Evaluation of [^{18}F]N-methyl-lansoprazole as a tau PET imaging agent in first-in-human studies

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1. Chemistry Investigations

Using non-radioactive experiments we found that heating of both, the reference compound as well as the difluoroenol precursor in presence of potassium carbonate and [2.2.2]cryptand (kryptofix® 222, crypt-222) lead to a rapid degradation of the material (Figure S1). This process was markedly inhibited by purging the reaction mixture with N_2 prior to heating resulting in a threefold higher yield of the desired product.* Since the formation of the non-radioactive 1,1,1-trifluoroethyl ether was observed in absence of any added fluoride ion in our control experiment, we concluded that the degradation process must lead to release of fluoride ion from the starting material ultimately leading to dilution of the radiolabeled [^{18}F]NML with its non-radioactive analogue. Interestingly, we observed degradation of the labeled product under the reaction conditions, complete with formation of traces of the enol-ether elimination product, which illustrates the reversibility of the labeling reaction under the labeling conditions giving rise to extensive apparent isotopic exchange. By limiting the reaction time to three minutes at 90 °C we were able to reduce formation of the non-radioactive product to 9.5-13 µg (34 nmol) per batch.

* It is unknown exactly why N_2 improved the yield, but it is presumably a function of oxygen dissolved in the solution in the reaction vial. Reflecting this, for other substrates a denser gas like Ar does work better, but we have no data on synthesis of [^{18}F]NML using Ar. We continue our methodology work around this reaction, and plan to report additional findings in due course.

