

## Supporting Information

### Selectivity determination of a small molecule chemical probe using protein microarray and affinity capture techniques

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## Identification of Protein Interactors from the Protein Microarray

<sup>3</sup>H-PF-06652474 was profiled on ProtoArray® Human Protein Microarrays containing more than 9,000 human proteins. All assays included <sup>3</sup>H-estradiol (1.0 nCi/μL) for positional mapping, and a negative control assay was run in parallel using <sup>3</sup>H-estradiol alone. <sup>3</sup>H-PF-06652474 was assayed at one concentration in the absence or presence of 150 mM NaCl. Through ionic interactions and reorganization of water molecules in the solvation shell at the protein surface, sodium chloride may perturb binding interactions observed in the absence of high salt concentrations. Subtle differences in the interactions between the small molecule of interest and proteins on the array may result in altered binding constants and, therefore, differences in the observed binding interactions.

The arrays were washed to remove unbound small molecule, exposed on a tritium-sensitive phosphor screen for 14 days and then scanned at 600 dpi. The high resolution images were then processed through Adobe Photoshop. Signal intensity data are subsequently determined with microarray acquisition software (GenePix Pro 6). The files containing these signal intensity data are then processed through the ProtoArray® Prospector software (Invitrogen, free to download).

The Prospector software calculates several values that can be used to evaluate whether a protein on the array exhibited a significant interaction with the radiolabeled small molecule probe. All possible protein interactors were evaluated and compared to the negative control assay. A protein was defined as exhibiting a significant interaction with <sup>3</sup>H-PF-06652474 if it met the following conditions on at least one array probed with <sup>3</sup>H-PF-06652474 in the absence of competitor:

- (1) A Signal Used value greater than 1,000 RLU
- (2) A Z-Score greater than 3.0, indicating a signal greater than 3 standard deviations above the mean signal from all human proteins on the array
- (3) A replicate spot CV of less than 50% for the corresponding protein

Applying these selection criteria to the data set generated in Phase 2 identified one protein interactor, DcpS. This protein was identified in assays both with and without 150 mM NaCl. The Prospector data for this protein's interaction with <sup>3</sup>H-PF-06652474 are presented in Table S1.

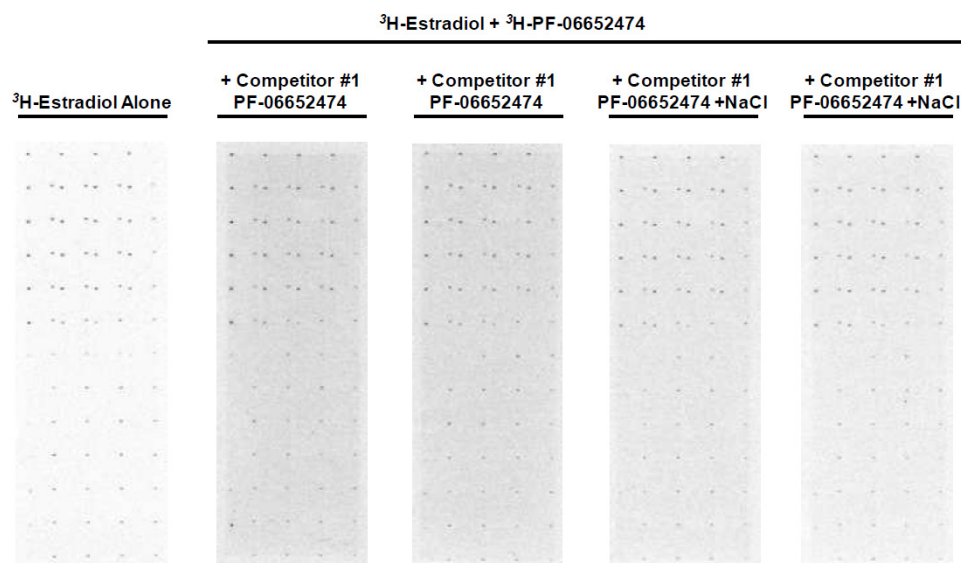
**Table S1.** Interacting proteins identified in microarray assays with <sup>3</sup>H-PF-06652474.

	ORF ID	Neg control	Signal used	Z-factor	Z-score	Replicate spot CV	Inter-assay CV	Protein description
Without NaCl	IOH12798	219	2621	0.50	19.49	13%	8%	DcpS
With NaCl	IOH12798	219	3091	0.50	24.51	12%	13%	DcpS

The data presented include the background subtracted pixel intensity value (the "Signal Used" value) from the negative control array (Neg Signal Used) and from the arrays probed with <sup>3</sup>H-PF-06652474 in the absence and presence of NaCl. Additional data include the Z-Factor, Z-Score, replicate spot CV, calculated from the replicate spots for the protein, and inter-assay CV. Only the data from the array with the higher Signal Used value is shown. The identified protein (DcpS) met the selection criteria on both replicate arrays.

## Competitor Effects

The effect of small molecule competition was calculated as a “percent competition” using the Signal Used value from the array with no competitor, compared to the Signal Used value from the array with competitor (see Figures 2 and S1). This value was calculated by the equation: percent competition = 1-(average Signal Used value with competitor/ Signal Used value without competitor) x100. Using this equation, no inhibition would have a value of 0%, while complete inhibition would have a value of 100%. PF-06652474 percent competition = 100%.



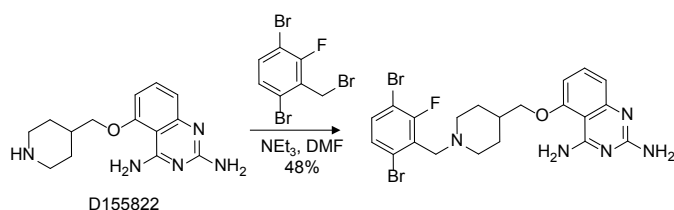
**Figure S1.** Images of arrays probed with 100 nM <sup>3</sup>H-PF-06652474 plus 10 μM PF-06652474, with and without 150 mM NaCl. (Note the absence of signal in these images from the same protein as highlighted in Figure x [red box]).

