Sequential preparation of two different PET radiotracers employing the Advion NanoTek synthesis system

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Why do we want to do a Back to Back Synthesis?

To prove that at least two tracers could be made without impacting yield, purity and specific activity

- we chose FLT and FMISO as test tracers
  • these compounds use the most common radiosynthesis methodologies:
    » Incorporation of Fluoride
    » Hydrolysis to remove protecting groups
    » HPLC purification
[F-18]FLT and FMISO

Radiosynthesis

3-N-Boc-5’-O-dimethoxytrityl-3’-fluorothymidine

3-N-Boc-5’-O-dimethoxytrityl-3-O-nosyl-thymidine

1-Fluoro-3-(2-nitro-imidazol-1-yl)-propan-2-ol (FMISO)
System Components

Base Module.
Stores and delivers cold reagents.

Reactor module.
Isotope delivery and reaction.

Concentrator Module
Traps and concentrates F-18.

Distribution valves

Syringe pumps. 48,000 steps

Pressure sensors

Four independent reactors

Temperature controllers

Heated vial
Dominant effects in Microfluidics

- Faster thermal diffusion
- Laminar flow (Low Reynolds number)
- Surface forces (Capillary phenomenon)
- Liquid evaporation - smaller volume so evaporation has a larger effect
- Gas bubbles - have effects on compressibility
Vapour Pressure

Pressure of Common Solvents

Temperature (ºC)

- Water
- Acetonitrile
- DMSO
- Dimethylformamide
Discovery Mode

• In discovery mode a small aliquot (10-20 µl) from the original solution of the radioisotope and precursor is mixed in the reactor.
  • This allows the testing of 10-30* different reaction conditions, such as
    • reaction temperature,
    • flow rate,
    • pressure,
    • reagent ratios.

• This can also be done for 2-step reaction.

* The number of reactions are dependent on the volume of solutions loaded to the loops and the size of the bolus used in the reactions
E-mail info@advion.com to request a copy of the animation.
Optimization of [F-18] Incorporation

- 48 Individual Run Experiments
  Precursor Concentration = 20 mg/ml,
  450 µl / run = 9 mg of precursor / run

% Incorporation vs temperature
Reactor Flow Rate = 40 µl/min

170°C study repeated to determine repeatability
Optimization of [F-18] Incorporation

Precursor Concentration = 20 mg/ml, 450 µl / run = 9 mg of precursor / run

Effect of Reactor Flowrate on % Incorporation of [F-18] Fluoride

![Bar chart showing the effect of reactor flowrate on % incorporation of [F-18] fluoride.](chart.png)
Optimization of [F-18] Incorporation

Precursor Concentration varied from 5 to 40 mg/ml, Flow rate through reactor = 200 µl/min, Reactor residence time ~5 seconds

% Incorporation of [F-18] Fluoride vs Precursor Concentration

40 mg/ml study repeated to determine repeatability
Individual Conditions for [F-18]FLT and FMISO

Optimum conditions for FLT

- Reactor Conditions
  - 150-180°C, 150-200 µl/min, 100µm X 2 m

- Reagent Concentrations
  - Precursor 15-40 mg/ml (6-16 mg/run)
  - 2 N HCl, 95°C, 6 minutes
  - 3M NaOAc, 2 minutes, RT

Optimum conditions for FMISO

- Reactor Conditions
  - 150-180°C, 150-200 µl/min, 100µm X 2 m

- Reagent Concentrations
  - Precursor 5-10 mg/ml (2-4 mg/ run)
  - 1 N HCl, 100°C, 1 minutes
  - 1 N NaOH, 2 minutes, RT
Requirements for Back to Back Synthesis

Each reaction occurs in an independent flow path

- Fluoride is split into 2 independent flow paths and mixed with precursor
- Reaction occurs in two different reactors
- Hydrolysis occurs in two separate reaction vials
- HPLC purification occurs on two separate HPLC columns
  - Column selector chooses correct column
  - One column for each compound
- HPLC injector and all associated flow paths are cleaned automatically between tracers
- System can be cleaned fully between runs and low carryover between runs
Modifications to Standard NanoTek
Back to Back Radiosynthesis

• To Perform a B2B radiosynthesis

  – The user must install prior to starting the Radiosynthesis
    • Kryptofix / carbonate solution and fluoride trap on Concentrator 1
    • 2N Hydrochloric Acid, 3M Sodium Acetate, 1N Sodium Hydroxide, Water
    • FLT precursor on Pump 1
    • FMISO precursor on Pump 2
    • HPLC solvents (70% ethanol / water, 10% ethanol / water, 5% ethanol / water, 8% ethanol / phosphate buffered saline

  – The system will prompt the User for
    • When [F-18]Fluoride is ready and has been transferred to hotcell
    • To inject the crude [F-18]FLT on to the HPLC and to collect the pure [F-18]FLT
    • To inject the crude [F-18]FMISO on to the HPLC and to collect the pure [F-18]FMISO
Purification of [F-18]FLT

As read on the Nanotek system from analogue inputs
Results of B2B Radiosynthesis

B2B Yield when compared with independent reactions (Non-decay corrected yields)

- FLT = 20 ± 3% vs 18.9 ± 3% (B2B)
- FMISO = 40 ± 5% vs 38 ± 6% (B2B)

Specific Activity >2 Ci/µmol

Runs performed over the range of 50 mCi to 1 Ci

Analysis of the solutions for radioactive and chemical carryover.

The decay corrected radioactive carryover of

- 0.065% for FLT and 0.031% for FMISO

All other peaks attributable to the chemical carryover resulted in

- 0.07% for FLT and 0.09% for FMISO
Conclusion

- Two radiotracers were prepared sequentially in
  - Reasonable yield
  - High purity (>99%)
  - No impact on specific activity
  - Tested to a starting activity of 1 Ci
  - System was able to be cleaned with <0.1% carryover
  - Minimum interaction from the end user

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