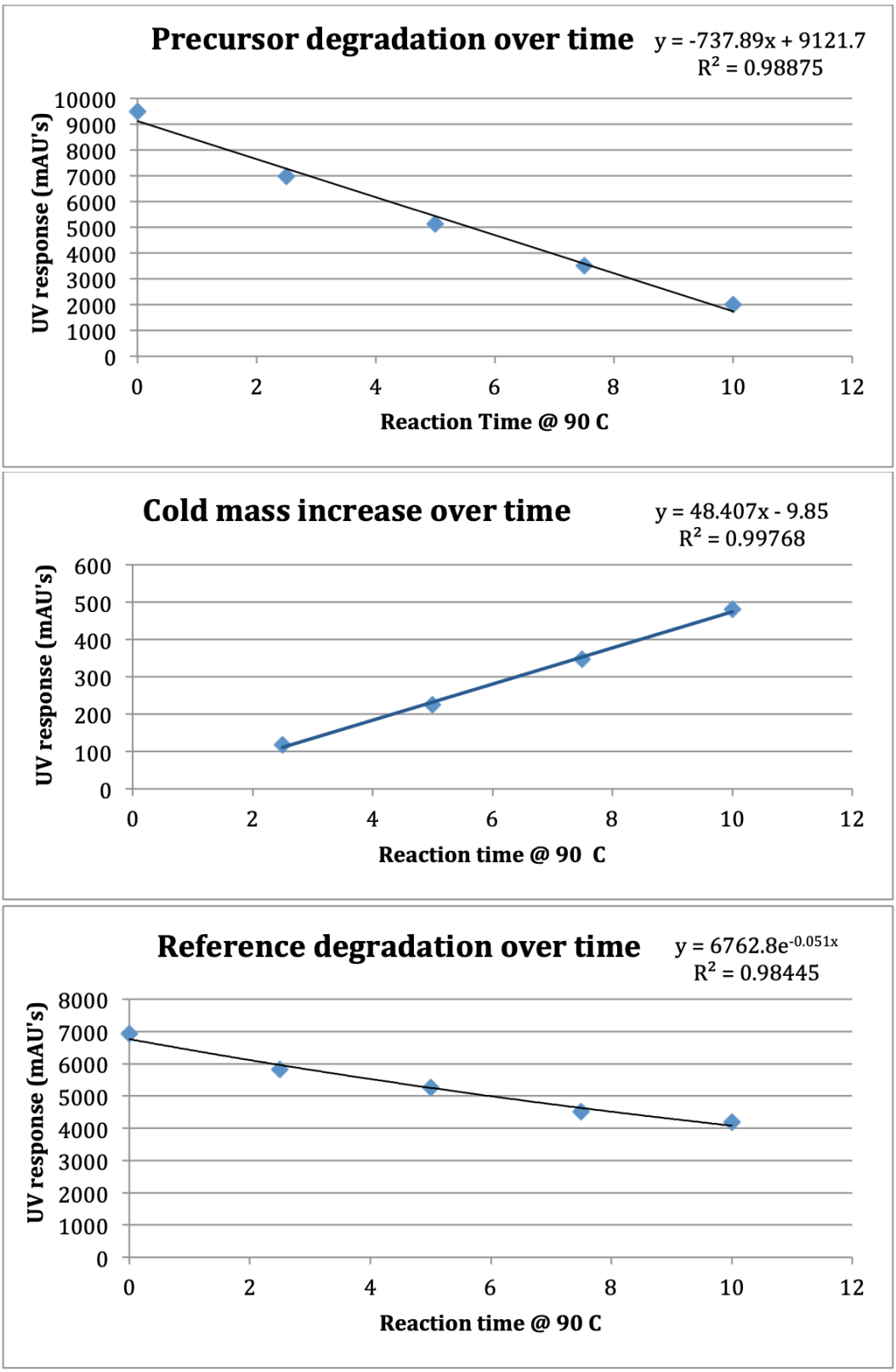

Figure S1: Degradation of precursor over time (top), formation of non-radioactive reference as a function of precursor degradation (middle), and evidence of product degradation over time (bottom).

2. Biodistribution Studies

Biodistribution studies were conducted at 5,30, 60 and 120min post-injection of [^{18}F]NML. Briefly, male (n = 8) and female (n = 8) Sprague-Dawley rats (weighing 181 - 270 g) were anesthetized with isoflurane (5% induction, 1-2% maintenance), injected *i.v*. with [^{18}F]NML (952.8 ± 114.5 kBq for 5 min; 2081.2 ± 92.5 kBq for 30 min; 3792.5 ± 140.1 kBq for 60 min; 3487.3 ± 293.5 kBq for 120 min) via the lateral tail vein and allowed to awaken until sacrifice. 2 M and 2F rats were sacrificed at each time point (5, 30, 60 and 120 min post-injection). Tissues and organs were collected, weighed and counted for their radioactivity with a Packard 5550 autogamma counter. Results were averaged for each time point and expressed as % injected dose/gram of tissue for each organ (Table S1 and Figure S1).

Table S1: Rodent Biodistribution data (%i.d/g)[†]