## m<sup>7</sup>GTP agarose pulldown assay

10 million human PBMCs cells were lysed by sonication in 1 mL lysis buffer (25 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM MgCl<sub>2</sub>, 1 mM EDTA, 1% NP-40 and 5% glycerol) supplemented with protease inhibitors. After centrifugation, supernatant was transferred to a new tube, and incubated with DMSO, D156844 or m<sup>7</sup>GTP at room temperature for 60 minutes. 100 μL 7-methyl-GTP Sepharose<sup>®</sup> 4B (GE Healthcare) was added to the mixture and further incubated at 4 °C for 60 minutes. Beads were then washed three times with 1 mL cold PBST (PBS with 0.1% tween-20), and eluted by boiling with NuPAGE LDS sample buffer. The eluates were separated by SDS-PAGE and then analyzed by immunoblot using antibodies of cap-associated proteins, including DcpS (Abcam), CBP20 (Abcam), CBP80 (Cell Signaling), and EIF4E (Cell Signaling).

## Synthesis of <sup>3</sup>H-PF-06652474

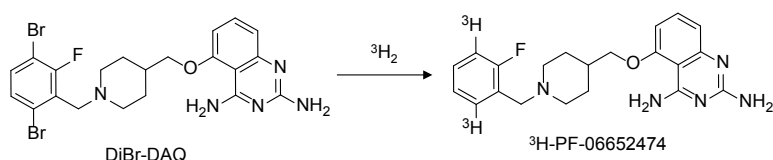
### 5-((1-(3,6-dibromo-2-fluorobenzyl)piperidin-4-yl)methoxy)quinazoline-2,4-diamine (DiBr-DAQ)



A 20 mL scintillation vial was equipped with a magnetic stirring bar, and the benzyl bromide (1.6 mmol, 556 mg), piperidine derivative D155822 (1.34 mmol, 365 mg), trimethylamine (5.34 mmol, 744  $\mu$ L) and DMF (6.7 mL) were added. The vial was capped and heated to 60°C for 28 hours. The reaction was cooled to room temperature, concentrated and purified on the combiflash system (0-10% MeOH/DCM, 0.1% ammonium hydroxide) on a 24 g gold column to provide 348 mg of the desired product; <sup>1</sup>H NMR (CD<sub>3</sub>OD, 400MHz)  $\delta$  7.72 (m, 1H), 7.54 (m, 1H), 7.41 (m, 1H), 7.02 (m, 1H), 6.95 (m, 1H), 4.18 (m, 2H), 3.71 (m, 2H), 3.07 (m, 2H), 2.36 (m, 2H), 2.05 (m, 1H), 1.86 (m, 2H), 1.48 (m, 2H); LCMS (ESI) 540 [M+H]<sup>+</sup> (ELSD 91% pure).

### <sup>3</sup>H-PF-06652474

Prepared by PerkinElmer Life & Analytical Sciences, 549 Albany Street, Boston, MA 02118

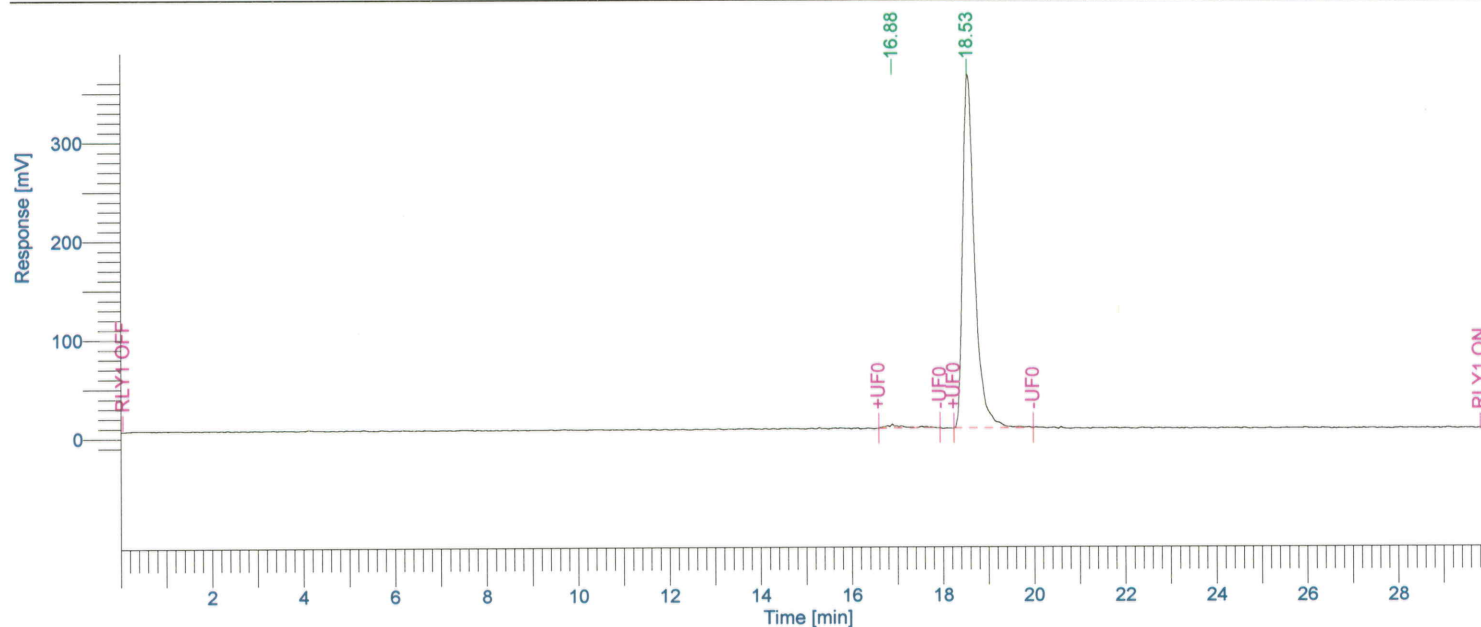


In a 10 mL tritium reaction flask containing a magnetic stir bar, DiBr-DAQ (10 mg) was dissolved in 3 mL of DMF (Sigma Cat # 227056) and 10% Pd/C (Sigma Cat # 205699, 7 mg) was added. 30 pSi tritium gas was introduced into the reaction mixture and stirred at room temperature for 1 hour. The mixture was filtered and transferred into another 10 mL reaction flask connected to a vacuum line and tritium gas was removed using methanol (3 x 5mL). HPLC analysis of the crude reaction mixture showed 75% conversion. The crude product was purified by preparative HPLC using Semi-prep Brownlee C18 column (250 x 10 mm, 5 $\mu$ m) using Solvent A: 0.3% Trifluoroacetic acid in water and Solvent B: 0.3% Trifluoroacetic acid in Acetonitrile, 10% B to 30% B for 60 min, hold 30% B for 20 min. Pure fractions of the product were collected and combined. The solvent was removed using a rotary evaporator and the product was re-constituted in ethanol. Yield = 30 mCi. The product ID was confirmed by HPLC (co-elution with a co-injected sample of PF-06652474) and MS. Specific activity was determined by MS – see attached analytical data.

