SwRI Contract Number 01-87479
SwRI Project No. 01.25728

FINAL REPORT

SYNTHESIS OF NOVEL TOPICAL ANESTHETICS WITH ENHANCED THERAPEUTIC PROFILE

August 31, 2021

for

Pendleton Wickersham, M.D.
Chief Science Officer
PTC Innovation, LLC
1400 Patriot Boulevard, Suite 152
Glenview, IL 60025

by

Shawn Blumberg, Ph.D.
Senior Research Scientist

Southwest Research Institute
6220 Culebra Road, P.O. Drawer 28510
San Antonio, Texas 78228-0510

Darrel Johnston
Senior Program Manager

for **Joe McDonough, Ph.D**
Director
Pharmaceuticals and Bioengineering Department
Chemical and Chemical Engineering Division

TABLE OF CONTENTS

INTRODUCTION

The ultimate objective of this work was to find new local anesthetic drug candidates that reduce the dose-related side-effects of the current compounds available on the market. PTC Innovations provided Southwest Research Institute® (SwRI®) the following compounds based on a preliminary analysis of the chemical space (Figure 1).

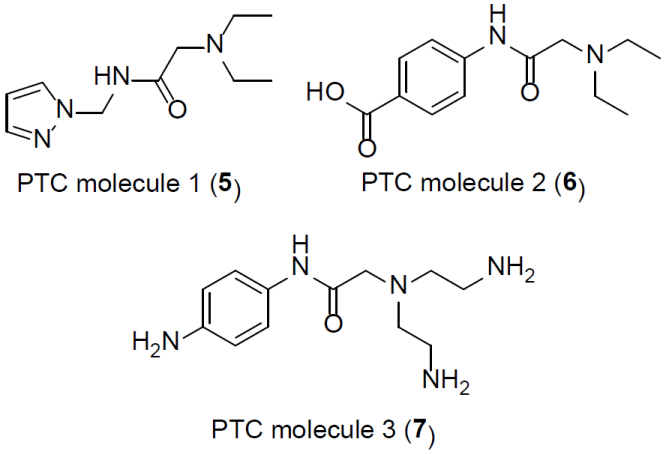


Figure 1. PTC Innovation Molecules Provided to SwRI

Although compounds **6** and **7** were relatively easy to synthesize, compound **5** is more difficult and it is likely hydrolytically unstable. Development of new drug lead compounds traditionally requires screening hundreds of compounds and with so few molecules, this invites a high likelihood of failure. In order to maximize the likelihood of discovering a new lead compound, SwRI performed a thorough virtual analysis to develop a virtual model of drug binding. The crystal structures of several Na_V proteins have recently been published, so SwRI used our proprietary docking platform Rhodium™ to screen a larger virtual library based on PTC Innovation compounds **5**, **6**, and **7**. By prioritizing the skin expressed $Na_V1.7$ and 1.8 proteins over the other Na_V ion channels, we tailored the candidate selection toward compounds that should give pain relief while minimizing side effects. We used Stardrop™ to further down-select the candidates to tune the pharmokinetic properties (such as LogP and Pka) that are optimal for a topical anesthetic. In this way, the candidates that have passed these virtual screens have the best chance for activity without any of the detrimental side-effects that previous compounds encountered.

BACKGROUND

Local anesthetics have been used by humans since antiquity, using opium extracts or cocaine for pain relief (Figure 2). Opiates and cocaine have significant psychoactive affects in addition to their pain reliving properties, so efforts to find derivatives which only have the pain relieving effects were highly desirable. Significant progress toward these ends were made in the early 1900s with benzocaine. Since then, several derivatives have come to market, including lidocaine and mepivicaine. All local anesthetics work by binding to ligand-gated ion channels, which mediate

neuron polarization. Key qualities that distinguish local anesthetics from general ones is that the diffusion of the compounds remain limited to the area of application and clearance in the blood stream is rapid.

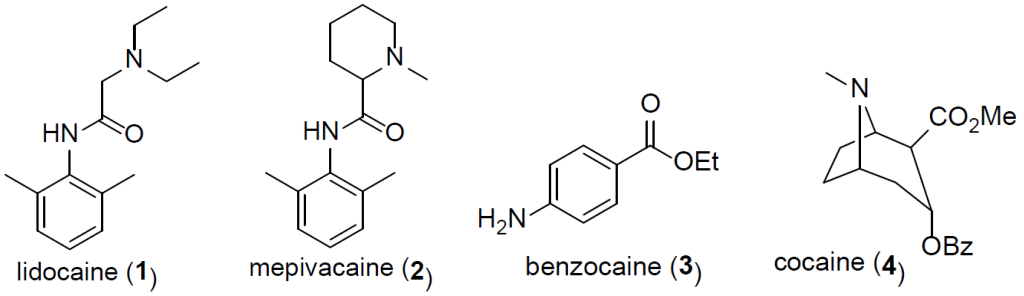


Figure 2. Current and Outdated Topical Anesthetics

Voltage-gated sodium channels control the flow of sodium ions across cellular membranes and are critical to the initiation and propagation of electrical impulses in excitable cells. There are nine different human isoforms of sodium channels ($Na_V1.1 - Na_V1.9$) with varying tissue expression patterns in neurons and cardiac and skeletal muscle.[1]

Nonselective Na_V inhibitors such as lidocaine (**1**) are non-specific binders of the proteins and thus demonstrate dose-limiting side effects associated with modulation of non-pain related Na_V subtypes. $Na_V1.7$ and 1.8 are expressed in the skin and thus selective inhibitors of these Na_V subtypes are highly desirable.

TECHNICAL APPROACH

Task 1: Generation of a Virtual Compound Library and High-Throughput Virtual Screening

In order to maximize the likelihood of success for this discovery program, prudent down-selection of candidate molecules is of maximum importance. To accomplish this, we generated a virtual library of candidate compounds that incorporate some of the design elements provided to us by PTC innovations as well as strategic modifications based on both exploration of chemical space and medicinal chemist expertise. A virtual binding model of the Na_V protein structures were developed using our propriety software Rhodium, using several Na_V protein crystal structures and known ligands available in the literature and protein binding database (PBD). Once the virtual model has been made and validated, the virtual library were screened against the Na_V protein structures. Pharmokinetic properties of the virtual library, such as cLogp, cPKa as well as toxicological red flags such as hERG and CYP inhibition were evaluated using Stardrop's automated Derek Nexus algorithm. A composite score for each molecule were made, based on several factors: 1) high predicted binding efficacy for $Na_V1.7$ and $Na_V1.8$ over the other Na_V proteins from the Rhodium docking study; 2) Low chance for metabolic instability, hERG and/or CYP inhibition; and 3) optimum pharmokinetic properties such as a cLogp. Compounds with the

highest score had the highest of desirable activity and the top 15-20 candidates were selected for synthesis.

Task 2: Synthesis of Anesthetic Candidates

A synthetic plan was generated based on the top 15-20 candidates generated in the virtual screen. The expected synthesis of these compounds is depicted in Figure 3 and is anticipated to take ~ 2 steps. The compounds were synthesized, characterized and stored in the freezer until a suitable anesthetic assay to evaluate effectiveness is found, to be determined by PTC innovations.

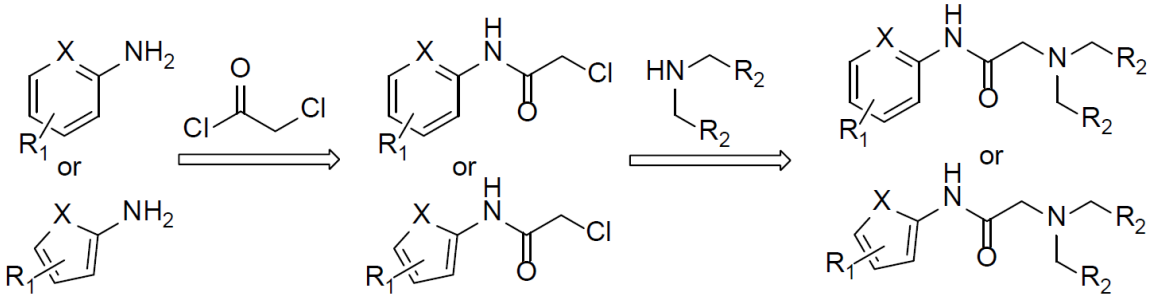


Figure 3. SwRI's Planned Synthesis of Anesthetic Derivatives based on PTC Compounds 5-7

Task 3: Patch Clamp Screening

Patenting virtual compounds are very difficult, even if coupled with synthetic efforts. For a much stronger patent in the area of novel topical anesthetics, the synthetic data needs to be coupled to preliminary bioactivity data. To that end, up to 15 candidate molecules were screened against $Na_V1.5$ and 1.7 using patch clamp electrophysiology. A final report was provided to summarize the results of the screening.

