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Evaluation of an unshielded luminescence flow-through radio-HPLC detector for LC quality control and preparation of PET radiopharmaceuticals

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HIGHLIGHTS

- We evaluate a novel unshielded luminescence flow-through radio-HPLC detector (fLumo) which is only sensitive to the positron and insensitive to gamma rays for applications in PET radiopharmaceuticals analysis and preparation.
- The fLumo detector exhibits a low limit of detection as activities as low as 4 kBq are detected (HPLC and UPLC radiodetectors).
- The fLumo detector demonstrates excellent linearity (0.2 to 2500 MBq/ml, $r^2 > 0.995$) and reproducibility.
- Thanks to its compactness and absence of shielding, the fLumo has been installed in a production shielded "hot" cell to detect radiocompounds during a semi-preparative HPLC purification.
- This work demonstrates the value of the fLumo luminescence flow-through radio-HPLC detector for applications in PET tracers radiochemistry.

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ABSTRACT

Radio-HPLC is an essential method to assess the purity of PET radiopharmaceuticals. The usual NaI scintillator radiodetector requires heavy, costly and cumbersome lead shielding. The luminescence LB 500 fLumo detector has been developed to tackle these drawbacks and achieve high sensitivity. The fLumo uses a photon counting detector combined with a flow-through cell modified with a solid melt-on scintillator only sensitive to the positron. This study demonstrates the usefulness of the fLumo for analysis and purification of PET radiopharmaceuticals.

1. Introduction

Positron-emission tomography (PET) is a major imaging modality that is used to provide quantitative information on in vivo physiological and biochemical process in human subjects and animals. It uses short-lived positron emitting radionuclides to track labeled molecules in vivo (fluorine-18, half-life=109.7 min, carbon-11, half-life=20.4 min, gallium-68, half-life=68.0 min). The short physical half-lives means that PET radiopharmaceuticals must be rapidly produced under good manufacturing practice (GMP) compliances on a daily basis (Ametamey et al., 2008). Furthermore, each production is followed by a rapid quality control that allows the radiopharmaceutical to be released for clinical use. Currently, analysis by radio-HPLC is a vital tool to assess radiochemical and chemical purity. Typically, the HPLC system is attached to an UV and a NaI scintillator-based radioactivity detector. This detector requires heavy, costly and cumbersome lead shielding to reduce background noise and reach an acceptable limit of detection. Flow through detectors based on coincidence detection have been developed to tackle these drawbacks and achieve high sensitivity and low background interference. However, the cost of these radiodetectors is often prohibitive. In this work, we evaluate a novel, unshielded, luminescence flow-through radio-hplc detector. The luminescence flow-through monitor LB 500 fLumo is commercially available (Berthold Technologies) and represents the latest development in flow through measurement of luminescent and radioactivity samples. Using an extremely sensitive photon counting detector combined with various measuring cells the instrument offers excellent performance and flexibility (Fig. 1). A special flow-through cell with unique positron sensitivity was used

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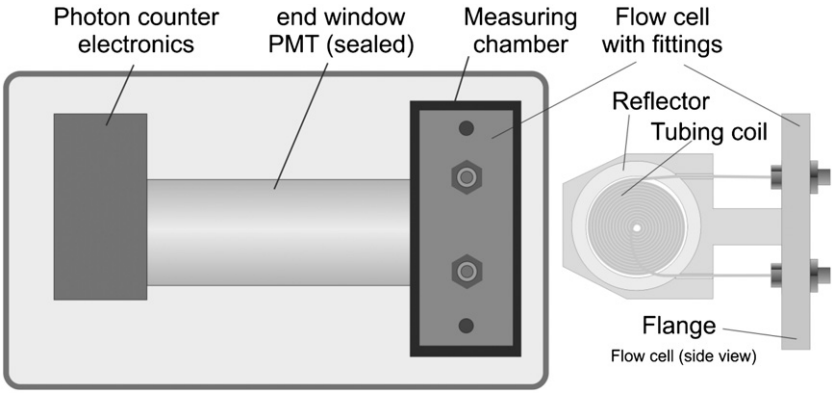