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Sample Amount : 1.000000  
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Channel : A  
Operator : mcclaisg  
Dilution Factor : 1.000000

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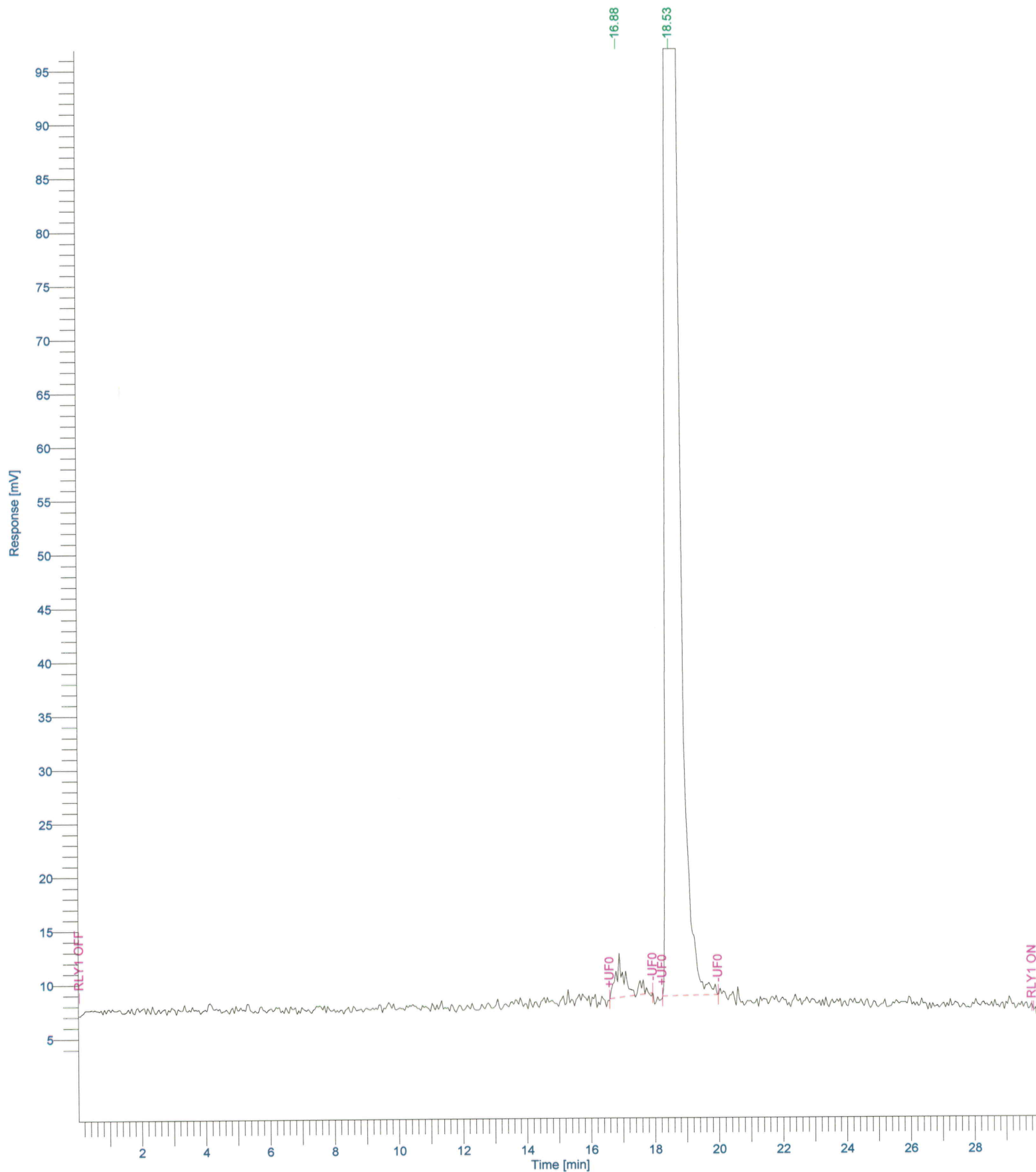
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Column: Brownlee C18 column 5 micron 250 x 4.6mm  
Flow Rate: 1.0 mL/min  
Run Time: 30 min  
Channel A: Radioactivity Detection:,Pico-fluor 40  
Channel B: UV Detection: at 220 nm

Peak #	Time [min]	Area [%]
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2	18.533	98.71
		100.00

# Chromatogram

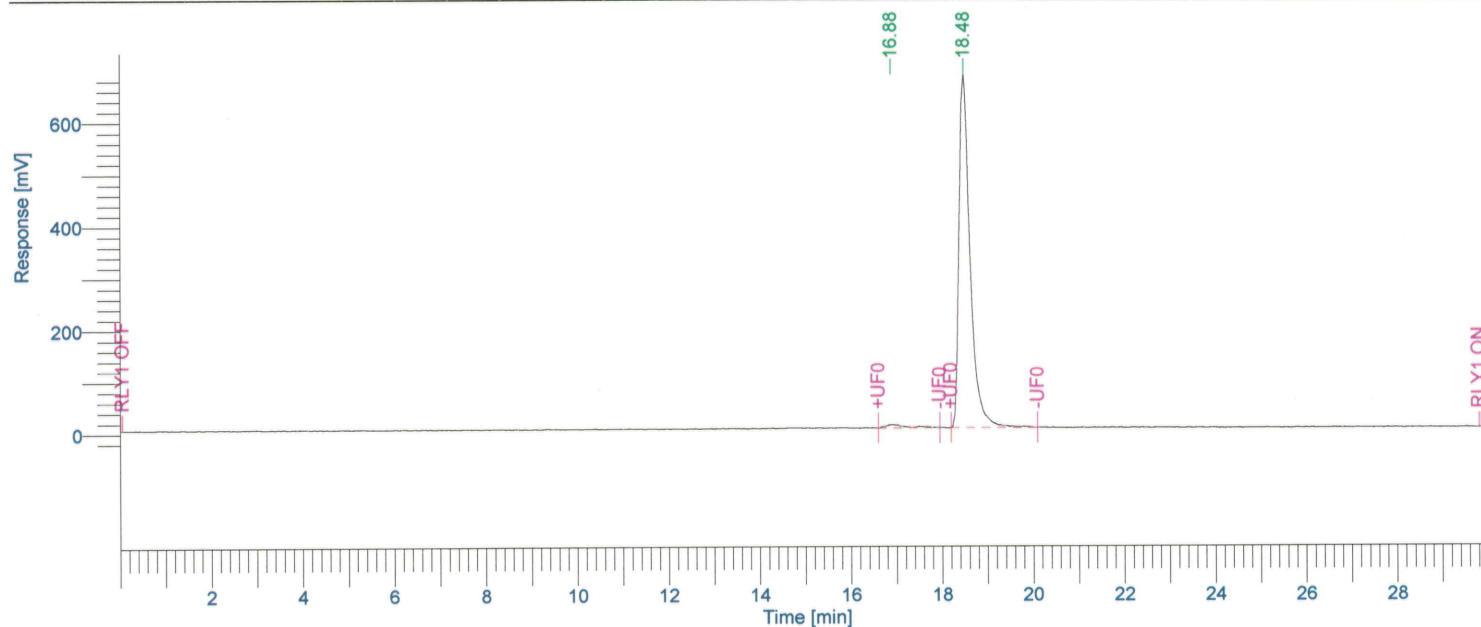
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Plot Offset: 3.60 mV      Plot Scale: 93.3 mV