RESULTS

Task 1: Generation of a Virtual Compound Library and High-Throughput Virtual Screening

Constructing a predictive model for identifying potent $Na_V1.7$ antagonists can accelerate the timeline for a hit-to-lead discovery program. To identify a reliable predictive model, seven crystal structures from the Royal Society of Chemistry (RCS) protein database were chosen to generate a docking model (Table 1). Known $Na_V1.7$ inhibitor PF-05089771 and its derivatives were docked to all 7 crystal structures of various $Na_V1.7$ sodium-gated voltage channels. The most reliable crystal structure for predicting potency in the sulfonamide chemical series was 5EK0, a human $Na_V1.7$-VSD4-Na_VAb in complex with GX-936. GX-936 is a sulfonamide that is structurally similar to PF-05089771 (Figure 4).

Table 1. Nav1.7 Sodium-Gated Ion Channels Screened for Docking Model

PDB	Description of Crystal Structure
3RVY	Na_VAb voltage-gated sodium channel mutation I217C
5EK0	$hNa_V1.7$-VSD4-Na_VAb in complex with GX-936
6J8G	Structure of human voltage-gated sodium channel $Na_V1.7$ in complex with auxiliary beta subunits, huwentoxin-IV and saxitoxin (Y1755 up)
6J8H	Structure of human voltage-gated sodium channel $Na_V1.7$ in complex with auxiliary beta subunits, huwentoxin-IV and saxitoxin (Y1755 down)
6N4Q	CryoEM structure of $Na_V1.7$ VSD2 (actived state) in complex with the gating modifier toxin ProTx2
6N4R	CryoEM structure of $Na_V1.7$ VSD2 (deactived state) in complex with the gating modifier toxin ProTx2
6NT4	Cryo-EM structure of a human-cockroach hybrid Na_V channel bound to alpha-scorpion toxin AaH2.

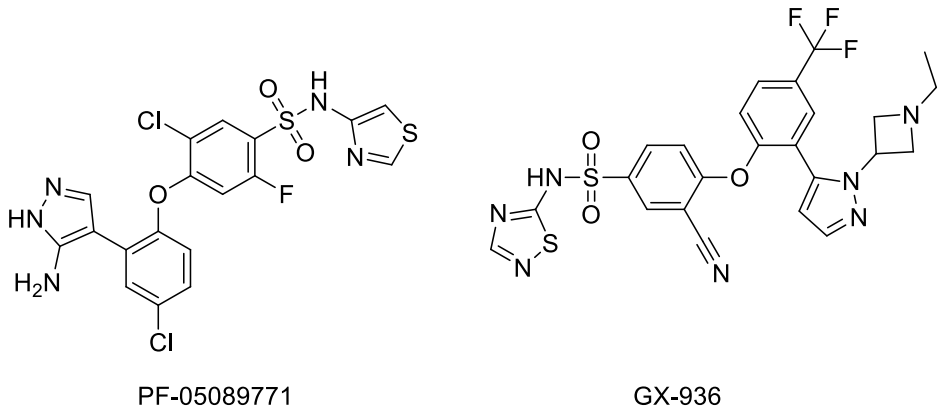


Figure 4. Structures of known Nav1.7 inhibitor PF-05089771 and GX-936 sulfonamide

The aim of the preliminary docking results was to build a correlative dataset for the *in vitro* inhibition of known $Na_V1.7$ sulfonamide antagonists, a variety of non-selective "caine" drugs, known $Na_V1.5$ antagonists, and the PTC analogs provided by the client. The docking results summarized in Figure 5 show the values for CAVOC (cavity filling score) plotted against the population score (statistical probability score). With these two docking score parameters, we determined that higher scores correspond to increased inhibition, and those criteria were set for further virtual screening and development of PTC-3.

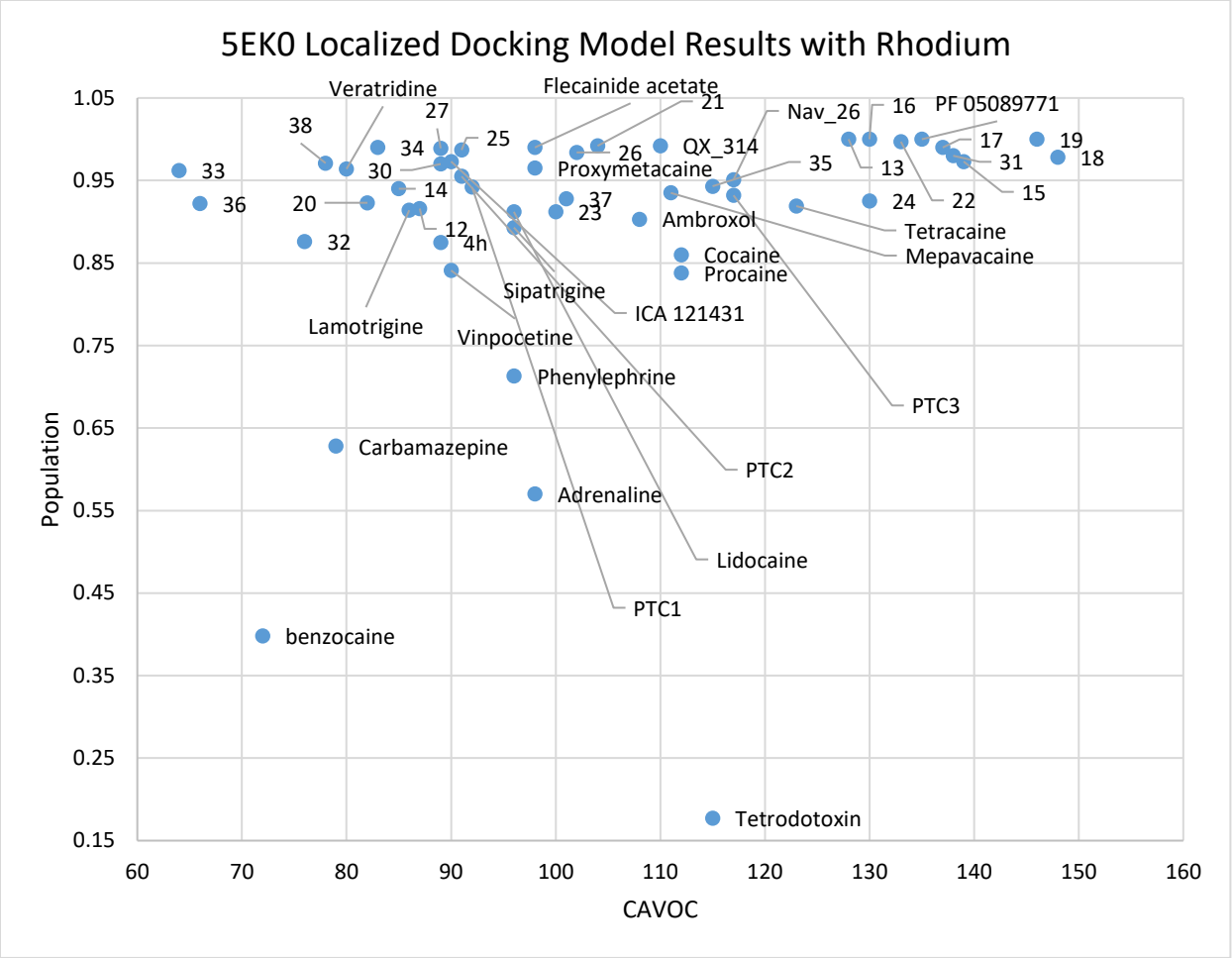


Figure 5. ICA 121431 is a selective inhibitor of Nav1.1 and Nav1.3. GS-458967 (4h) is a selective inhibitor of Nav1.5. PF-05089771 is a selective inhibitor of Nav1.7. Lidocaine is a common topical anesthetic (Nav1.7 inhibitor). Numbered compounds are sulfonamides (Nav1.7 inhibitors). PTC-3 is the compound of most interest.