Fig. 1. Schematic view of fLumo.

to directly measure positron-emission. This cell is specially designed with an external solid melt-on scintillator which is only sensitive to positrons and insensitive to gamma rays. This virtually eliminates the influence of the gamma background counts from 511 keV gamma emission, even without lead shielding. In this paper, we evaluate the performance of the fLumo as a radio-HPLC and a radio-UPLC detector for analysis of PET radiopharmaceuticals but also as a radiodetector in HPLC purification of PET radiocompounds.

2. Materials and methods

2.1. Instruments and conditions

The LB500 fLumo chemiluminescence scintillation detector uses a highly sensitive head-on photon counting detector with a low background. Fig. 1 shows a schematic diagram of the detector unit. The dimensions of the detector unit are $150 \times 350 \times 180\ mm^3$ ($w \times d \times h$). In order to adapt to different flow rates and sample intensities, different cell types and volumes can be inserted in the measuring chamber right in front of the detector. For this study we used a commercially available 20 μl MX-cell. This cell is equipped with a scintillator which is mounted onto the outside of the cell tubing. The detection unit with low-noise head-on photomultiplier (PMT) is designed to directly measure the light created by the interaction of the positrons and the scintillator. No energy windows are set as the PMT measures the entire spectrum.

The detector unit is connected to the high energy radio monitor (HERM) electronics. The dimensions of the electronics box are $210 \times 60 \times 120\ mm^3$ ($w \times d \times h$). Fig. 2 shows a connection diagram of the detector unit and HERM electronics. The HERM electronics function either in stand-alone mode or can be connected to a PC through the use of a terminal program. The data can be transferred in a number of ways: using an 0–1 V analogue output, a transistor-transistor logic (TTL) signal or via data telegram (USB connection) directly to a PC.

In this study, the data are transmitted to the HPLC system (Empower 2, eSatin Module) as analogue signals ranging from 0 to 1 V. Among the available parameters, the "calvolt" parameter is particularly critical for the export of data to the HPLC system. Indeed, this parameter defines the number of counts/s that corresponds to an output signal of 1 V. The highest value which can be measured by the PMT is 10,000,000 cps, (6 to 7 decades of linearity are typical for PMT's). Above this, the detector will saturate. Thus, it is important that this parameter is set at a value sufficiently high to avoid major peaks appearing saturated. Unfortunately, high values of the "calvolt" parameter result in poor resolution at the lower end. Alternatively, the detector can be used in "counts per second" mode to avoid such signal saturation issues. In this mode, data can be processed in dedicated software or in simple spreadsheet software.

A Waters 2695 Alliance HPLC separation module was used for the HPLC analysis. 20 μl of solution were injected through an autosampler. For [^{18}F]FPRGD2 analysis, an X-Bridge C18 column from Waters ($250 \times 4.6\ mm^2$, 3.5 μM) with CH_3CN and H_2O containing 0.1% TFA mixture was used (1 ml/min, $t_{0-2\ min}$ 95% H_2O 0.1% TFA/5% MeCN 0.1% TFA, $t_{32\ min}$ 35% H_2O 0.1% TFA/65% AN 0.1% TFA).

A Waters Acquity UPLC System was used for UPLC analysis. For [^{18}F]FPRGD2 analysis, an acquity uplc BEH C8 column from Waters ($2.1 \times 100\ mm^2$, 1.7 μm) with CH_3CN and H_2O containing 0.1% TFA mixture was used (0.8 ml/min, 50 °C, $t_{0-1\ min}$ 95% H_2O 0.1% TFA/5% MeCN 0.1% TFA, $t_{4\ min}$ 10% H_2O 0.1% TFA/90% AN 0.1% TFA). 10 μl of solution was injected through the autosampler of the UPLC system.

