ***5*** and ***6***). Interestingly, as we used potassium hydroxide (30 µmol) with 3% (v/v) water (solution ***7***), the [^{18}F]fluoride recovery was significantly lower in comparison to K_2CO_3 and $K_2C_2O_4$; a decrease to 2% (v/v) water in solution ***8*** led to the lowest observed recovery of [^{18}F]fluoride (33%). For further optimization we applied QMA-light® columns containing 45 mg of anion exchanger, in that case only 0.7 ml of solution ***2*** was needed for achieving 95-98% [^{18}F]fluoride recovery. In conclusion, solutions ***2*** and ***3*** containing K_2CO_3 and ***5*** and ***6*** containing $K_2C_2O_4$ were found to be optimal with regard to the recovery of [^{18}F]fluoride from the anion exchanger.

Subsequently the solutions ***1-8*** were evaluated in terms of reactivity by subjecting them to the nucleophilic aliphatic substitution with 1,2-ethylene glycol-bis-tosylate to form[^{18}F]FETs under standard ^{18}F-labeling conditions. In detail the [^{18}F]fluoride containing solutions were reacted with 7 mg (19 µmol) 1,2-ethylene glycol-bis-tosylate precursor for 15 min at 100°C. The radiochemical conversion (RCC) to [^{18}F]FETs as determined by radio thin layer chromatography is also displayed at Table 1 (see column "RCC to [^{18}F]FETs"). It became obvious that solutions ***2*** and ***4*** showed the highest yields in the reaction to [^{18}F]FETs (RCC 70-88% and 79-80%, respectively).

Table 1. Recovery of [^{18}F]fluoride by elution with base/K_{222}/acetonitrile/water mixtures and RCC into [^{18}F]FETs

	Vol. mL	cont. K_2CO_3 [µmol]	cont. $K_2C_2O_4$ [µmol]	cont. KOH [µmol]	cont. K_{222} [µmol]	cont. MeCN [µL]	cont. H_2O [µL/% (v/v)]	[^{18}F]fluoride recovery [%]	RCC to [^{18}F]FETs #) [%]
solution ***1***	1.0	30	-	-	60	970	30 (3%)	64-97 (n=3)	57-61 (n=3)
solution ***2***	1.0	30	-	-	60	980	20 (2%)	84-98 (n=22)	70-88 (n=17)
solution ***3***	1.0	15	-	-	30	980	20 (2%)	85-98 (n=6)	64-70 (n=3)
solution ***4***	1.0	7	-	-	15	980	20 (2%)	71-77 (n=3)	79-80 (n=2)
solution ***5***	1.0	-	30	-	60	980	20 (2%)	95-98 (n=6)	26-43 (n=3)
solution ***6***	1.0	-	15	-	30	980	20 (2%)	94-96 (n=3)	52-79 (n=2)
solution ***7***	1.0	-	-	30	60	970	30 (3%)	33-74 (n=2)	70-71 (n=2)
solution ***8***	1.0	-	-	30	60	980	20 (2%)	15-33 (n=2)	66-71 (n=2)
solution ***2*** QMA (45mg)	0.7	21	-	-	42	686	14(2%)	95-98 (n=9)	76-96 (n=2)
Gomzina [20]		12	-	-	25	1920	80 (4%)	n.d.	80-85 *)
Friebe [34]		4	-	-	13	200/ 750§	50 (5%)	79	48
Kolb [37]		40	-	-	134	950	50 (5%)	95	95 **)
Wessmann [29]		-	-	84	125	500	0	92	70-90 **)

#) 15 min, 90°C; *) evaporation of eluent but no azeotropic drying; **) RCC to [^{18}F]FDG and other radiotracers; §) acetonitrile/butanol

Notably, the $K_2C_2O_4$ containing solutions ***5*** and ***6***, on the one hand having excellent [^{18}F]fluoride recovery, showed a lower RCC of 26-43% and 52-79%, respectively, into [^{18}F]FETs in comparison to K_2CO_3. Finally, the KOH containing solutions ***7*** and ***8*** resulted only in a 70% radiochemical conversion to [^{18}F]FETs and, taking the low [^{18}F]fluoride recovery into account, showed the lowest overall RCC. Accordingly, solution ***2*** was found to be the most suitable in terms of recovery and reactivity and was used for all further radiolabeling reactions. Of note, 0.7 mL of solution ***2*** was applied for 45 mg QMA-light® column.