To develop PTC-3, several generations of molecular scaffolds with structural similarity were explored. Over 19,000 compounds were generated with StarDrop Nova, which produced the following general transformations of PTC-3: functional group modification, linker modification, atom deletion, ring addition, ring deletion, ring modification, and terminal group exchanges. To manage the large library of compounds related to PTC-3, selection criteria were determined based on docking scores and physicochemical properties for topical drug-like small molecules. PF-05089771 is a selective human-Nav1.7 isoform inhibitor (currently in Phase II clinical trials for wisdom tooth removal and primary erythromelalgia) that is designed as an orally available therapeutic. The aim of our virtual screening efforts also focused on compounds that mimic the physicochemical properties of existing commercially-available and FDA-approved local anesthetics. These criteria for desirable compounds trended toward moderate cLogP (2-3), lower topological polar surface areas (< 85 $Å^2$), and molecular weights between 350-450 g/mol. The

general structures for the results of the virtual screen are shown in Figure 96. The summaries of the down-selected compounds based on favorable screening criteria are shown in Figure 7 and Figure 8.

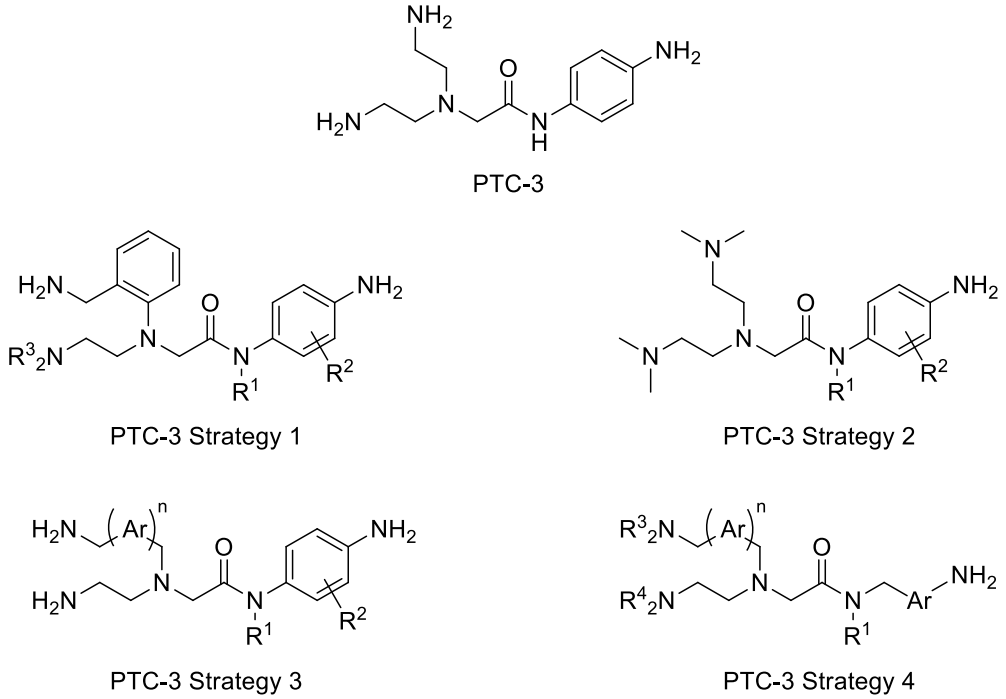


Figure 6. Four different strategies toward drug-likeness were condensed from a screen of 19,000+ compounds from a library generated by StarDrop Nova and characterized by PCP and docking scores. These general structures are predicted to be $Na_V1.7$ inhibitors.

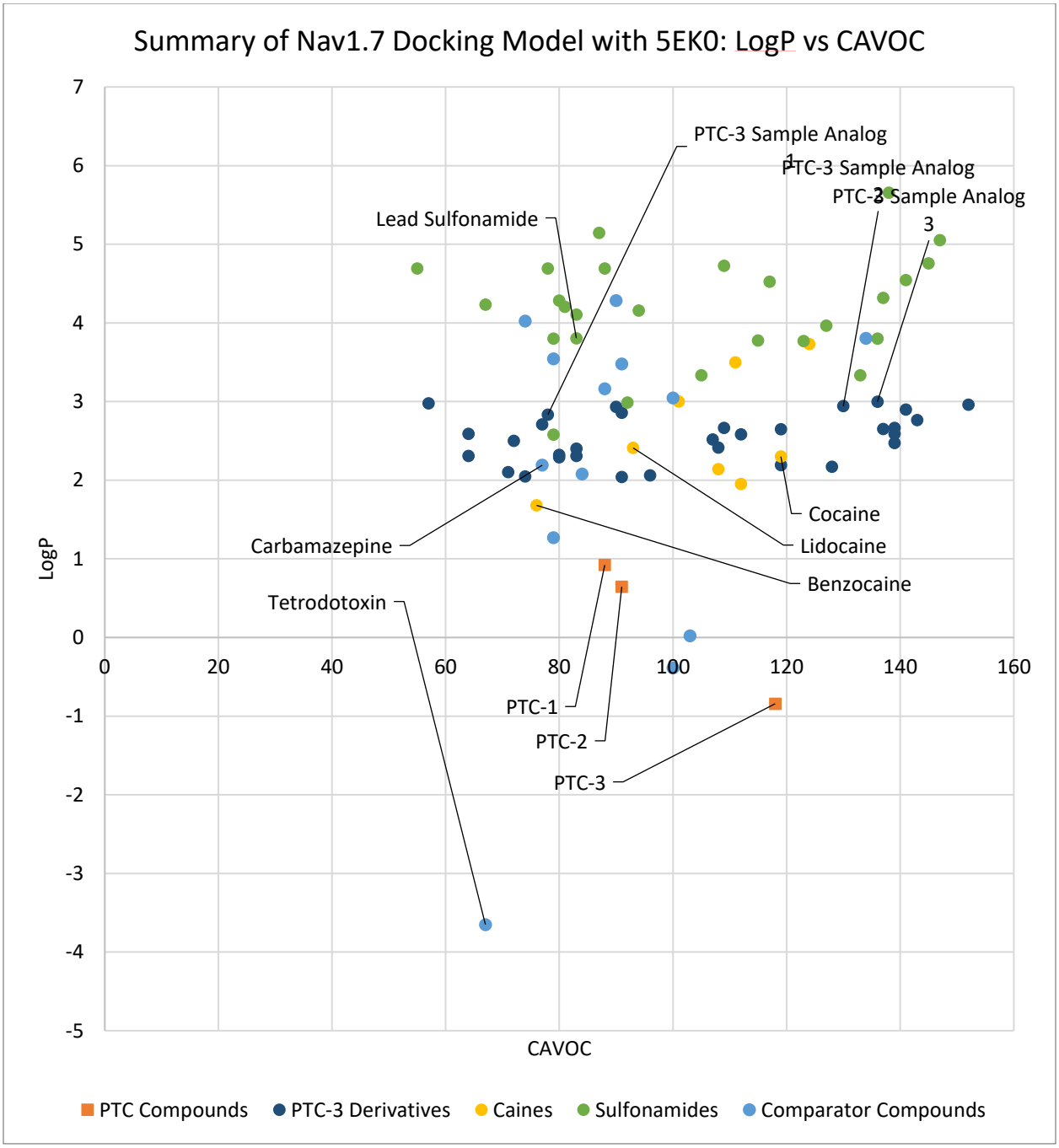


Figure 7. Compounds that do not score high cavity filling scores (CAVOC) should still be investigated if they possess desirable PCP. Improvements on PCP of PTC-3 analogs by creating a focused group of compounds.