The eSatin sampling rate was set up to 10 samples per second.

The fLumo HPLC radiodetector ($22\ cm \times 14\ cm \times 9\ cm$) was connected to the outlet of the photodiode array (PDA) detector with a short opaque peak tubing (~20 cm). Opaque tubing was also used to connect the outlet of the fLumo radiodetector to the waste bottle (Fig. 2). The fLumo cell tubing is made of Teflon.

A Waters 1515 binary HPLC pump was used for semi-preparative HPLC purifications. For [^{18}F]FPRGD2 purification, the crude [^{18}F]FPRGD2 solution was transferred to the HPLC system through an automatic switching valve (Rheodyne two-position, six-port switching valve). [^{18}F]FPRGD2 was purified on a Phenomenex Jupiter Proteo column (4 ml/min, $t_{0-2\ min}$, isocratic, 95% H_2O 0.1% TFA/5% MeCN 0.1% TFA, $t_{2\rightarrow 32\ min}$, linear gradient, 35% H_2O 0.1% TFA/65% MeCN 0.1% TFA). An automatic switching valve (Rheodyne two-position, six-port switching valve) allows remote collection of the peak corresponding to [^{18}F]FPRGD2. The symmetry of peaks was measured according the European Pharmacopeia (2.2.46)

2.2. Chemicals

All solvents and chemicals were of analytical grade and used without further purification. [^{18}O]-enriched water was purchased from Cambridge Isotope Laboratories. [^{18}F]FPRGD2 was synthesized as described by Thonon et al. (2011), [^{18}F]FDOPA was prepared as described by Libert et al. (2011). Gallium-68 chloride solution was obtained by elution of a ^{68}Ge/^{68}Ga-generator (Eckert Ziegler generator) and diluted to achieve the required volumic activity (Ocak et al., 2010).

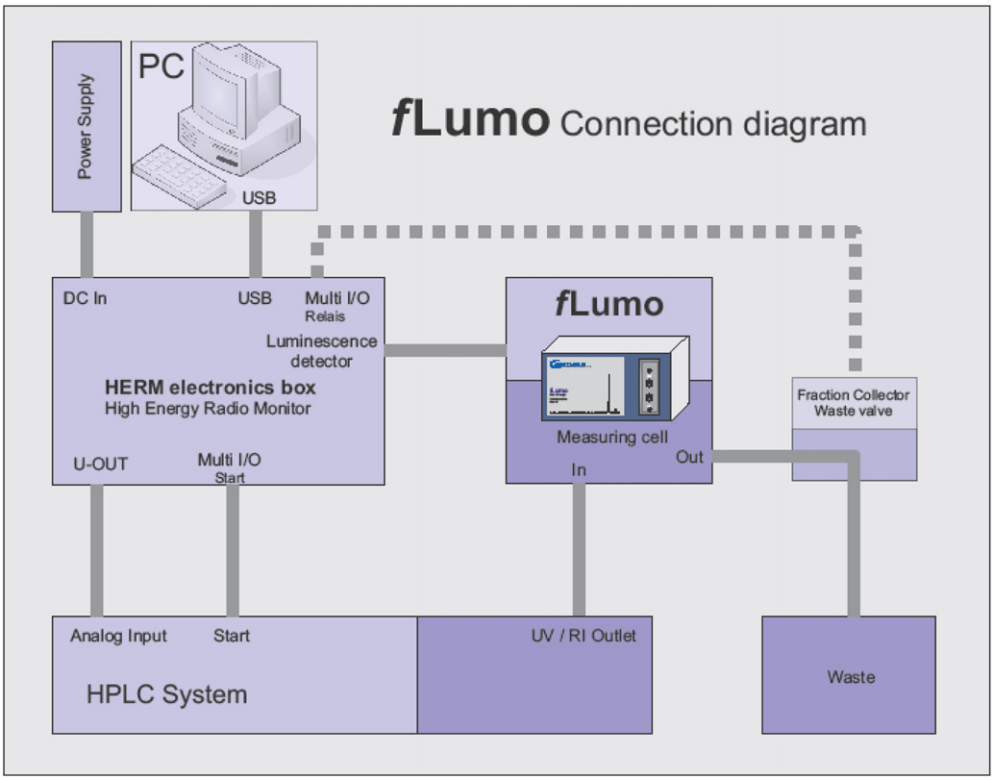

Fig. 2. fLumo connection diagram.

