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Faster analysis of radio pharmaceuticals using UPLC™ in combination with low volume radio flow cell

INTRODUCTION

Positron emission tomography (PET) has now established itself as a dominant imaging modality in nuclear medicine and drug research. The most commonly used PET radionuclides are carbon-11 and fluorine-18 with relatively short physical half-lives (^{18}F: half-life = 109.7 min, ^{11}C: half-life= 20 min). The short physical half-lives of the positron emitters require that the analysis time of the PET radiopharmaceutical be kept as short as possible. The time constraint imposed on the quality control is very critical especially for carbon-11 radiolabeled pharmaceuticals. Currently, radio-HPLC (High Pressure Liquid Chromatography) and radio-TLC (Thin Layer Chromatography) are the methods of choice for the analysis of the labeled compounds. Typically, the HPLC system is attached sequentially to a UV- and a radioactivity detector with NaI scintillator. Radio-HPLC has a higher sensitivity as well as higher resolution but requires a "relative" long analysis time. Radio-TLC is much more time consuming than radio-HPLC due to the time required for sample preparation and TLC-plate development. Therefore, fast and more sensitive analytical methods are needed to meet the requirements of short-lived positron emitters. Ultra Performance Liquid Chromatography (UPLC™) is a widely accepted analytical method because of its improved detection limits, resolution and speed when compared to HPLC. UPLC™ has not yet been established as an analytical method for analyzing PET radiopharmaceuticals.

In this study, we investigated whether the combination of an UPLC™ with a radioactivity detector would result in faster analyses of PET radiopharmaceuticals with a higher degree of sensitivity and efficiency.

MATERIALS AND METHODS

Preparation of samples

The radiolabeled compound was synthesized by a simple nucleophilic substitution of a leaving group with ^{18}F-fluoride in an automated synthesizer and diluted to give approximately 1-4 MBq in 100 µl.

Analytical equipment

Waters Acquity UPLC™ System with an attached Berthold FlowStar LB513 radioactivity flow through detector (coincidence detection) was used.

Evaluation of flow cell

Measurements were performed with a ^{18}F-labeled compound to evaluate the speed, sensitivity and peak shape of the radioactivity signal in the Berthold FlowStar detector. Different cell volumes were tested; standard 100 µl volume cell and specially manufactured cell with 50 µL and 20 µl cell volumes. Each sample was injected twice to receive reproducible data. The new low background cells used did not require any additional lead shielding.

UPLC™ condition

System:	Waters ACQUITY UPLC™ System with ACQUITY UPLC™ Column Manager, ACQUITY UPLC PDA, eSatin Kit 1, Empower 2
Column:	Waters, ACQUITY UPLC BEH C_{18}, 2.1 x 50 mm, 1.7 µm
Col. Temp.:	30°C
Sample rate:	20 Hz
Mobile Phase A:	0.01 M Na_2HPO_4 in Water pH 7.4

Mobile Phase B:	Acetonitrile
Flow Rate:	0.7 ml/min
Injection Volume:	1 µl
Gradient:	as indicated in chromatogram (Figure 1)
Detection:	UV @ 330 nm
Resolution:	1.2(nm)
Sample rate:	20 Hz

Radio detector:	Berthold Flowstar LB513
Cell:	extra low background MX-cell
Activity:	^{18}F-labeled molecule 1-4 MBq in 100 µl

RESULTS

The improvement observed in the radioactivity signal with the extra low background cell is shown in Figure 1. The peak of the labeled radiotracer was identified in PDA spectra by co-injection of cold reference and compared to the associated radiochromatogram.
In developing this analytical method, we could reduce the analysis time from 12 minutes to 3 minutes. The short gradient of 1 minute led to faster elution of the compounds and sped up the conditioning of the column. The radioactive signal, compared to the PDA signal is imperceptively bigger with an excellent signal/noise ratio. The detection limit of the radio detector is < 40 kBq and as shown in Figure 1 we could observe a low background of the radio detector.
The peak shape of the radio signal depends on the volume of the radio flow cell, increasing the cell volume gave an increased tailing phenomenon. A cell volume of 100 µL showed a peak width of 1.4 minutes, whereas a cell flow volume of 50 µl gave less tailing and a peak width of 0.5 minutes. The best results were observed using a 20 µl cell volume which resulting in a peak width of 0.2 minutes.

In addition, external radiation sources did not influence any of the measurements; when a 10 MBq sample was placed 20 cm from the unshielded Berthold radio detector no significant background signal or change in the peak was observed.
Figure 2 shows the setup of the radio-UPLC system. It consists of the Waters Acquity UPLC™ System and the Berthold FlowStar LB513.