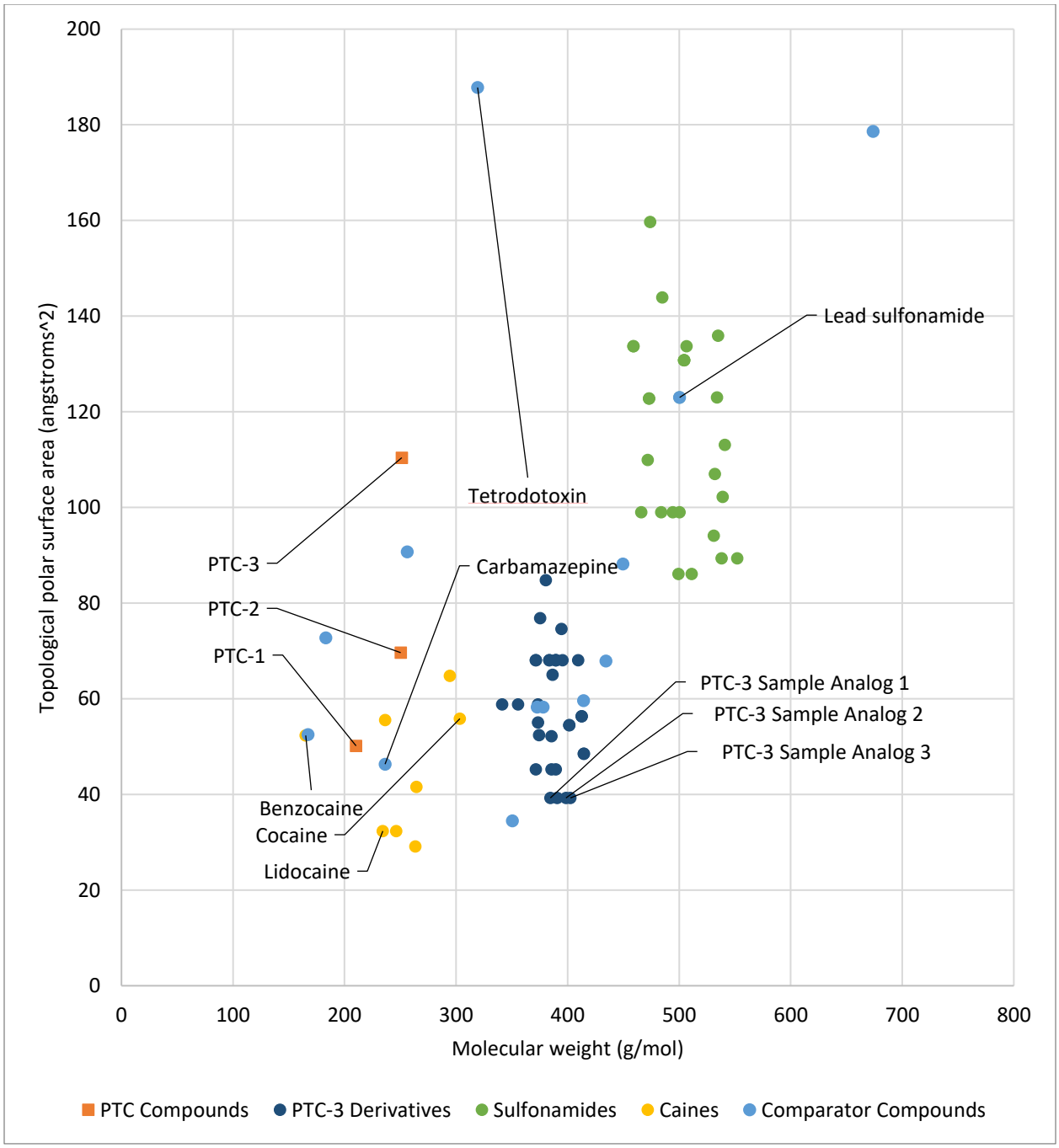


Figure 8. Proposed PTC-3 Derivatives show PCP that more closely resembles currently available topical anesthetics. The proposed analogs are not as polar (TPSA) or as high in molecular weight as the previously reported sulfonamides, which were originally designed for oral bioavailability in mind and are highly potent and selective for $Na_v1.7$ over other sodium voltage channel human isoforms.

This task was completed on May 21, 2020 and 25 candidate molecules were identified (Figure 9). A composite score for each candidate molecule was generated based on the following: 1) high predicted binding efficacy for $Na_V1.7$ and $Na_V1.8$; 2) low chance for metabolic instability, hERG, and/or CYP inhibition; and 3) optimum pharmacokinetic properties, such as cLogP. Using the composite scores, 15 candidate molecules were selected for synthesis in Task 2.

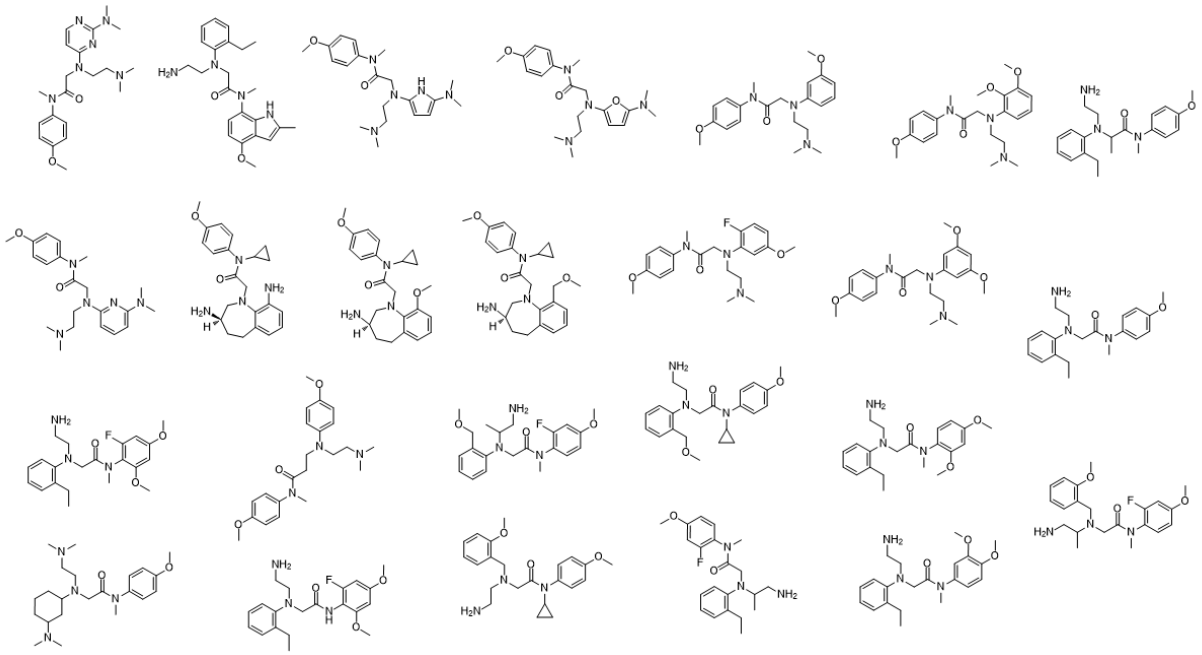


Figure 9. 25 candidate molecules from virtual screening

Task 2: Synthesis of Anesthetic Candidates

With a small virtual library, we next began to strategize which candidates to make and what structure activity relationships to explore. An analysis of the virtual library suggested that there were only two structural motifs represented, which we designated as PTC structural class A and PTC structural class B (Figure 10). The PTC structural class A encompassed a full 88% of the structural diversity of the virtual library and was anticipated to be much easier to synthesize, which would allow for the generation of more analogues to test for activity. Of the PTC structural class A, 68% of them contained a para-methoxy group pendant to the analide moiety. Using this structural feature, we further simplified the candidate structures, which would allow for the structure-activity relationship (SAR) exploration of distal nitrogen and the other phenyl group.