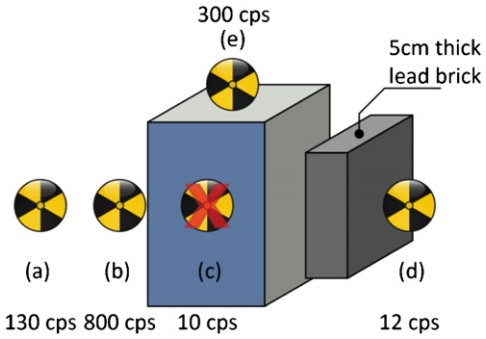

Fig. 3. Influence of a 10 MBq fluorine-18 source on background signal, (a) at 20 cm from the detector cell, (b) adjacent to the detector cell, (c) no source in the room, (d) 20 cm from detector behind a 5 cm thick lead brick, (e) on the top of the detector box.

3. Results and discussion

3.1. Background signal

In the absence of radioactivity, the background activity was approximately 10 counts per second (CPS). The use of transparent tubing to connect the fLumo detector to the HPLC system must be avoided as this is a significant source of noise (light entry). For example, the use of non-opaque teflon tubing increased the background counts to 50–100 CPS. A low background (~10 CPS) could be restored by wrapping the tubing in aluminum foil or removing ambient light.

Placing an unshielded 10 MBq radioactive source at 20 cm from the detector cell increased the background signal to 130 CPS. The influence of a 10 MBq fluorine-18 source on background counts as a function of position with respect to the detector is shown in Fig. 3.

As injection of 20 µl of a 10 MBq/100 µl sample produced a signal in excess of 15,000 CPS, the shielding of the fLumo detector from gamma rays is unnecessary. Clearly, this represents a great advantage over classical NaI detectors which required extensive lead shielding.

3.2. Detection limit, linearity, linear range (gallium-68 and fluorine-18)

The detection limits and linearity of the fLumo detector for fluorine-18 and gallium-68 (two of the most commonly used nuclides for PET) were evaluated using $[^{18}F]F^-$ and $[^{68}Ga]GaCl_3$ solution. The detection limits [signal-to-noise (S/N) ratio = 3] were 0.2 MBq/ml per 10 µl injection volume (0.1 MBq/ml per 20 µl injected, HPLC system) for fluorine-18 and 0.04 MBq/ml for gallium-68 per 10 µl injected on the UPLC system. The difference in detection limit between gallium-68 and fluorine-18 is most likely related to the difference in the positron energy between these radioisotopes (Emax 0.63 and 1.90 MeV). These values correspond to an injected activity of 2 kBq for fluorine-18 and 0.4 kBq for gallium-68 at the detection limit of the fLumo radiodetector.

These detection limit values are at least 10 times lower than those obtained with iodide scintillation crystal detectors in our laboratory (NaI(TI) SPA-3 2″ × 2″ with associated electronics from Eberline with 7 cm lead shielding). The linearity of the detector was tested in liquid chromatography runs using activity concentrations ranging from 0.2 -2500 MBq/ml. The detector response was linear over this range with correlation coefficients (r^2) of more than 0.995 (Fig. 4).