In comparison to literature reported methods avoiding the azeotropic drying step the following conclusions can be drawn. Gomzina et al. applied a similar K_{222}/K_2CO_3 solution as ***3*** containing 4% (v/v) water in acetonitrile; the authors observed after [^{18}F]fluorination an increased RCC to [^{18}F]FETs, however there was an additional evaporation step performed.[20] Friebe et al. applied a base and K_{222} concentration similar to solution ***4*** with 5% (v/v) water in an acetonitrile/butanol solvent mixture and performed a predrying step on a Na_2SO_4-SPE-column before [^{18}F]fluoride elution; however, the authors achieved only a moderate RCC to [^{18}F]FETs.[34] Wessmann et al. used an anhydrous K_{222}/KOH solution containing high amounts of K_{222} resulting in high RCC for [^{18}F]FDG, [^{18}F]FLT and other radiotracers as well.[29] However, the use of the strong base KOH can be disadvantageous for sensitive precursor molecules and resulted in our case in the lowest RCC's. Finally, Kolb et al. applied 5% aqueous acetonitrile and K_{222}/K_2CO_3 successfully to get 70-95% RCC for several radiotracers.[37] Our results showing high [^{18}F]fluorination yields in aqueous acetonitrile confirm this finding, and additionally demonstrate that the high K_{222} concentrations applied by Kolb et al. are not necessarily needed.

2.4. Impact of Cs_2CO_3 in synthesis of [^{18}F]FETs. Cs_2CO_3 is a common base for the deprotonation of phenols in case of [^{18}F]fluoroethylation. Moreover it was found thirty years ago that addition of Cs_2CO_3 facilitates the nucleophilic substitution of [^{18}F]fluoride.[38] For documentation if this effect will also be true in case of the formation of [^{18}F]FETs we performed a number of radiosyntheses with and without addition of 20 µmol of Cs_2CO_3 to the 1,2-ethylene glycol-bis-tosylate precursor. The course of reaction was monitored by radio TLC samples taken after 5, 10, and 15 min. As shown in Figure 2 there is significant higher reaction rate and radiochemical yield of [^{18}F]FETs in that samples where Cs_2CO_3 was present. This may originate from the formation of intermediate Cs[^{18}F]F leading to higher labeling yields what also was described by other authors.[39]

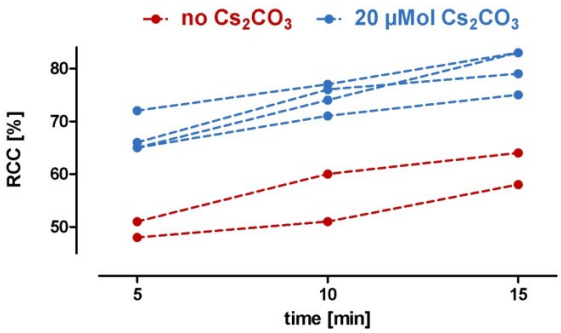


Figure 2. Effects of Cs_2CO_3 on the formation of [^{18}F]FETs for 0 µmol (n=2) and 20 µmol (n=4) Cs_2CO_3

2.5. Progress of smart [^{18}F]fluoroethylation. Smart [^{18}F]fluoroethylation of the three hydroxyl precursors **4**, **6** and **8** to form the corresponding ^{18}F-labeled COX-2 inhibitors [^{18}F]**5**, [^{18}F]**7**, and [^{18}F]**9** was done by addition of 20 µmol of hydroxyl precursor deprotonated with 20 µmol of Cs_2CO_3 in DMF to the vial containing the [^{18}F]FETs and subsequent heating to 110°C for 10 min. Figure 3 displays an overview on the work flow of smart [^{18}F]fluoroethylation without azeotropic drying. In brief, the [^{18}F]fluoride was loaded on the QMA column by elution with target water. After drying the column with acetonitrile, the [^{18}F]fluoride was transferred by the K_{222}/K_2CO_3/acetonitrile/water solution into the reaction vial loaded with 1,2-ethylene glycol-bis-tosylate and Cs_2CO_3. After 15 min heating, the phenol precursor with Cs_2CO_3 in DMF was added and heating was continued for 10 min for [^{18}F]fluoroethylation. Finally, the raw product was subjected to semi-preparative HPLC purification.