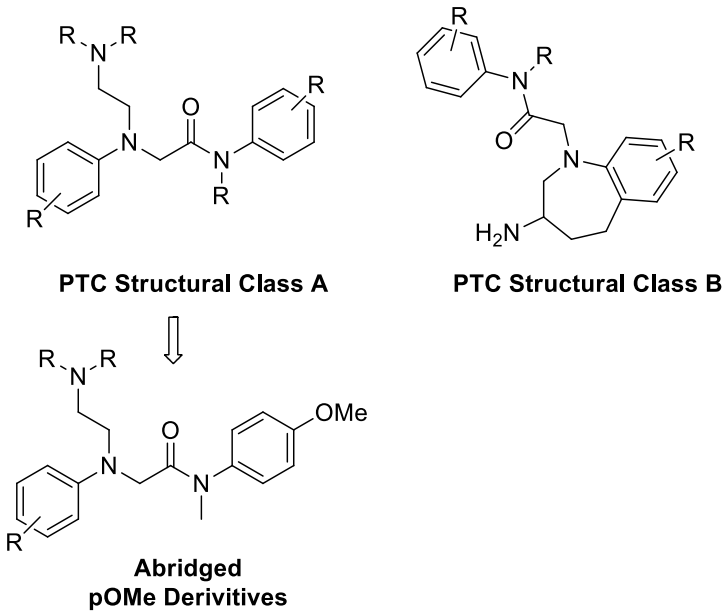


Figure 10. Structural simplification for efficient SAR exploration and general synthesis of the abridged pOME analogues

A synthesis for these analogues was devised at SwRI and executed at the Center for Innovative Drug Discovery (CIDD) (Figure 11). The non-varying fragment **9** was synthesized in bulk by acylation of aniline **8** with bromoacetyl bromide. Various anilines **10** were then alkylated with β-haloamines to form the varying fragment **11**, which were then combined with the non-varying fragment **9** to generate the analogues. A total of 15 analogues were synthesized by CIDD, dissolved in DMSO, and sent to Charles River for analysis via patch clamp assay (Figure 12).

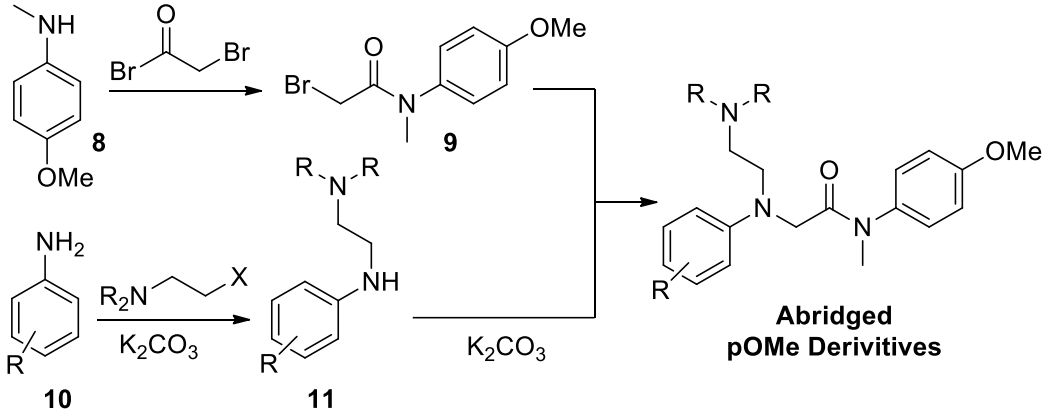


Figure 11. General synthesis of the abridged pOME analogues

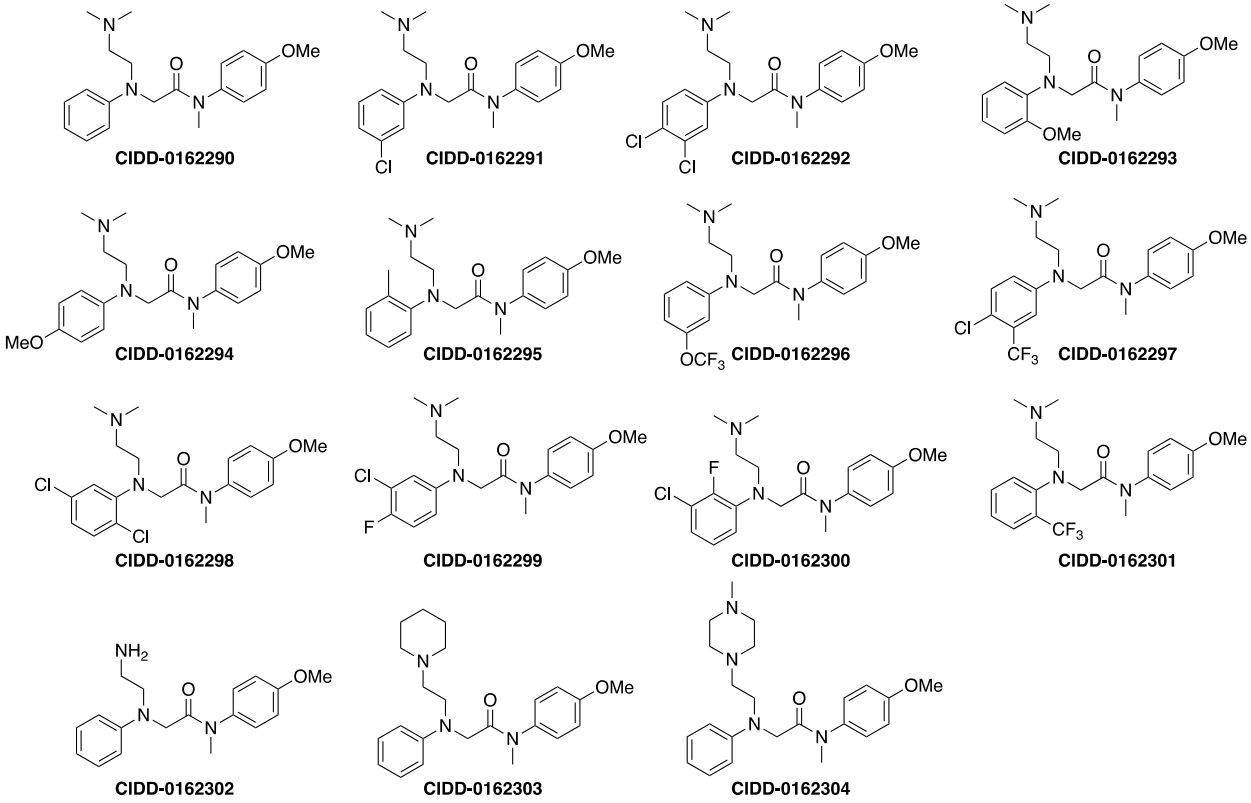


Figure 12. Abridged pOME analogues synthesized by CIDD

Task 3: Patch Clamp Screening

An examination of the *in vitro* effects of the 15 test articles on ion channels $Na_V1.5$ and $Na_V1.7$ was performed by Charles River in Cleveland, OH. The test articles were analyzed at concentrations of 1000, 300, 100, 30, 10, 3, 1, 0.3 µM. Lidocaine was included as a positive control at concentrations of 3000, 1000, 300, 100, 30, 10, 3, and 1 µM. All test and control article solutions contained 0.3% DMSO. The test article solutions were loaded into a 384-well polypropylene compound plate using an automated liquid handling system (Integra Assist Plus, Integra) and then placed in the plate well of SyncroPatch 384PE (SP384PE; Nanion Technologies, Livingston, NJ) immediately before application of the cells.

IC_{50} values of the channel current inhibition for each test article are provided in Table 2 ($Na_V1.5$) and Table 3 ($Na_V1.7$). The derivatives seem to inactivate $Na_V1.5$, but less so than $Na_V1.7$ and no more than lidocaine. These derivatives, however, are significantly less lipophilic than lidocaine and thus would be predicted to penetrate the bloodstream to a much lesser extent. Most of the candidates screened had activity against $Na_V1.7$, with one of them being about 1.6x more potent than lidocaine (CIDD-0162303). Interestingly, unlike lidocaine, these derivatives show inhibitory activity independent of the $Na_V1.7$ activation mode, suggesting that it's binding in an allosteric site.