3.3. Peak shape, reproducibility

Fluorine-18 radiopharmaceuticals were used to study the performance of the fLumo detector on analytical HPLC or UPLC

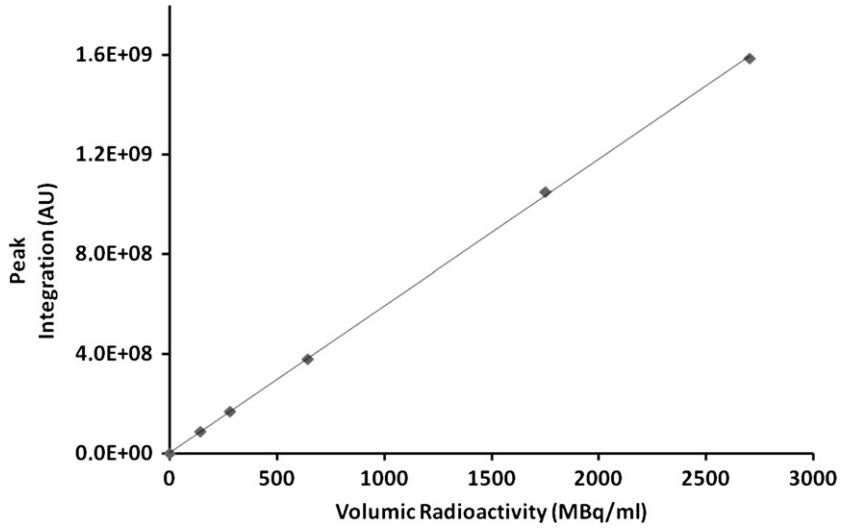

Fig. 4. Linearity of fLumo from 0.2 to 2500 MBq/ml ($r^2 > 0.995$).

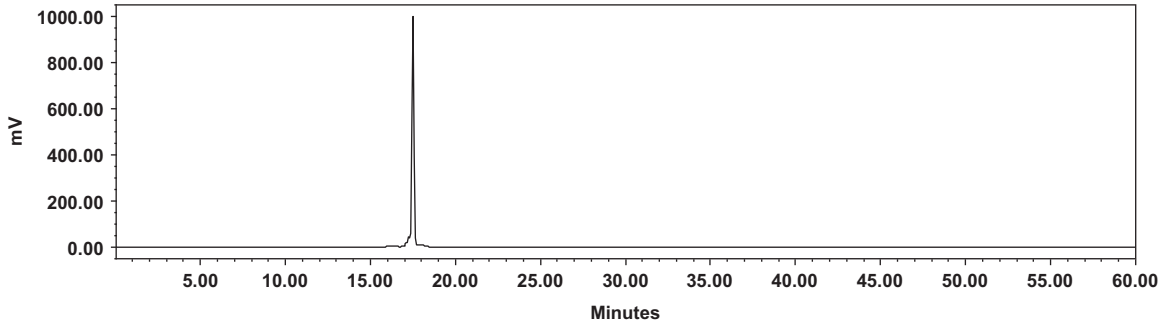

Fig. 5. HPLC radiochromatogram of [^{18}F]FPRGD2 (100 MBq/ml, 20 μl injected, calvolt 10,000 counts/s/volt).

systems. The HPLC chromatograms obtained are shown in Fig. 5. The peaks obtained were symmetrical (Symmetry factor = 0.87) and no significant tailing was observed with the 20 μl flow cell used. Typically, in the conditions used, the peak width was around 0.35 min. This value is similar to the peak width observed on the PDA signal for the corresponding non radioactive compound (0.20–0.25 min). Considering that the fLumo detector is installed downstream of the UV detector, the peak width obtained in HPLC mode appears sufficiently small.