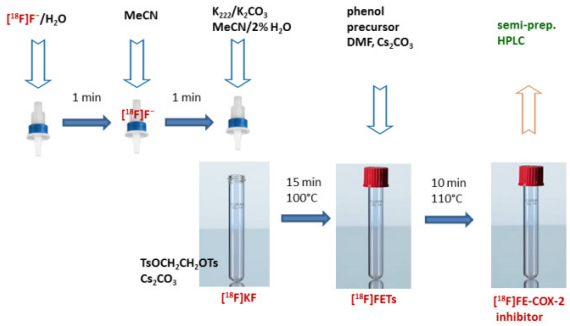


Figure 3. Radiosynthesis of COX-2 inhibitors by smart [^{18}F]fluoroethylation

To observe the radiochemical conversion (RCC) of [^{18}F]FETs over the time we monitored the course of the [^{18}F]fluoroethylation in 5 min periods by radio-TLC. Figure 4 displays the amount of [^{18}F]fluoride, [^{18}F]FETs, and [^{18}F]fluoroethylated COX-2 inhibitor at each time point exemplarily for two independent radiosyntheses of pyrazolo[1,5-*b*]pyridazine [^{18}F]**7**. It is obvious that 58-72% of the [^{18}F]FETs was formed already after 5 min and a maximum of 79-83% was reached after 15 min whereas the content of [^{18}F]fluoride has dropped to 21-17% continuously. After addition of the phenol **6** and Cs_2CO_3 in DMF at t = 15 min, a 42-71% radiochemical conversion of [^{18}F]FETs occurred within 5 min to [^{18}F]**7** (20 min total reaction time). After overall 30 min, the yield of [^{18}F]**7** had increased to 72-88%, whereby the content of [^{18}F]FETs dropped to 10% resp. was completely consumed. Notably the ratio of [^{18}F]fluoride was steadily decreased during the [^{18}F]fluoroethylation process; 22% to 16% and 16% to 12%, respectively. This suggests that formation of [^{18}F]FETs is pursued during the whole reaction sequence and stands for an additional benefit of the one-pot reaction.

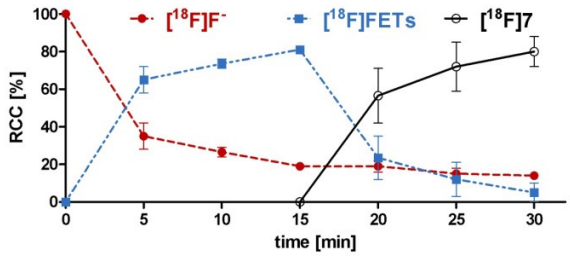


Figure 4. Time-dependent RCC of [^{18}F]fluoride, [^{18}F]FETs in the formation of [^{18}F]**7** (mean of n=2, bars indicate lowest and highest value)

2.6. Comparison of smart [^{18}F]fluoroethylation with the conventional approach. As known from radiopharmaceutical practice there exists always a significant gap between radiochemical conversion (RCC) as expressed from samples taken and analyzed by radio TLC or radio HPLC and the isolated overall decay corrected radiochemical yield (RCY) of a radiotracer, a value including all purification and formulation steps.

For direct comparison of the smart [^{18}F]fluoroethylation with the conventional method we performed a set of experiments where in the first block [^{18}F]FETs was synthesized according to the well-known azeotropic drying procedure of [^{18}F]fluoride (i.e., 90°C, N_2-stream, addition of acetonitrile, 90°C, vacuum) and reacting the dried [^{18}F]fluoride at 100°C with the ethylenglycole-1,2-bistosylate precursor to form [^{18}F]FETs. This was followed by [^{18}F]fluoroethylation of the hydroxyl precursors **4**, **6**, and **8**, respectively, and additional semi-preparative HPLC purification. All these steps were performed in an automated synthesizer (Tracerlab$_{FXN}$). In a second block of experiments the [^{18}F]FETs was synthesized without azeotropic drying in manual mode and smart [^{18}F]fluoroethylation was performed by addition of the corresponding hydroxyl precursor and Cs_2CO_3 as shown in Figure 3. The purification of the radiotracers [^{18}F]**5**, [^{18}F]**7**, and [^{18}F]**9** was realized in both cases by semi-preparative HPLC (conditions see supporting info) and subsequent solid phase extraction from the eluent. The overall decay corrected radiochemical yield of the isolated

corresponding COX-2 inhibitor was based in both experimental blocks on the starting activity of [^{18}F]fluoride.