Table 2. IC_{50} Values for $Na_V1.5$ Ion Channel Inhibition with Test Compounds and Lidocaine

TA #	TA ID	IC_{50}, mM		
		TP1A	TP2A	TP25B
1	CIDD-0162290	>1000	>1000	>1000
2	CIDD-0162291	808.5	740.3	692.5
3	CIDD-0162292	207.7	215.7	193.1
4	CIDD-0162293	>1000	>1000	>1000
5	CIDD-0162294	>1000	>1000	>1000
6	CIDD-0162295	>1000	>1000	>1000
7	CIDD-0162296	254.4	235.9	221.6
8	CIDD-0162297	95.1	91.2	72.7
9	CIDD-0162298	249.4	240.3	204.0
10	CIDD-0162299	515.2	449.0	504.5
11	CIDD-0162300	327.2	307.0	259.2
12	CIDD-0162301	265.3	242.5	154.0
13	CIDD-0162302	124.1	96.8	75.9
14	CIDD-0162303	16.6	13.5	9.5
15	CIDD-0162304	>1000	880.4	670.2
PC	Lidocaine	453.2	15.8	68.7

TP1A - Tonic Block; TP2A - Inactivated State-Dependent Block
TP25B - Use-Dependent Block

Table 3. IC_{50} Values for $Na_V1.7$ Ion Channel Inhibition with Test Compounds and Lidocaine

TA #	TA ID	IC_{50}, mM		
		TP1A	TP2A	TP25B
1	CIDD-0162290	>1000	>1000	>1000
2	CIDD-0162291	561.4	463.1	490.4
3	CIDD-0162292	164.8	162.1	166.5
4	CIDD-0162293	>1000	>1000	>1000
5	CIDD-0162294	>1000	>1000	>1000
6	CIDD-0162295	>1000	>1000	>1000
7	CIDD-0162296	265.4	231.2	210.5
8	CIDD-0162297	74.7	66.2	54.4
9	CIDD-0162298	272.5	228.6	220.5
10	CIDD-0162299	392.6	315.0	334.3
11	CIDD-0162300	546.4	672.8	625.8
12	CIDD-0162301	302.1	275.3	235.0
13	CIDD-0162302	70.2	77.9	55.4
14	CIDD-0162303	15.0	14.5	13.1
15	CIDD-0162304	>1000	>1000	>1000
PC	Lidocaine	407.8	23.7	112.7

TP1A - Tonic Block; TP2A - Inactivated State-Dependent Block
TP25B - Use-Dependent Block

DISCUSSION

Model Validation: Predicting the Structure of Biologically Active Local Anesthetic Candidates

To validate the predictive capabilities of Rhodium, the PTC-3 derivatives were re-docked to the crystal structure that we determined were useful for predicting the potency of selective $Na_V1.7$ inhibitors. Two models based on 5EK0 were constructed: 1) the global model that considers the surface of the entire protein structure including the ion pore channel and the voltage-sensing domain, and 2) the local model that considers the exact location the co-crystallized ligand GX-936 was reported to be the active site of inhibition of $Na_V1.7$. The results in Figure 13 are significant ($p < 0.05$) with a Pearson's correlation coefficient for this sample size. The non-linear activity cliff is demonstrated by localization of binding at the voltage sensing domain IV (VSD4).

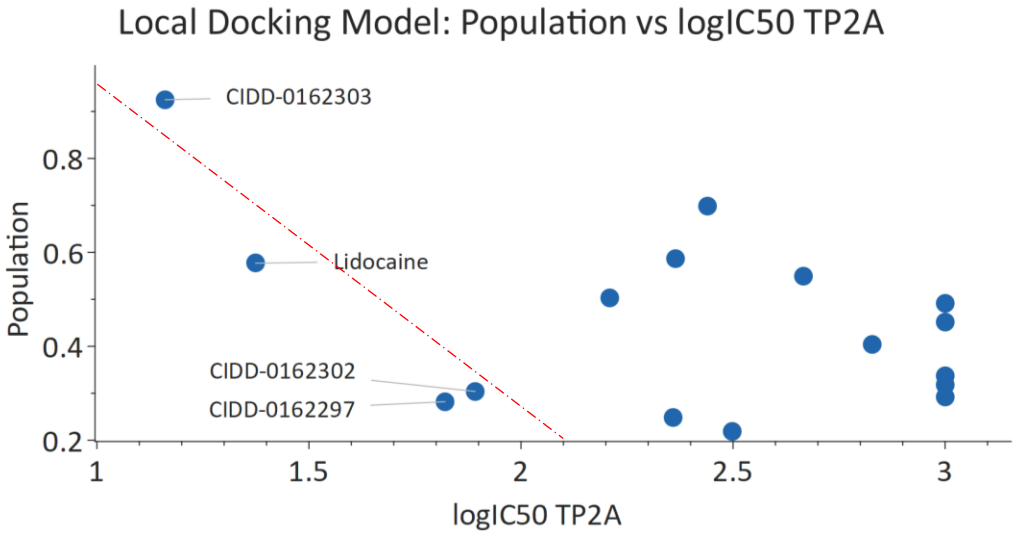


Figure 13. Derivatives of PTC-3 were locally re-docked to 5EK0 to co-crystal site of GX-936 in the VSD4. The population (statistical probability) is plotted against Nav1.7 inhibition ($logIC_{50}$) of PTC-3 derivatives for test protocol TP2A. The dotted red line is a visual guide for the eye.

The localized docking was performed with a single conformer of each test article (abridged PTC-3 derivatives) where each conformer represents the lowest-energy conformation calculated with Balloon (conformer generator program). It is reasonable that the lowest energy conformer is the most reliable model of a bioactive conformation *in vitro* compared to other conformers of higher energy. In Figure 13, the population is a statistical probability score ranging from 0→1, whose output is the sum of a search optimization algorithm. For our model, the result of the optimization algorithm is obtained for a localized grid space, i.e. desired binding site, and the population score is plotted against *in vitro* $Na_V1.7$ inhibition expressed in $logIC_{50}$ of the micromolar (μM) inhibition (IC_{50}) values.

For a meaningful predictive model at this stage, R^2 values can range from 0.2–0.6. Values of R^2 greater than 0.8 are the upper limit for assessing quantitative structure-activity relationships (QSAR) models and values less than 0.2 are increasingly insignificant. It is important to note that outliers should not be dismissed on the basis of not being significant. The plots in Figure 13 assume that the mechanism of action (MOA) is within the voltage-sensing domain IV (VSD4), though further analysis suggests the possibility of competitive inhibition at an allosteric site. This would result in the activity cliffs observed for the abridged chemical series of PTC-3. The activity cliff appears to be connected to pose-localization score (i.e. the population score).

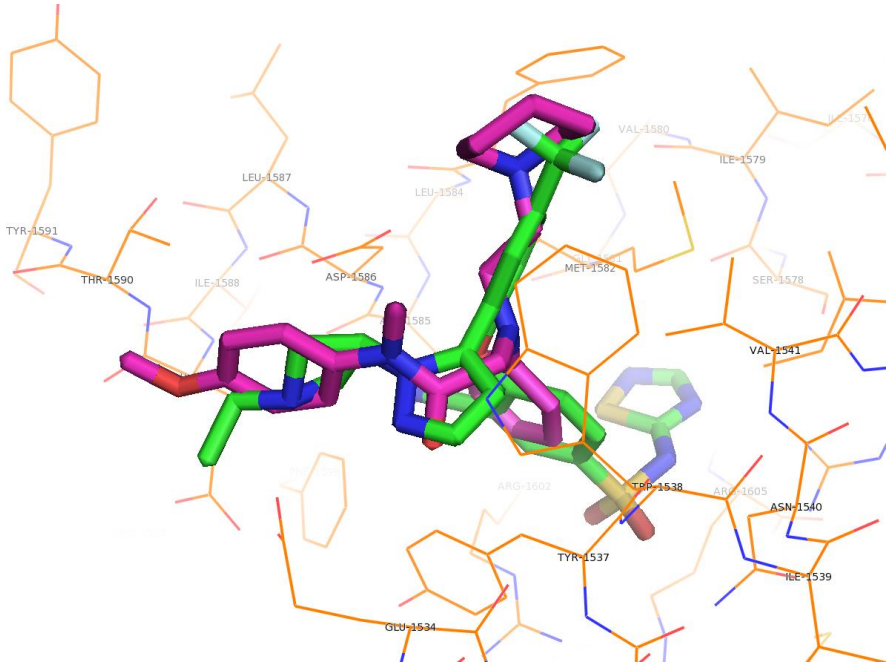


Figure 14. Overlay of CIDD-0162303 (green) and PF-05196233 (magenta; GX-936) from a local docking model with 5EK0 (orange).