The UPLC chromatograms obtained are shown in Fig. 6. As in HPLC mode, the peak shape was satisfactory and no excessive tailing was observed (Symmetry factor 1.62). Typically, the peak width was around 0.25 min, similar to the value of 0.20 min reported by Franck et al., obtained with a UPLC system equipped with a coincidence detector and a 20 μl cell (Franck et al., 2009). Contrary to the observations in HPLC, in UPLC the peak width of the radioactive channel is undoubtedly higher than the width obtained on the UV channel (0.05–0.08 min). This enlargement is most likely linked to the difference in size between the detection cells (20 μl for the fLumo detector and 500 nl for the PDA cell). Up to now, no smaller cell is available for the fLumo detector. In the future, it will be very interesting to evaluate the influence of smaller cells on the UPLC peak width and the limit of detection. In any case, the peak width obtained with the fLumo detector is at worst similar to the peak width obtained with the NaI detector available in our laboratory (NaI(Tl) SPA-3 $2'' \times 2''$ with associated electronics from Eberline with 7 cm lead shielding). Thus, the peak width obtained is sufficient for most PET radiopharmaceutical analyses. Reproducibility was examined by performing five repeated injections using a [^{18}F]FPRGD2 solution (195 MBq/ml, 10 μl injected.). The relative standard deviation obtained for radioactive integration (corrected for fluorine-18 decay) was 3%.

3.4. Preparative applications

Many PET radiopharmaceuticals require semi-preparative HPLC purification after radiosynthesis (Ross et al., 2011). Due to pharmaceutical, radioprotection and cost constraints, these radiocompounds are produced in shielded "hot cells" where space is limited. The fLumo detector could easily be integrated into such GMP production hot cells thanks to its compactness and light weight (The HERM electronics box is installed in a technical area outside the hot cell). As the detector cell is not disposable, we dedicate one radiodetector cell per radiopharmaceutical produced. Typically, the radioactivity that crosses the detector is between 1000 MBq and 50 GBq. Thus, we decided to study the performance of the fLumo detector for this application in terms of signal saturation and peak shape. First, the standard fLumo detector was installed in the semi-preparative HPLC system. For this range of activities, the "calvolt" parameter, which defines the count rate corresponding to the maximal analogue output of 1 V, was set to 10,000,000 CPS, the maximum value that the PMT can measure. This value was selected in order to use the maximum measurable range for the LB500 and to postpone saturation of the analogue signal as long as possible. [^{18}F]FPRGD2, developed to visualize and quantify αv-integrin expression in tumor tissues, was injected in the HPLC system to determine the "upper" limit of the radioactive detection. The radioactive peaks were collected

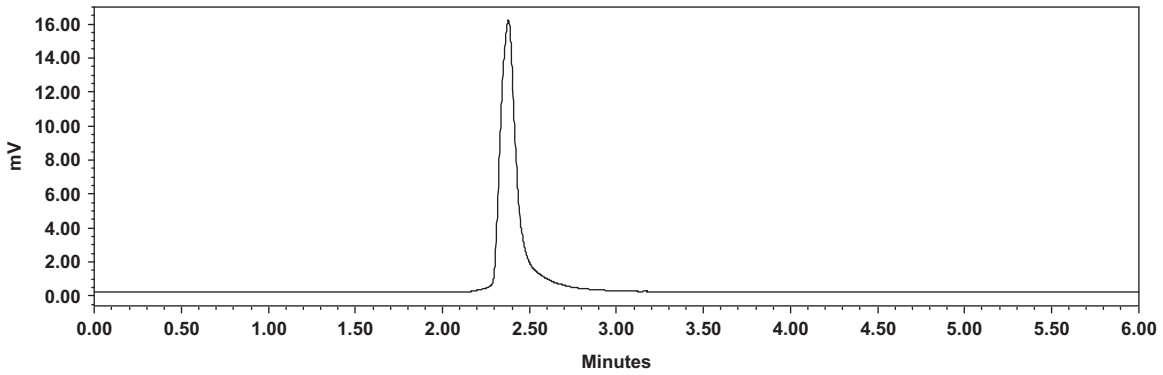

Fig. 6. UPLC radiochromatogram of [^{18}F]FPRGD2 (260 MBq/ml, 10 μl injected, calvolt 1,000,000 counts/s/volt).