The results of the competition between conventional and smart [^{18}F]fluoroethylation are shown at Table 2. It becomes obvious that the overall RCY of [^{18}F]fluoroethylated COX-2 inhibitors prepared according to the conventional method did not exceed 8% for [^{18}F]**5** and 4,4% for [^{18}F]**9**. In contrast, by application of smart [^{18}F]fluoroethylation the RCY has increased to 34% for [^{18}F]**5** and 15% for [^{18}F]**9** by using same starting materials. In a similar way the isolated RCY has increased for [^{18}F]**7** by factor five from 4.9% to 26%. Consequently smart fluoroethylation gives a major benefit for radiolabeling of weak phenols with [^{18}F]FETs as demonstrated for the indomethacin [^{18}F]**9**. Furthermore it is noteworthy that for smart [^{18}F]fluoroethylation lower [^{18}F]fluoride starting activities (1.5 – 2.0 GBq) can be used to yield 100-300 MBq of radiotracer, instead of 5-8 GBq applied for conventional [^{18}F]fluoroethylation. Additionally the radiolabeling time by smart [^{18}F]fluoroethylation is diminished from 40 min to 25 min (Figure 1), a point that is of ongoing interest in radiotracer synthesis.

Table 2. Comparison of RCY by conventional and smart [^{18}F]fluoroethylation

	conventional [^{18}F]fluoroethylation			**smart [^{18}F]fluoroethylation**		
	A_{start} [MBq]	$A_{isolated}$ [MBq]	**RCY** [%]	A_{start} [MBq]	$A_{isolated}$ [MBq]	**RCY** [%]
[^{18}F]**5**	4700	204	**7.2**	1400	320	**34**
	8600	420	**7.8**	1320	271	**31**
[^{18}F]**7**	2700	105	**6.1**	1260	237	**29**
	4700	132	**4.9**	3000	533	**26**
[^{18}F]**9**	6400	113	**2.9**	1420	117	**12**
	5900	156	**4.4**	1040	108	**15**

3. CONCLUSIONS

We have developed a simple general procedure for [^{18}F]fluoroethylation of small molecules, comprising, i) synthesis of [^{18}F]FETs without the azeotropic drying step by elution of a [^{18}F]fluoride loaded QMA column with a K_2CO_3/K_{222}/acetonitrile solution containing 2% (v/v) water direct to the 1,2-ethylene glycol-bis-tosylate precursor, and ii) subsequent reaction with hydroxyl precursor and base. The effectiveness of so called 'smart [^{18}F]fluoroethylation' was demonstrated for the radiosynthesis of three [^{18}F]fluoroethylated COX-2 inhibitors having a cyclopentene, a pyrazolo[1,5-*b*]pyridazine and an indomethacin core structure. The superiority of smart [^{18}F]fluoroethylation was demonstrated by a minimum loss of [^{18}F]fluoride since azeotropic drying is omitted, by factor 5 improved overall radiochemical yields compared to the conventional [^{18}F]fluoroethylation, and by about 20 min shortened reaction time. Since smart [^{18}F]fluoroethylation is based upon a one-vessel method and elaborated procedures for the purification of the intermediate [^{18}F]FETs are avoided, the radiolabeling can be performed with a minimum reservoir of equipment what makes smart [^{18}F]fluoroethylation extremely attractive for research applications. The small reaction volume of 1.2 mL and the omitting of the azeotropic drying warrants an effective exploitation of [^{18}F]fluoride and holds promise for further optimization, e.g. by microwave based heating whereby the reaction time can be significantly reduced.[40]

Whether smart [^{18}F]fluoroethylation is applicable for radiolabeling on other heteroatoms, e.g. an amino, imide or thiol group, and in which extent the K_2CO_3/K_{222}/acetonitrile/2% (v/v) water solution is generally usable for nucleophilic [^{18}F]fluorinations without azeotropic drying has to be further evaluated; preliminary experiments with mannose triflate, a precursor for [^{18}F]fluorodeoxyglucose [^{18}F]FDG were successful.

Finally we believe that smart [^{18}F]fluoroethylation is a valuable tool to get quick access to [^{18}F]fluoroethylated radiotracers and to translate methoxy and ethoxy substituted high affine drugs in potential PET radiopharmaceuticals for preclinical application.

ASSOCIATED CONTENT

Supporting Information

Synthesis procedures as well as NMR and UPLC data of compounds **1-9**, detailed protocols of smart and conventional [^{18}F]fluoroethylation, results of radio TLC in the formation of [^{18}F]FETs, and results of radio-semi-preparative HPLC and analytical radio-UPLC of the [^{18}F]fluoroethylated species [^{18}F]**5**, [^{18}F]**7** and [^{18}F]**9** are available. This material is available free of charge via the Internet at http://pubs.acs.org.

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