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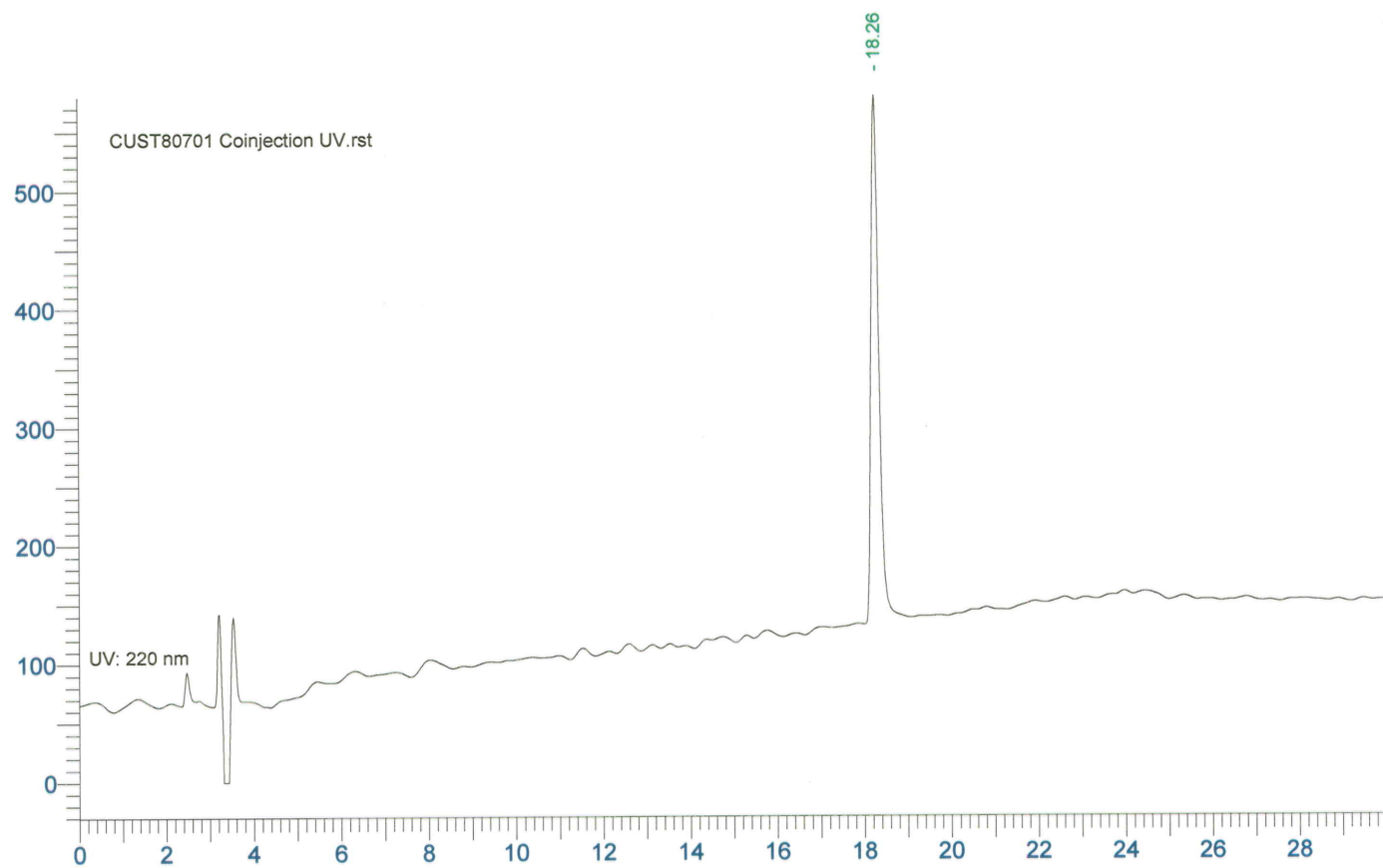
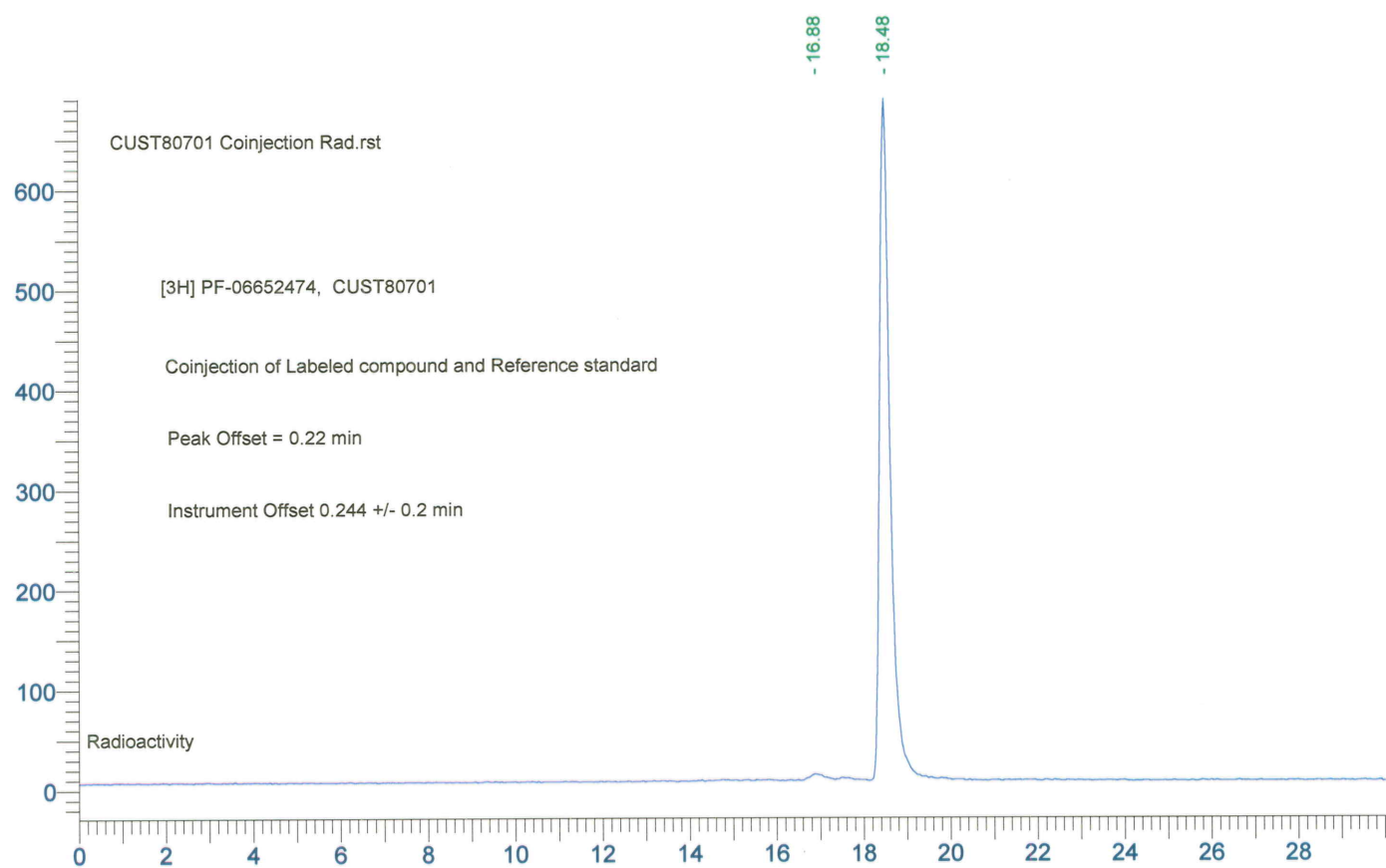
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## PerkinElmer Analytical Report