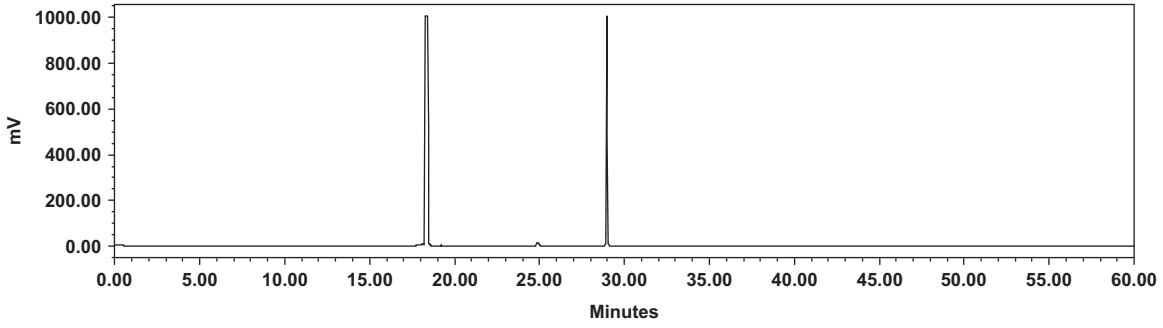

Fig. 7. Semi-preparative purification of [^{18}F]FPRGD2 with fLumo standard cell (5000 MBq of [^{18}F]FPRGD$_2$ collected, calvolt at maximum).

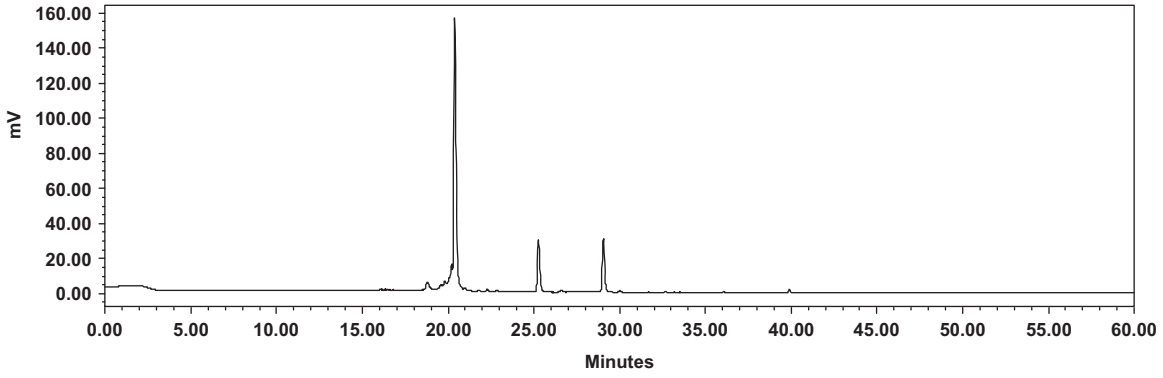

Fig. 8. Semi-preparative purification of [^{18}F]FPRGD2 with fLumo cell equipped a light filter (6300 MBq of [^{18}F]FPRGD$_2$ collected, calvolt at 1,000,000).

and counted to assess the corresponding radioactivity. A range of activities between 3700 and 20 GBq was used. The background signal was around 10,000 CPS for the 20 GBq injection, in the absence of shielding at the injector, column and FLumo detector. This background signal was not problematic as it represented less than 0.1% of the counts obtained at the maximum of the radiocompound peak collected (the distance between the HPLC loop and the radiodetector is ~70 cm). However, in this arrangement and for this range of activities, the resulting peak counts were in excess of the maximal value of 10,000,000 cps and the HPLC peak appears "saturated" (Fig. 7). To solve this problem, an OD2 Schott DG9 filter was mounted between the cell and the PMT. This filter decreases the light that reaches the PMT by a factor of 100. This shifts the measuring range of the LB500 by 2 decades to higher activities. In this configuration, no peak saturation was observed in the range of activity investigated (3700–20,000 MBq, Fig. 8). This detector configuration is currently used in our laboratory for the routine production of [^{18}F]FPRGD2. The symmetry factors of the obtained peaks were between 1 and 1.16 (Fig. 8).