	5 min			30 min			60 min			120 min		
	Mean ± SD			Mean ± SD			Mean ± SD			Mean ± SD		
BRAIN	0.183283	±	0.093522	0.06776	±	0.035213	0.120988	±	0.018491	0.113468	±	0.02337
EYEBALLS	0.13632	±	0.059326	0.045145	±	0.023048	0.078675	±	0.012555	0.081085	±	0.016046
HEART	0.38981	±	0.185317	0.096288	±	0.051894	0.134688	±	0.020828	0.12127	±	0.021784
LUNG	0.34547	±	0.156645	0.098553	±	0.051339	0.135325	±	0.016834	0.123593	±	0.019048
LIVER	1.311453	±	0.483217	0.222673	±	0.112751	0.248693	±	0.028682	0.21582	±	0.02245
PANCREAS	0.60523	±	0.285249	0.096835	±	0.050686	0.131783	±	0.015798	0.119583	±	0.017816
SPLEEN	0.366393	±	0.181331	0.07787	±	0.042441	0.114045	±	0.01602	0.104435	±	0.018421
ADRENAL	1.325248	±	0.880979	0.134873	±	0.080009	0.159795	±	0.027456	0.146283	±	0.036369
KIDNEY	0.915023	±	0.344208	0.223158	±	0.156301	0.202533	±	0.018989	0.166208	±	0.011036
ADIPOSE	0.123758	±	0.046332	0.065968	±	0.034135	0.084465	±	0.013469	0.093418	±	0.034291
STOMACH	0.673058	±	0.268503	0.25002	±	0.133711	0.398165	±	0.091819	0.319818	±	0.054636
CONTENTS OF STOMACH	0.29768	±	0.340555	0.28261	±	0.090181	0.67109	±	0.354124	0.937318	±	0.62891
SMALL INTESTINE	0.720463	±	0.349824	0.386495	±	0.220944	0.408108	±	0.086978	0.345648	±	0.0332
CONTENTS OF SMALL INTESTINE	0.567013	±	0.35213	0.89076	±	0.249936	1.18608	±	0.370512	1.46517	±	0.539034
CAECUM	0.269135	±	0.121267	0.089395	±	0.048281	0.1306	±	0.024555	0.138518	±	0.0386
CONTENTS OF CAECUM	0.188293	±	0.25895	0.05222	±	0.027233	0.122918	±	0.031823	0.151538	±	0.030286
LARGE INTESTINE	0.631805	±	0.605407	0.084125	±	0.044748	0.118565	±	0.012854	0.122493	±	0.024147
CONTENTS OF LARGE INTESTINE	0.099435	±	0.045414	0.049638	±	0.028615	0.126808	±	0.027649	0.177698	±	0.021501
OVARY	0.50265	±	0.059934	0.04151	±	0.010988	0.127945	±	0.007955	0.12033	±	0.028808
UTERUS	0.38029	±	0.016716	0.04471	±	0.012431	0.11638	±	0.00502	0.114225	±	0.034387
MUSCLE	0.11568	±	0.033856	0.072468	±	0.039342	0.106423	±	0.011325	0.093098	±	0.014085
BONE	0.168228	±	0.075584	0.044445	±	0.026703	0.071855	±	0.010277	0.066248	±	0.007709
BLOOD	0.349005	±	0.168919	0.088618	±	0.048022	0.125483	±	0.016643	0.113298	±	0.015769
TESTES	0.09926	±	0.03783	0.08376	±	0.020379	0.094895	±	0.005862	0.087115	±	0.008535

[†] Data shows some accumulation within the brain at 5 min, followed by washout within 30 min, then an increase at 60-120 min which may suggest the presence of late brain-penetrating metabolites in rodents. However, we have seen no evidence of brain-penetrating metabolites in any of the human data presented in the main manuscript.