CONCLUSION

This study demonstrates the feasibility of using Waters Acquity UPLC™ System with Berthold FlowStar LB513 radioactivity detector for sensitive analysis of radiotracers. The time of analysis can be reduced by a factor of 10 using the Waters UPLC™ system. The tailing phenomenon mentioned above can be explained by the different cell volumes of UPLC™ PDA detector (0.500 µl) and radio detector (50-100 µl): a factor of roughly 200 times. Reducing the radio flow cell volume from 100 µl to 20 µl eliminates the tailing of the radioactivity peak and produces a narrow peak.
The influence of an external radiation source on the actual radioactivity signal was not significantly detectable. The observed low background is a product of the sharp and tall signal of the radio detector. With further optimization studies using this analytic UPLC™ method it should be possible to reduce the analysis time to 1 minute.

In comparison with common Radio-HPLC systems (NaI detector), we could detect much lower activities with UPLC™ and the Berthold FlowStar radio detector (0.04 MBq in 1 µl injected sample volume). This is regarded as very important in radiation protection safety as the radiation dose to the operator is reduced significantly, thus creating a safer working environment.
With this faster analysis method precious time is saved during sample analysis. There is also a gain in efficiency, accuracy and the sensitivity of the results.
This system can also be used effectively for radio-metabolite studies where low levels of radioactivity are analyzed and increased sensitively is paramount.

To summarize, a 6-fold faster analyses times with much lower backgrounds were achieved using the combination of UPLC™ and Berthold FlowStar radioactivity detector. The measurements were not affected by external radiation sources in the vicinity of the instrument.

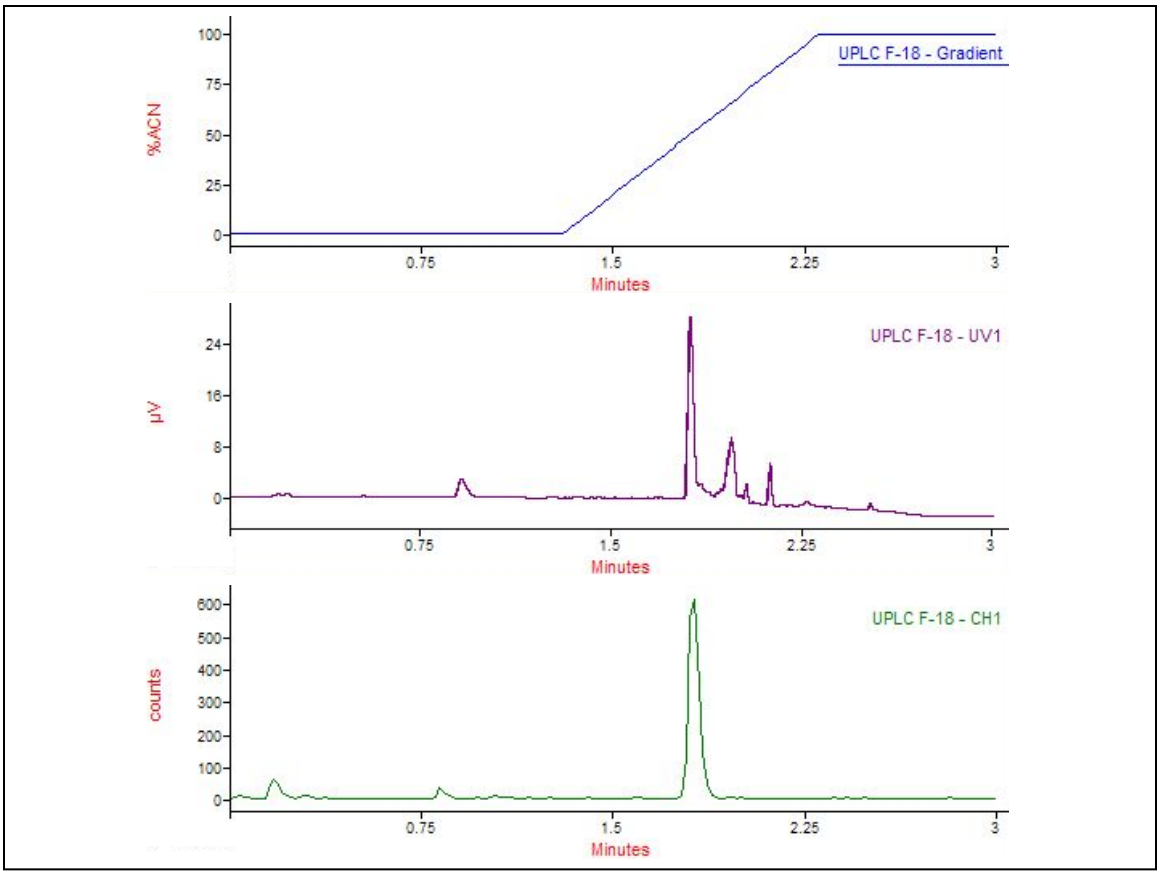

Figure 1: The combined PDA and radiochromatogram show a narrow and high radio peak at 1.920 minutes. The gradient is given in blue color in the last graph.

Figure 2: The radio-UPLC consists of the Waters Acquity UPLC™ System and the Berthold FlowStar LB513.