Sample: [3H]PF-06652474  
Catalog Number: CUST80701  
Mobile Phase: Pump A= 0.3% Trifluoroacetic acid in water : Pump B= 0.3%Trifluoroacetic acid in acetonitrile  
Gradient: 10%B to 30%B in 20 min, hold at for 10 min  
Column: Brownlee C18 column 5 micron 250 x 4.6mm  
Flow Rate: 1.0 mL/min  
Run Time: 30 min  
Channel A: Radioactivity Detection:,Pico-fluor 40  
Channel B: UV Detection: at 220 nm

Peak #	Time [min]	Area [%]
1	16.883	1.54
2	18.483	98.46
		100.00

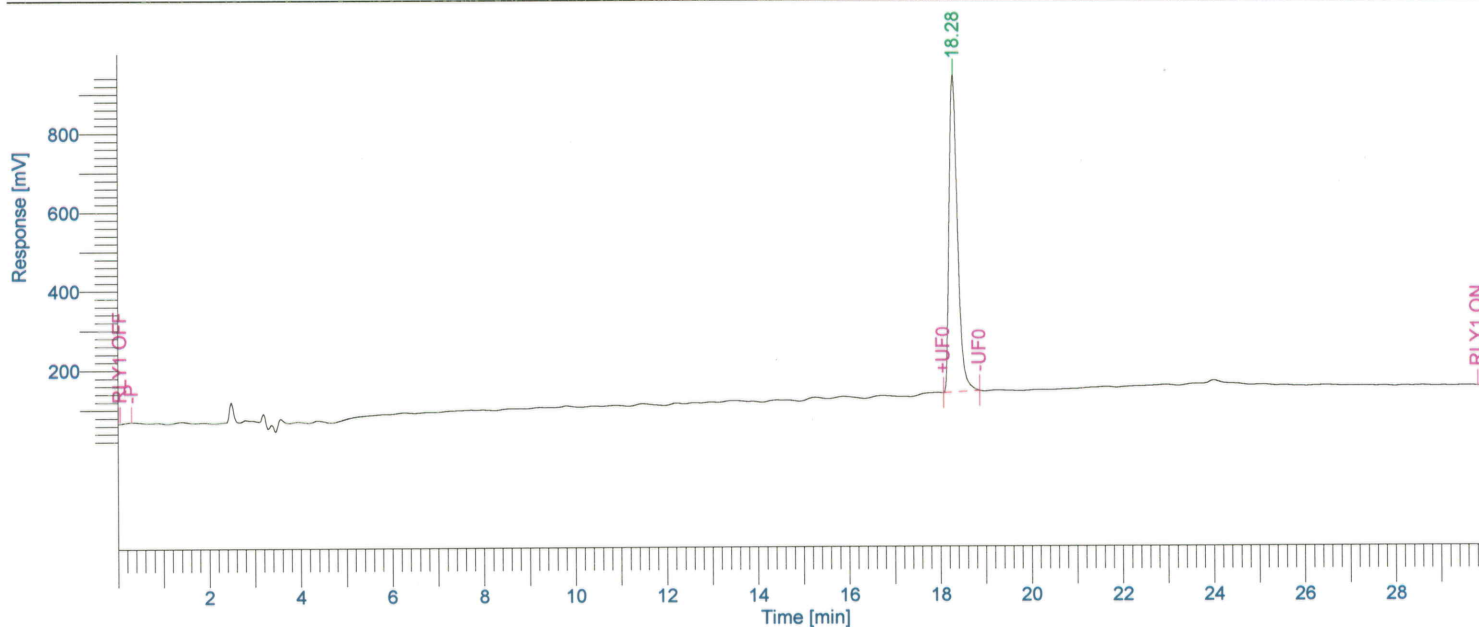




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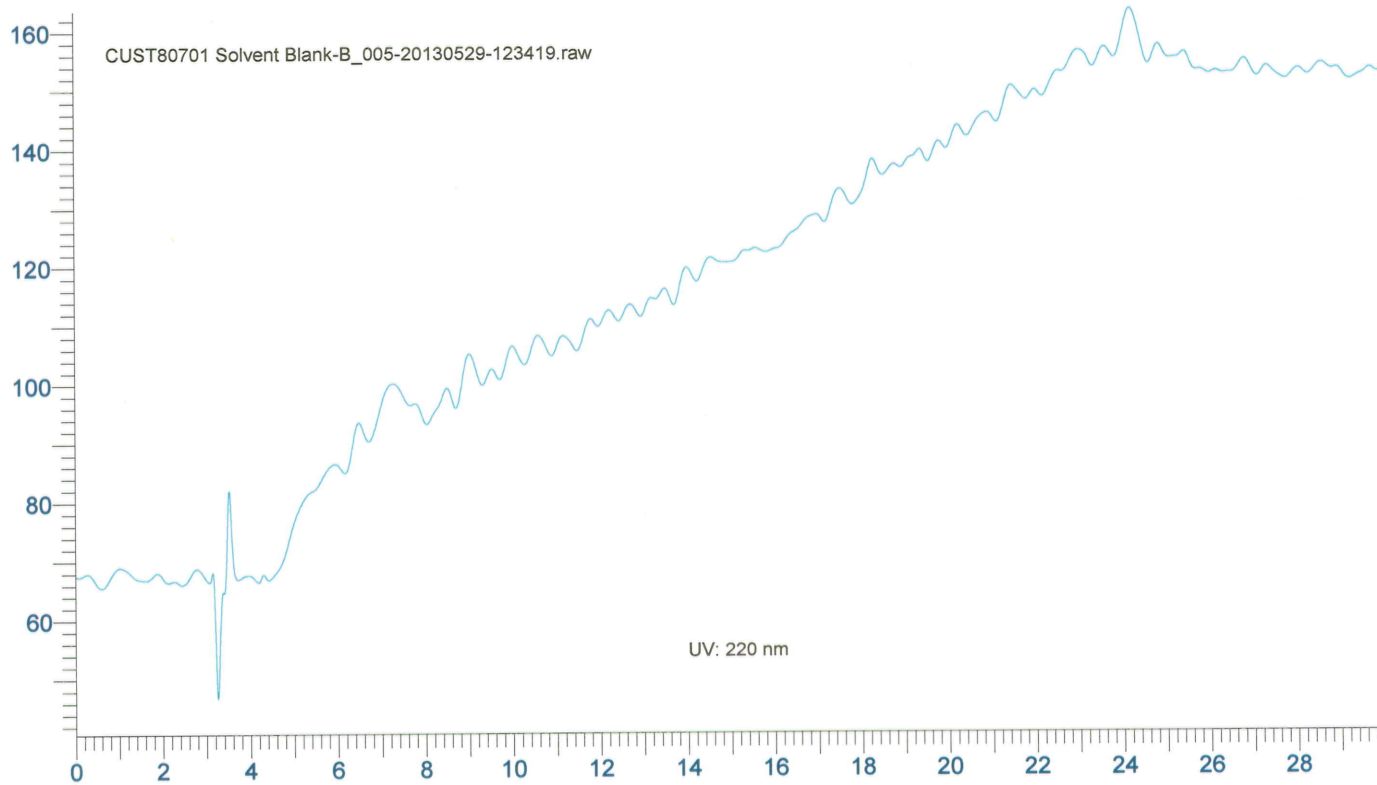
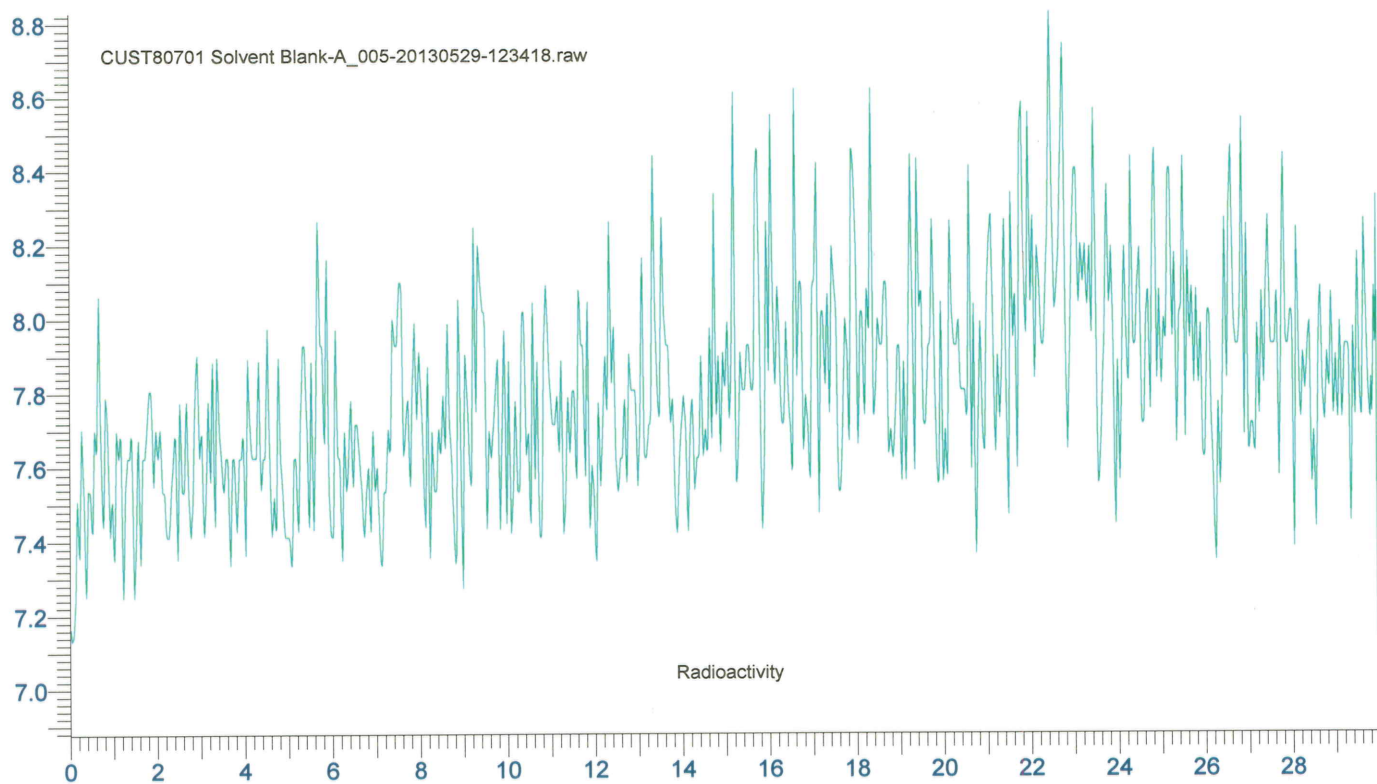
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## PerkinElmer Analytical Report