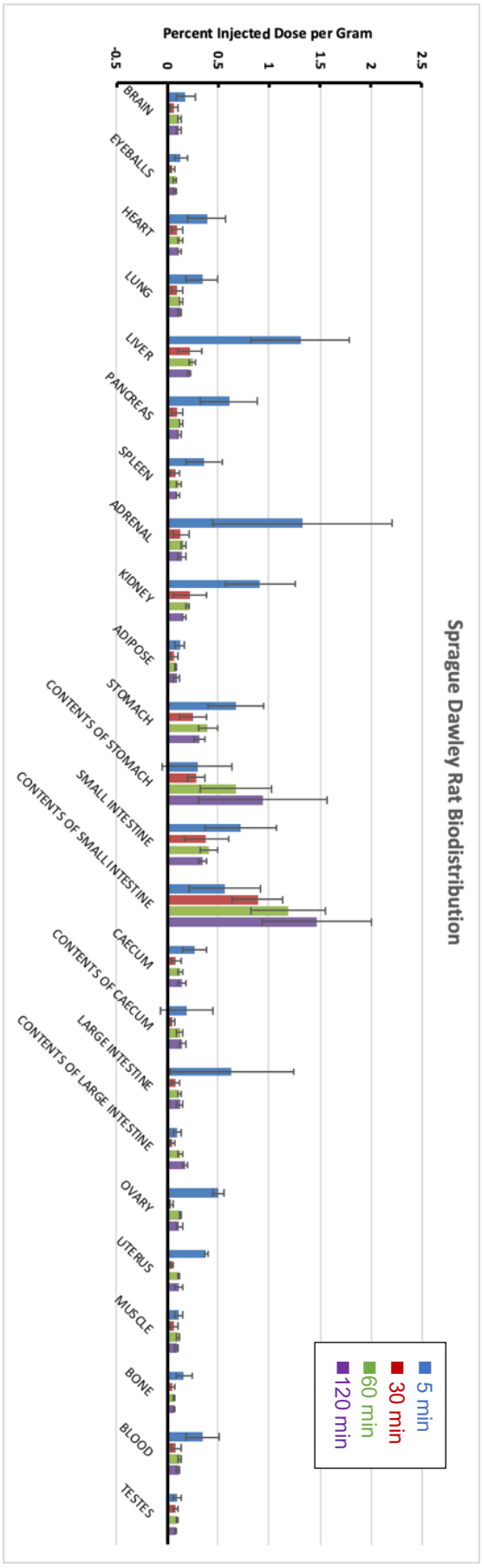

Figure S2: Rodent Biodistribution data (%i.d/g)

3. Human Dosimetry Estimates

Biodistribution studies were conducted in male and female Sprague Dawley rats as described above. Biodistribution data was used to determine radiation-absorbed-dose estimates using the OLINDA/EXM 1.0 software package.[1] Distribution to the urine was also determined by acquiring dynamic small animal PET scans from 0-120 min for male (n=2) and female (n=2) with bladder in frame, which allowed for calculation of radiation dose.

Table S2: Human Dosimetry Estimates

OLINDA - Organ Level INternal Dose Assessment Code (copyright Vanderbilt University, 2003)

NOTE: This code gives doses for stylized models of average individuals - results should be applied with caution to specific human subjects.
NOTE: Users should always carefully check input data (shown below) and critically review the reported results.

Organ Doses (mSv/MBq), Nuclide: F-18 (1.10E02 min), Adult Male

Target Organ	Alpha	Beta	Photon	Total	EDE Cont.	ED Cont.
Adrenals	0.00E000	2.64E-03	8.04E-03	1.07E-02	0.00E000	2.67E-05
Brain	0.00E000	1.21E-03	3.12E-03	4.33E-03	0.00E000	1.08E-05
Breasts	0.00E000	3.88E-03	4.55E-03	8.42E-03	1.26E-03	4.21E-04
Gallbladder Wall	0.00E000	3.88E-03	1.28E-02	1.67E-02	0.00E000	0.00E000
LLI Wall	0.00E000	1.55E-02	1.53E-02	3.08E-02	1.85E-03	3.70E-03
Small Intestine	0.00E000	4.31E-02	2.04E-02	6.35E-02	3.81E-03	1.59E-04
Stomach Wall	0.00E000	5.22E-03	9.44E-03	1.47E-02	0.00E000	1.76E-03
ULI Wall	0.00E000	4.31E-02	2.69E-02	7.00E-02	4.20E-03	1.75E-03
Heart Wall	0.00E000	1.70E-03	6.87E-03	8.57E-03	0.00E000	0.00E000
Kidneys	0.00E000	2.93E-03	8.70E-03	1.16E-02	0.00E000	2.91E-05
Liver	0.00E000	3.49E-03	7.94E-03	1.14E-02	0.00E000	5.71E-04
Lungs	0.00E000	1.69E-03	5.77E-03	7.46E-03	8.95E-04	8.95E-04
Muscle	0.00E000	3.88E-03	7.34E-03	1.12E-02	0.00E000	2.80E-05
Ovaries	0.00E000	3.88E-03	1.75E-02	2.13E-02	5.33E-03	4.27E-03
Pancreas	0.00E000	1.81E-03	8.95E-03	1.08E-02	0.00E000	2.69E-05
Red Marrow	0.00E000	2.76E-03	8.53E-03	1.13E-02	1.36E-03	1.36E-03
Osteogenic Cells	0.00E000	8.32E-03	7.96E-03	1.63E-02	4.89E-04	1.63E-04
Skin	0.00E000	3.88E-03	4.45E-03	8.33E-03	0.00E000	8.33E-05
Spleen	0.00E000	3.88E-03	8.00E-03	1.19E-02	0.00E000	2.97E-05
Testes	0.00E000	3.88E-03	7.21E-03	1.11E-02	0.00E000	0.00E000
Thymus	0.00E000	3.88E-03	6.47E-03	1.03E-02	0.00E000	2.59E-05
Thyroid	0.00E000	3.88E-03	6.48E-03	1.04E-02	3.11E-04	5.18E-04
Urinary Bladder Wall	0.00E000	3.11E-02	2.08E-02	5.19E-02	3.11E-03	2.59E-03
Uterus	0.00E000	3.88E-03	1.67E-02	2.05E-02	1.23E-03	5.13E-05
Total Body	0.00E000	4.56E-03	7.36E-03	1.19E-02	0.00E000	0.00E000
Effective Dose Equivalent (mSv/MBq)					2.39E-02	
Effective Dose (mSv/MBq)						1.85E-02