Concomitantly, our drug design strategy and synthetic chemistry efforts produced an abridged compound that mimics the pose of PF-05196233 at the VSD4 binding site, shown in Figure 14. Through the lens of the structure activity relationship (SAR), this design strategy provides a template for further drug-like development of local anesthetics that inhibit $Na_V1.7$ at the desired binding site or a novel active site.

Development for drug-like compounds with oral bioavailability and oral administration is well understood. Lipophilicity (logP) and topological polar surface area (TPSA) are physicochemical properties (PCP) that guide the development process for discovering drug-like compounds. To demonstrate the chemical space of the abridged chemical series, Figure 15 shows two PCP parameters plotted against the *in vitro* inhibition of $Na_V1.7$ from patch clamp assay data. Commercially-available and FDA-approved topical local anesthetics that inhibit $Na_V1.7$ were studied. We hypothesized that by mimicking the PCP's of the known $Na_V1.7$ inhibitors, we can

discover a biologically-active topical local anesthetic that is a subtype selective antagonist of $Na_V1.7$ as a candidate for further development.

In Figure 15, the regions shaded in blue are the targeted PCP-space that are the most similar to known $Na_V1.7$ inhibitors. In Figure 15A, CIDD-0162303 and CIDD-0162302 do not have the desired lipophilicity. The SAR and QSAR of CIDD-0162303 and CIDD-0162302 warrant further investigation for tuning the lipophilicity of the aliphatic amine substituent. In contrast, in Figure 15B, the TPSA for most of the abridged compounds is tolerated and falls within the desired TPSA. The aniline and methoxyphenyl-*N*-acetamide moieties also serve as valuable chemical space left to explore in tuning PCP and biological activities.

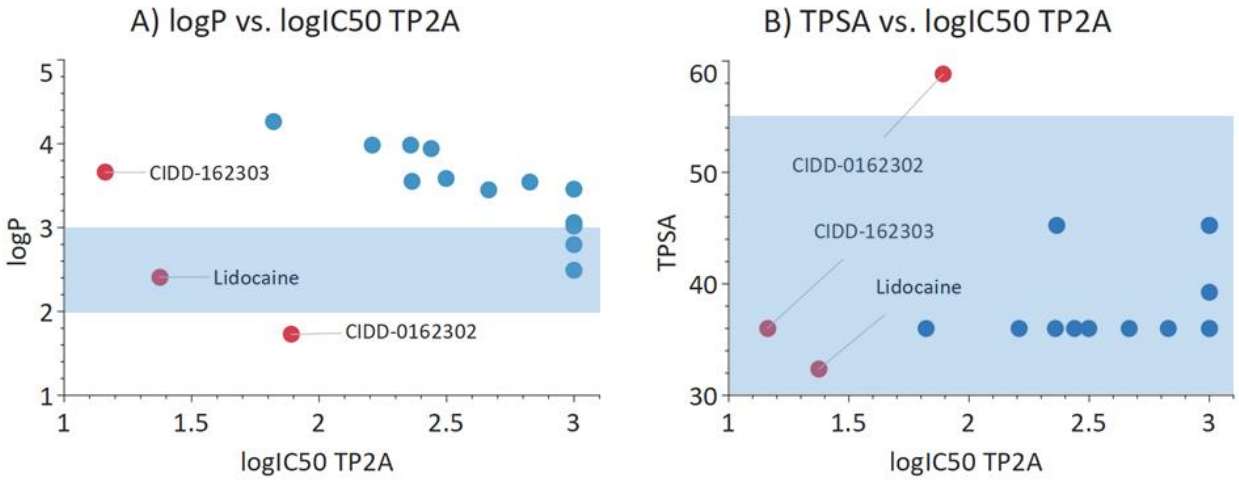


Figure 15. A) LogP vs. logIC50 of test protocol 2A (TP2A); B) TPSA vs. logIC50 of test protocol 2A (TP2A). The shaded blue regions are the desirable PCP ranges of our drug development program.

Structure-Activity Relationship (SAR) Analysis

In addition to an analysis of the virtual model, we also analyzed the compounds using a more traditional medicinal chemistry approach, namely the analysis of a homologous series (Figure 16). Limiting the modifications to only one portion of the molecule not only made the synthesis simpler, but also ensured that a series of compounds were generated which differed by only a single functional group. Looking at the collection of molecules, we can begin to see trends as to what structural modifications enhance or diminish binding, which can add an additional layer of design strategy for the elaboration of a lead compound. The most basic generalized template of the CIDD compounds is CIDD 0162290, which proved to be inactive. Compared to CIDD 0162290, CIDD 0162303 and CIDD 0162302 are much more active, suggesting that activity is very sensitive to the substituent off of the aliphatic nitrogen. Counterintuitively, however, removing methyl groups or adding alkyl bulk increase the activity, something that is thus far unexplained by the virtual model. Addition of an electron withdrawing group to the phenyl ring adds potency (CIDD

0162291) and additional withdrawing groups further add potency as seen with CIDD 0162292 and CIDD 0162297 vs. CIDD 0162290. Compared to the N-Alkyl effect, which is idiosyncratic, this effect seems general and additive.

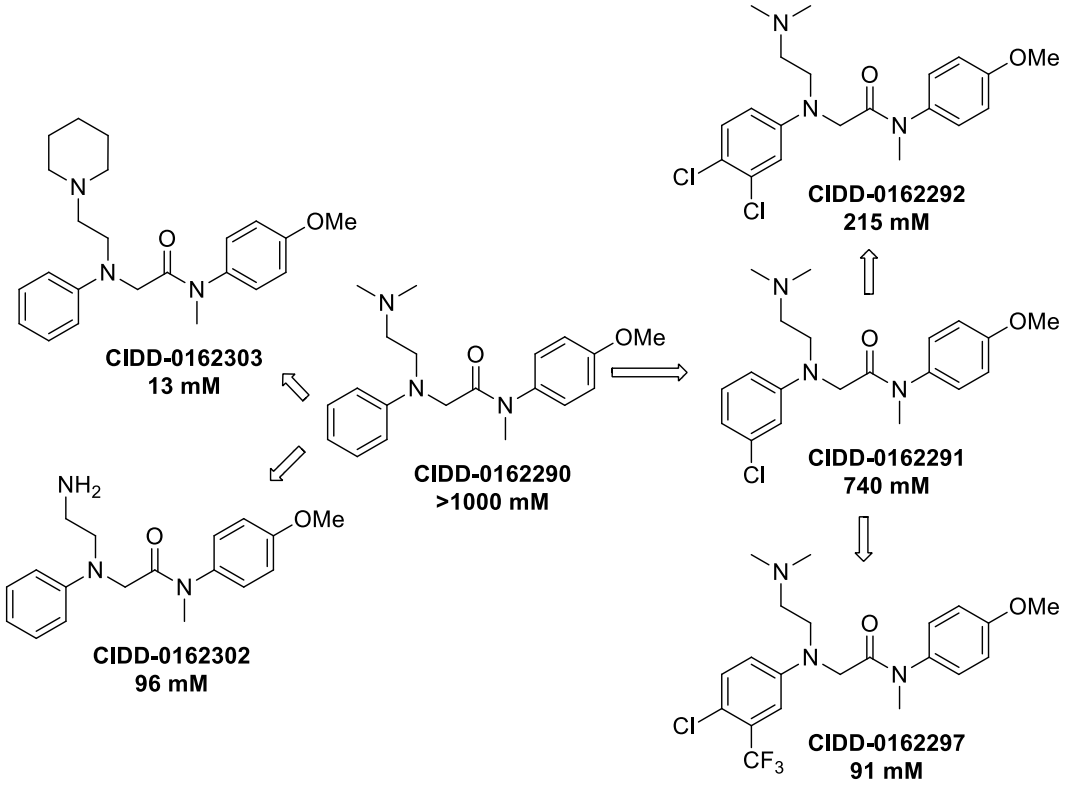


Figure 16. SAR analysis via homologous series.