Sample: [3H]PF-06652474  
Catalog Number: CUST80701  
Mobile Phase: Pump A= 0.3% Trifluoroacetic acid in water : Pump B= 0.3%Trifluoroacetic acid in acetonitrile  
Gradient: 10%B to 30%B in 20 min, hold at for 10 min  
Column: Brownlee C18 column 5 micron 250 x 4.6mm  
Flow Rate: 1.0 mL/min  
Run Time: 30 min  
Channel A: Radioactivity Detection: Pico-fluor 40  
Channel B: UV Detection: at 220 nm

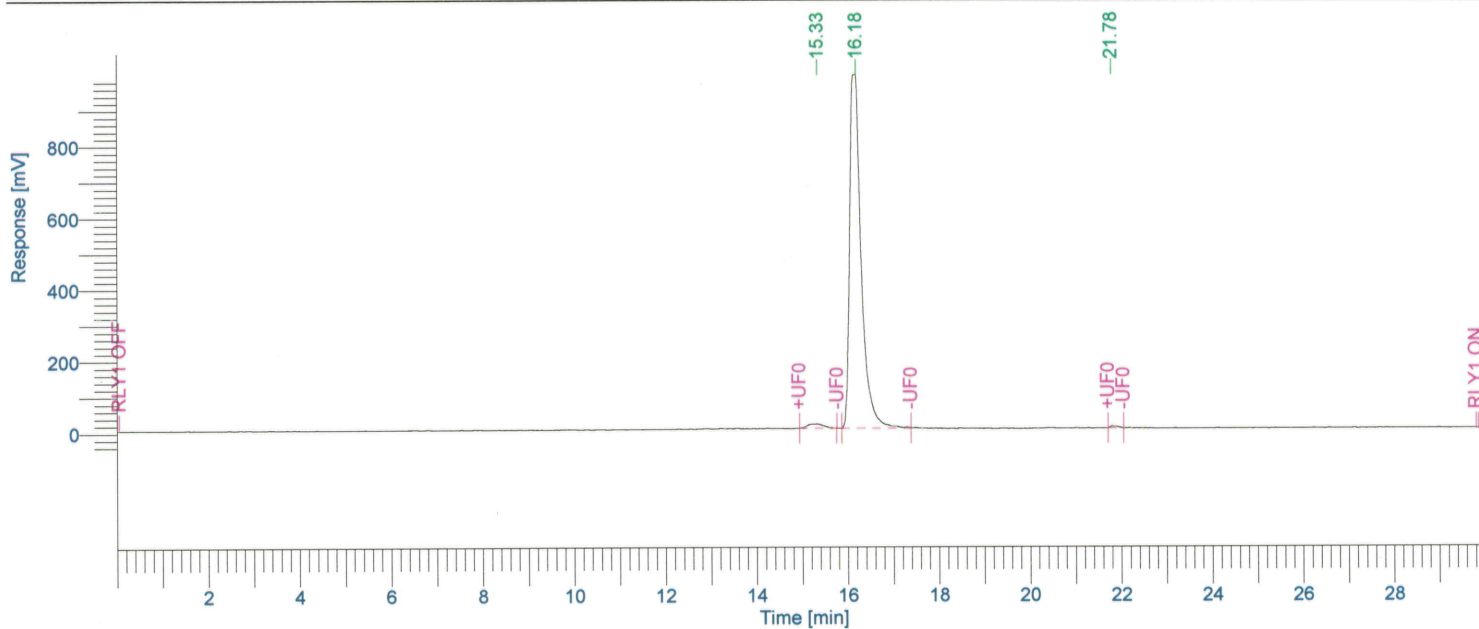
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		100.00