Organ Doses (rem/mCi), Nuclide: F-18 (1.10E02 min), Adult Male

Target Organ	Alpha	Beta	Photon	Total	EDE Cont.	ED Cont.
Adrenals	0.00E000	9.75E-03	2.97E-02	3.95E-02	0.00E000	9.87E-05
Brain	0.00E000	4.47E-03	1.15E-02	1.60E-02	0.00E000	4.00E-05
Breasts	0.00E000	1.43E-02	1.68E-02	3.12E-02	4.68E-03	1.56E-03
Gallbladder Wall	0.00E000	1.43E-02	4.74E-02	6.18E-02	0.00E000	0.00E000
LLI Wall	0.00E000	5.73E-02	5.66E-02	1.14E-01	6.84E-03	1.37E-02
Small Intestine	0.00E000	1.60E-01	7.55E-02	2.35E-01	1.41E-02	5.88E-04
Stomach Wall	0.00E000	1.93E-02	3.49E-02	5.42E-02	0.00E000	6.51E-03
ULI Wall	0.00E000	1.59E-01	9.97E-02	2.59E-01	1.55E-02	6.48E-03
Heart Wall	0.00E000	6.28E-03	2.54E-02	3.17E-02	0.00E000	0.00E000
Kidneys	0.00E000	1.09E-02	3.22E-02	4.30E-02	0.00E000	1.08E-04
Liver	0.00E000	1.29E-02	2.94E-02	4.23E-02	0.00E000	2.11E-03
Lungs	0.00E000	6.24E-03	2.14E-02	2.76E-02	3.31E-03	3.31E-03
Muscle	0.00E000	1.43E-02	2.71E-02	4.15E-02	0.00E000	1.04E-04
Ovaries	0.00E000	1.43E-02	6.46E-02	7.89E-02	1.97E-02	1.58E-02
Pancreas	0.00E000	6.70E-03	3.31E-02	3.98E-02	0.00E000	9.95E-05
Red Marrow	0.00E000	1.02E-02	3.16E-02	4.18E-02	5.02E-03	5.02E-03
Osteogenic Cells	0.00E000	3.08E-02	2.95E-02	6.03E-02	1.81E-03	6.03E-04
Skin	0.00E000	1.43E-02	1.65E-02	3.08E-02	0.00E000	3.08E-04
Spleen	0.00E000	1.43E-02	2.96E-02	4.39E-02	0.00E000	1.10E-04
Testes	0.00E000	1.43E-02	2.67E-02	4.10E-02	0.00E000	0.00E000
Thymus	0.00E000	1.43E-02	2.39E-02	3.83E-02	0.00E000	9.57E-05
Thyroid	0.00E000	1.43E-02	2.40E-02	3.83E-02	1.15E-03	1.92E-03
Urinary Bladder Wall	0.00E000	1.15E-01	7.69E-02	1.92E-01	1.15E-02	9.60E-03
Uterus	0.00E000	1.43E-02	6.16E-02	7.60E-02	4.56E-03	1.90E-04
Total Body	0.00E000	1.69E-02	2.72E-02	4.41E-02	0.00E000	0.00E000
Effective Dose Equivalent (rem/mCi)					8.83E-02	
Effective Dose (rem/mCi)						6.83E-02

Number of Disintegrations in Source Organs:

Adrenals	3.10E-04	MBq-h/MBq or uCi-h/uCi
Brain	1.23E-02	MBq-h/MBq or uCi-h/uCi
Breasts	0.00E000	MBq-h/MBq or uCi-h/uCi
Gallbladder Contents	0.00E000	MBq-h/MBq or uCi-h/uCi