A simplified approach to avoid saturation in preparative applications is to use a detector cell without scintillation substance. In this setup, the light pulses are not generated by the interaction of the positrons and the scintillator, but due to the Cherenkov light which is created by the interaction of the PMT material and high gamma radiation. With this scintillator-free cell, the background signal registered during the purification was comparable to the background measured with the classical analytical cell (~5000 counts/s for a 10 GBq injection).This outcome was expected as the background signal stems mainly from gamma photons. 200 to 10,000 MBq of [^{18}F]-FDOPA, a well-known PET radiopharmaceutical, were purified with this cell. For the 200 MBq injection, the background signal was around 200 CPS and the collected peak reached 16,000 CPS, indicating that detection of low radioactivity is still feasible with this cell configuration. For the 11,700 MBq injection, the signal reached a value close to 700,000 CPS (Fig. 9), far from saturation (which occurs at a value more than one hundred times higher). The fLumo detector equipped with this modified cell is thus also well suited to applications in HPLC purification of PET radiopharmaceuticals.

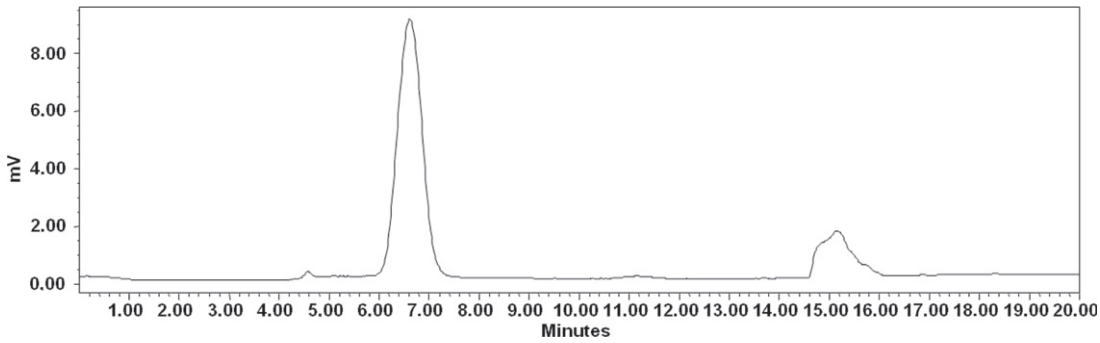

Fig. 9. Semi-preparative purification of [^{18}F]FDOPA with fLumo cell without scintillator (11700 MBq of [^{18}F]FDOPA collected, calvolt at 10,000,000).

4. Conclusions

This work demonstrates the value of the fLumo luminescence flow-through radio-HPLC detector for applications in PET radiochemistry. The performance of the fLumo was initially evaluated as a radiodetector for HPLC and UPLC systems in quality control applications. In both systems, the fLumo detector exhibited a very low limit of detection with activities as low as 4 kBq detected (detection limit 0.2 MBq/ml, 10 μl injected). The detector also demonstrated excellent linearity (0.2 to 2500 MBq/ml, $r^2 > 0.995$) and reproducibility. In HPLC, the shape of the peaks obtained was satisfactory (no tailing or enlargement). For applications in UPLC the peak width ($\sim$0.25 min) is tolerable. However, future developments should include a smaller cell volume to decrease the peak width and take full advantage of UPLC.

Thanks to its compactness and absence of shielding a fLumo detector with a modified detection cell was installed in a shielded production "hot" cell to detect radiocompounds on a semi-preparative HPLC system. In this "preparative" configuration the fLumo detector is currently used for the routine production of radiopharmaceuticals in our laboratory without difficulty.

Acknowledgments

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