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Sample Amount : 1.000000  
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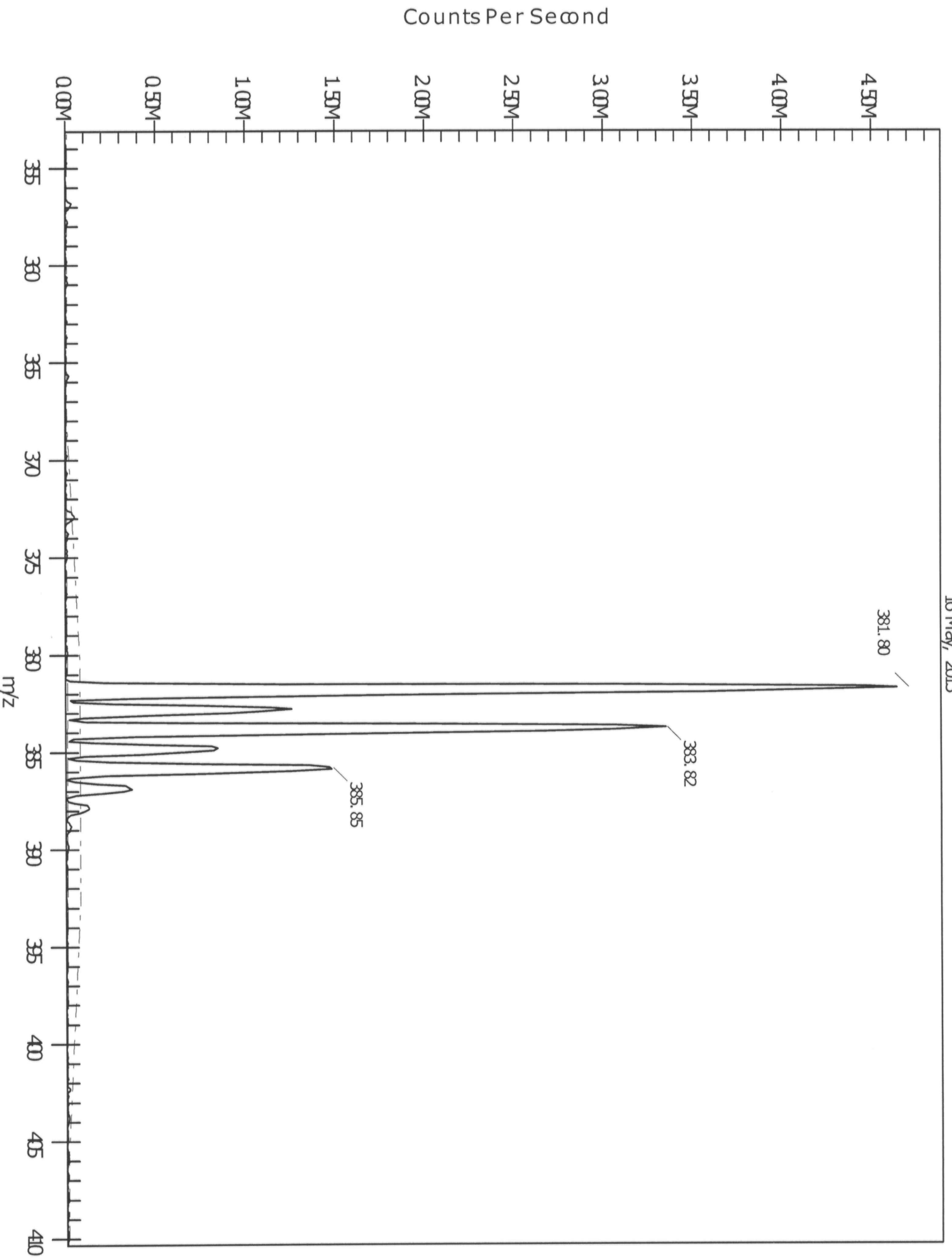
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Gradient: 10%B to 30%B in 15 min, 5 min gradient to 100% B hold for 10 min  
Column: Brownlee C18 column 5 micron 250 x 4.6mm  
Flow Rate: 1.0 mL/min  
Run Time: 30 min  
Channel A: Radioactivity Detection: Pico-fluor 40  
Channel B: UV Detection: at 220 nm

Peak #	Time [min]	Area [%]
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2	16.183	97.99
3	21.783	0.32
		100.00

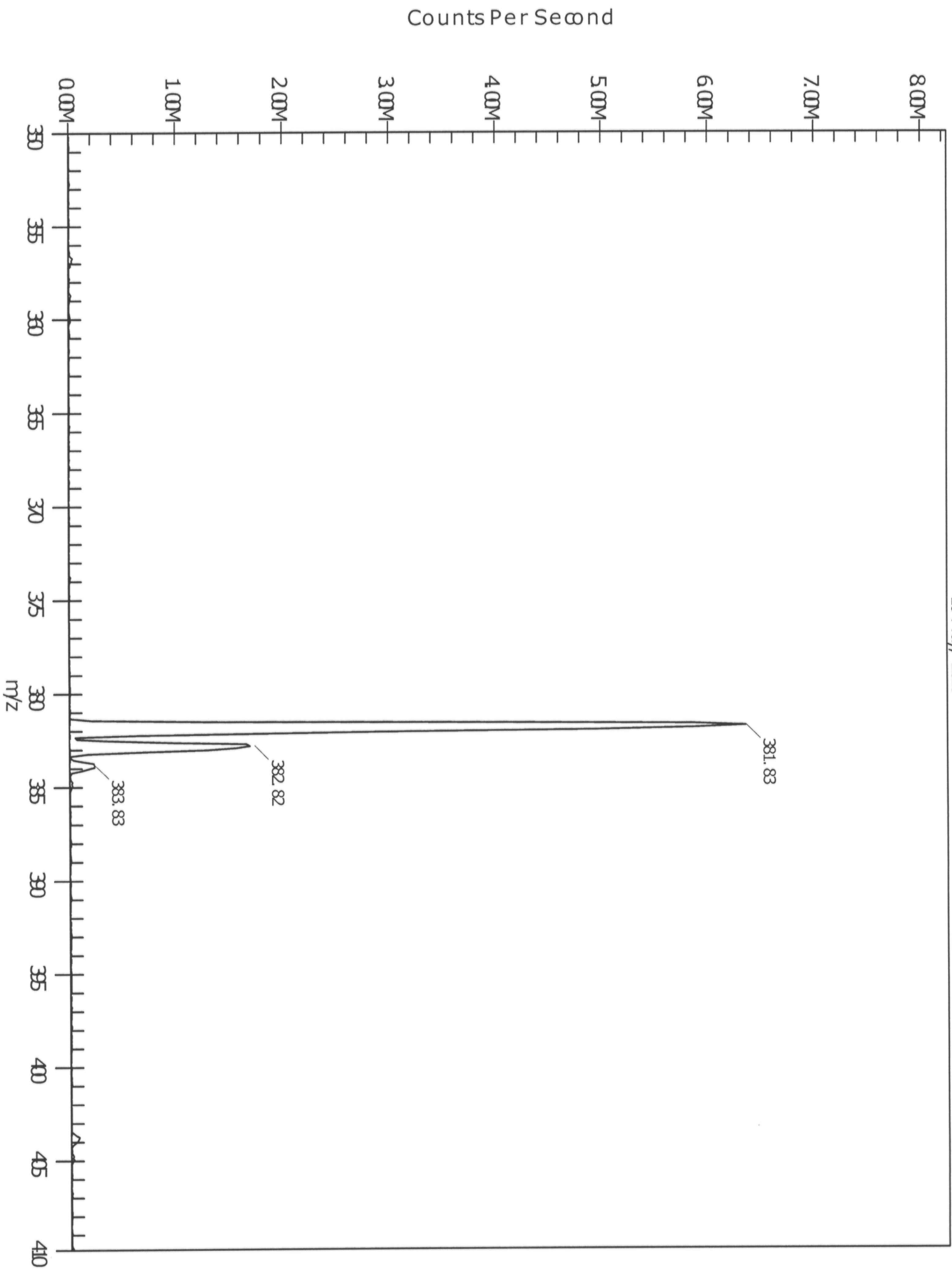
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16 May, 2013



# CUST80701-Cold-STD

16 May, 2013



**16 May 2013**

[illegible]