LLI	2.39E-02	MBq-h/MBq or uCi-h/uCi
Small Intestine	2.39E-01	MBq-h/MBq or uCi-h/uCi
Stomach	5.04E-03	MBq-h/MBq or uCi-h/uCi
ULI	1.31E-01	MBq-h/MBq or uCi-h/uCi
Heart Contents	0.00E000	MBq-h/MBq or uCi-h/uCi
Heart Wall	3.85E-03	MBq-h/MBq or uCi-h/uCi
Kidneys	6.31E-03	MBq-h/MBq or uCi-h/uCi
Liver	4.78E-02	MBq-h/MBq or uCi-h/uCi
Lungs	1.21E-02	MBq-h/MBq or uCi-h/uCi
Muscle	0.00E000	MBq-h/MBq or uCi-h/uCi
Ovaries	0.00E000	MBq-h/MBq or uCi-h/uCi
Pancreas	1.23E-03	MBq-h/MBq or uCi-h/uCi
Red Marrow	0.00E000	MBq-h/MBq or uCi-h/uCi
Cortical Bone	0.00E000	MBq-h/MBq or uCi-h/uCi
Trabecular Bone	0.00E000	MBq-h/MBq or uCi-h/uCi
Spleen	0.00E000	MBq-h/MBq or uCi-h/uCi
Testes	0.00E000	MBq-h/MBq or uCi-h/uCi
Thymus	0.00E000	MBq-h/MBq or uCi-h/uCi
Thyroid	0.00E000	MBq-h/MBq or uCi-h/uCi
Urinary Bladder Contents	8.25E-02	MBq-h/MBq or uCi-h/uCi
Uterus/Uterine Wall	0.00E000	MBq-h/MBq or uCi-h/uCi
Remainder	2.05E000	MBq-h/MBq or uCi-h/uCi

GI Model used:
Fraction entering SI : 0.15

Dynamic bladder model used:
Fraction: 0.05
T-bio: 0.337 hr
Void Interval: 4.0 hr

Target Organ Masses:

Adrenals	1.63E001 g
Brain	1.42E003 g
Breasts	3.51E002 g
Gallbladder Wall	1.05E001 g
LLI Wall	1.67E002 g
Small Intestine	6.77E002 g
Stomach Wall	1.58E002 g
ULI Wall	2.20E002 g
Heart Wall	3.16E002 g
Kidneys	2.99E002 g
Liver	1.91E003 g
Lungs	1.00E003 g
Muscle	2.80E004 g
Ovaries	8.71E000 g
Pancreas	9.43E001 g
Red Marrow	1.12E003 g
Osteogenic Cells	1.20E002 g
Skin	3.01E003 g
Spleen	1.83E002 g
Testes	3.91E001 g
Thymus	2.09E001 g
Thyroid	2.07E001 g
Urinary Bladder Wall	4.76E001 g
Uterus	7.90E001 g
Total Body	7.37E004 g

* Mass modified by user

Radiation Weighting Factors:

Alpha:	5.00E00
Beta:	1.00E00
Photon:	1.00E00

** Weighting factor modified by user

4. Radiosynthesis Data[a]

Run	Starting ^{18}F(GBq)	[^{18}F]NML (GBq)	RCY (%)	A_m (GBq/μmol)[b]
PM1	134.8	2.4	1.8	782.4
PM2	106.7	4.0	3.8	73.3
PM3	96.2	3.2	3.3	97.1
PM4	140.6	1.0	0.7	99.8
PM5	79.7	0.8	1.0	80.4
PM Average	111.6	2.3	2.1	226.6
PM SD	25.8	1.4	1.4	310.9
UM1	66.6[c]	1.8	2.7	43.7
UM2	66.6[c]	2.8	4.2	14.5
UM3	66.6[c]	3.4	5.0	46.0
UM4	66.6[c]	3.2	4.8	42.0
UM5	66.6[c]	3.9	5.8	91.7
UM6	66.6[c]	4.3	6.4	125.1
UM7	66.6[c]	6.6	9.9	111.6
UM8	66.6[c]	5.3	7.9	65.4
UM9	66.6[c]	5.3	5.0	108.1
UM10	66.6[c]	4.6	6.9	20.8
UM Average	66.6	3.9	5.9	66.9
UM SD	0.0	1.4	2.0	40.0
Overall Average	81.6	3.4	4.6	120.1
Overall SD	25.9	1.5	2.6	186.3

[a] PM = PositronMed; UM = University of Michigan; RCY = non-corrected radiochemical yield; A_m = molar activity; all runs passed quality control testing using the methods outlined in Section 5; [b] The apparent difference in molar activity between UM and PM is down to one outlier (Run - PM1). If this data point is omitted, the average A_m values between sites are more consistent (PM = 87.8 ± 12.9 GBq/μmol, UM = 66.9 ± 40.0 GBq/μmol); [c] starting [^{18}F]fluoride amounts estimated from known cyclotron production history.

5. Quality Control Testing of [^{18}F]NML

Quality control of [^{18}F]NML is carried out as described below:

Visual inspection

Doses are examined visually and must be clear, colorless and free of particulate matter.

Dose pH

The pH of [^{18}F]NML doses is analyzed by applying a small amount of the dose to pH-indicator strips and determined by visual comparison with the provided scale. Dose pH must be 4.5 – 7.5.

HPLC analysis

Radiochemical purity, NML concentration and molar activity are determined by radio-HPLC (column: Phenomenex Luna 5µ PFP(2) 11 Å column (150 x 4.6mm) (p/n 00F-4448-E0); mobile HPLC phase: 35% acetonitrile: 65% H_2O, flow rate: 2 ml / min; UV: 254 nm; retention time for the dose was approximately 6.5 minutes). Co-injection of the dose with unlabeled reference standard is performed to confirm compound identity.

Radioactive concentration and Radionuclidic identity

Radioactivity concentration is determined by measuring activity using a Capintec dose calibrator. To determine radionuclidic identity, half-life was calculated using Eq. (1). Calculated half-life must be 105–115 min.

$$T_{1/2} = -\ln 2(\text{Time Difference} / (\ln(\text{ending activity/starting activity}))) \quad (\mathbf{1})$$

Residual Solvent Analysis

Levels of residual solvents in [^{18}F]NML doses are analyzed using a gas chromatography and flame ionization detection. Limits of residual solvents are based upon the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines (MeCN: ≤410 ppm; DMSO: ≤5000 ppm).[2]

Residual Crypt-222

Residual crypt-222 (kryptofix-2.2.2) levels in [^{18}F]NML doses is analyzed using the established spot test. Strips of plastic-backed silica gel TLC plates saturated with iodoplatinate reagent are spotted with water (negative control), 50 µg/mL crypt-222 standard (positive control) and with the [^{18}F]NML dose. If

crypt-222 is present in a sample, a blue–black spot appears. Spots for the three samples are compared and a visual determination of residual crypt-222 in the dose is made. <50 μg/mL is acceptable.

Sterile filter integrity test

Sterile filter from dose (with needle still attached) is connected to a nitrogen supply via a regulator. The needle is then submerged in water and the nitrogen pressure gradually increased. If the pressure is raised above the filter acceptance pressure (44 psi) without seeing a stream of bubbles, the filter is considered intact.

Bacterial endotoxins

Endotoxin content in radiopharmaceutical doses is analyzed by a Charles River Laboratories EndoSafe® Portable Testing System and according to the US Pharmacopeia. Doses must contain <175 Endotoxin Units (EU).

Sterility

Culture tubes of fluid thioglycolate media (FTM) and tryptic soy broth (TSB) are inoculated with samples of [^{18}F]NML and incubated (along with positive and negative controls) for 14 days. FTM is used to test for anaerobes, aerobes and microaerophiles whilst TSB is used to test for non-fastidious and fastidious microorganisms. Culture tubes are visually inspected on the 3rd, 7th and 14th days of the test period and compared to the positive and negative standards. Positive standards must show growth (turbidity) in the tubes, and dose/negative controls must have no culture growth after 14 days to be indicative of sterility.

5. References

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