In addition to potency in binding to $Na_V1.7$, one of the other goals was improving on the selectivity of binding with respect to $Na_V1.5$, which is responsible for cardiotoxicity. To gain insight into the selectivity, the activity of the CIDD analogues and lidocaine toward $Na_V1.7$ was plotted against the activity to $Na_V1.5$ for the Tonic Block (TP1A, closed inactive), Inactivated State-Dependent Block (TP2A, open active) and the Use-Dependent Block (TP25B) (Figure 17). Lidocaine is more selective for $Na_V1.5$ under TP2A and TP25B, but shows higher affinity for $Na_V1.7$ under TP1A protocols, which is consistent with its reports of cardiotoxicity at high levels through this interaction. Two of the top compounds, CIDD 0162297 and CIDD 0162302, appear to be more selective for $Na_V1.7$ vs $Na_V1.5$ across all activation modes. The most potent compound, CIDD 0162303, shows a higher affinity for $Na_V1.5$ for TP2A and TP25B, but a preference for $Na_V1.7$ under the TP1A protocol.

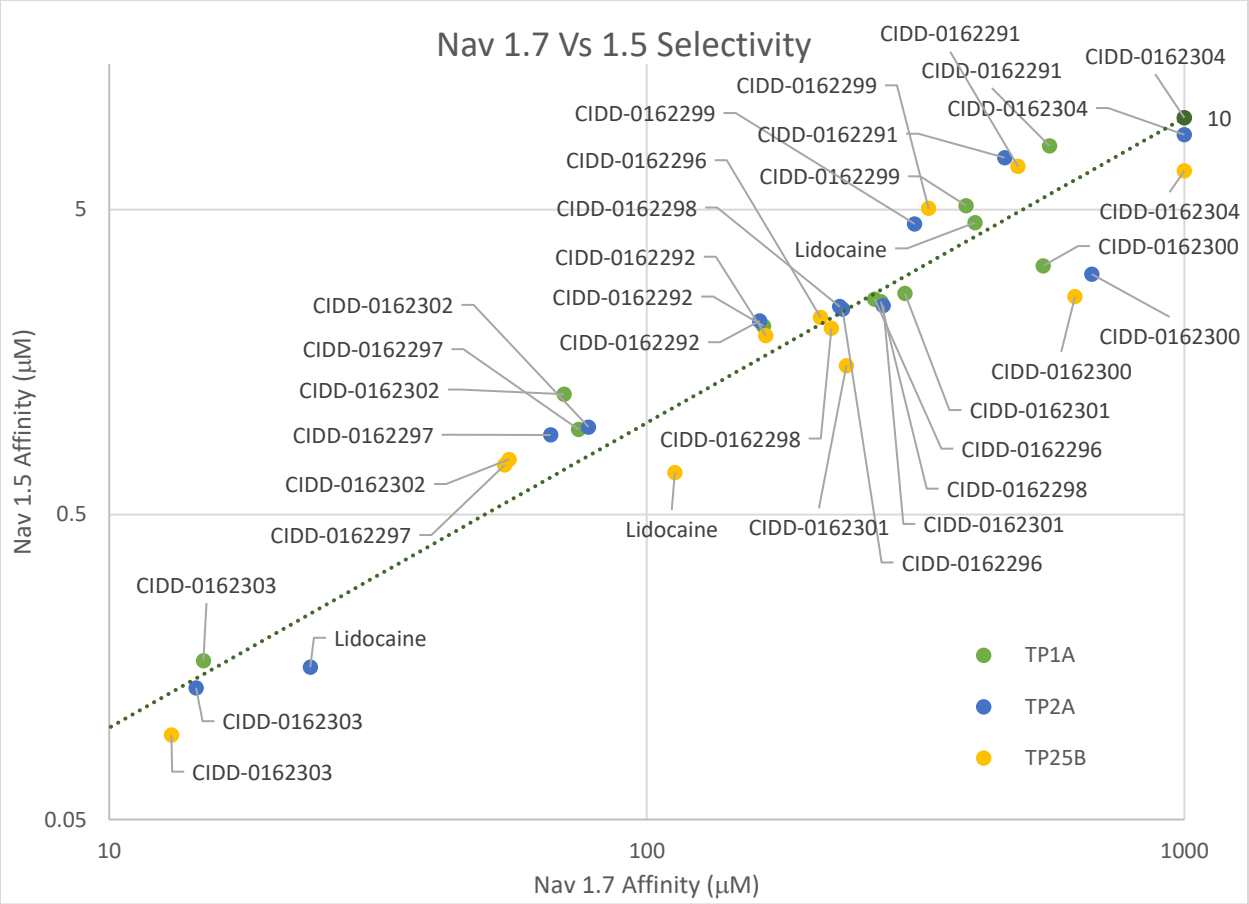


Figure 17. Plot of Nav1.7 activity vs Nav1.5 activity. The dotted line denotes equal affinity to both Nav1.7 and Nav1.5. Compounds Under the line are more selective for Nav1.5 and compounds over the line are more selective for Nav1.7.

SUMMARY AND FUTURE DIRECTIONS

A potent and selective sodium channel inhibitor has been discovered by virtual screening of over 19,000 compounds using the Rhodium docking model software and data extracted from the literature. Our drug development platform was guided by physicochemical properties of FDA-approved compounds for topical local anesthetics known to target Nav1.7. Using this virtual model, SwRI was able to design two novel scaffolds (PTC structural class A and PTC structural class B) *in silico*. PTC structural class A was chosen to synthesize an abridged series to validate both the *in silico* design principles and generate preliminary data to confirm activity. This 15-member library was screened against Nav1.7 and Nav1.5 for activity with lidocaine as a reference compound. All of the compounds tested indicated binding independent of activation mode, suggesting that they were binding at an allosteric site, which would be expected since the voltage sensing domain was used as the design constraint. Additionally, most of the compounds tested were more selective for Nav1.7 vs. Nav1.5. Two of the more potent compounds (CIDD 0162297 and CIDD 0162302) had activities of 66 and 78 µM (TP2A) against Nav1.7 respectively

and were also more selective, but are less potent than lidocaine (24 μM) (Figure 18). The most potent compound, CIDD 0162303, had an activity of 14 μM, making it 1.7X more potent than lidocaine. This derivative's selectivity, however, was higher for $Na_V1.5$, though to a lesser extent than lidocaine. The abridged series also gave some preliminary insight as to the structure activity relationship. Electron withdrawing groups provided an enhancement of activity while maintaining or even enhancing selectivity. This effect seemed to be generalizable and additive. The group that seemed to have the greatest impact to selectivity and potency, however, is the alkylgroups on the nitrogen atom. Yet, caution must be applied since both the addition and subtraction of alkyl groups at this position enhanced potency and greatly affected selectivity in ways that cannot be explained at this point. Taken together, two compounds emerge as lead candidates for PTC innovations, each with their own pros and cons: CIDD 0162302 and CIDD 0162303. CIDD 0162302 is less potent than lidocaine and lower than the desired clogP range (2-3) for topical anesthetics, indicating that it may have less penetration. However, it is much more selective for $Na_V1.7$ and is well within the TPSA range. CIDD 0162303 is more potent than lidocaine and above the desired clogP range (2-3) for topical anesthetics, indicating it would have more penetration. This compounds, however, shows a higher selectivity for $Na_V1.5$, though less so than lidocaine.

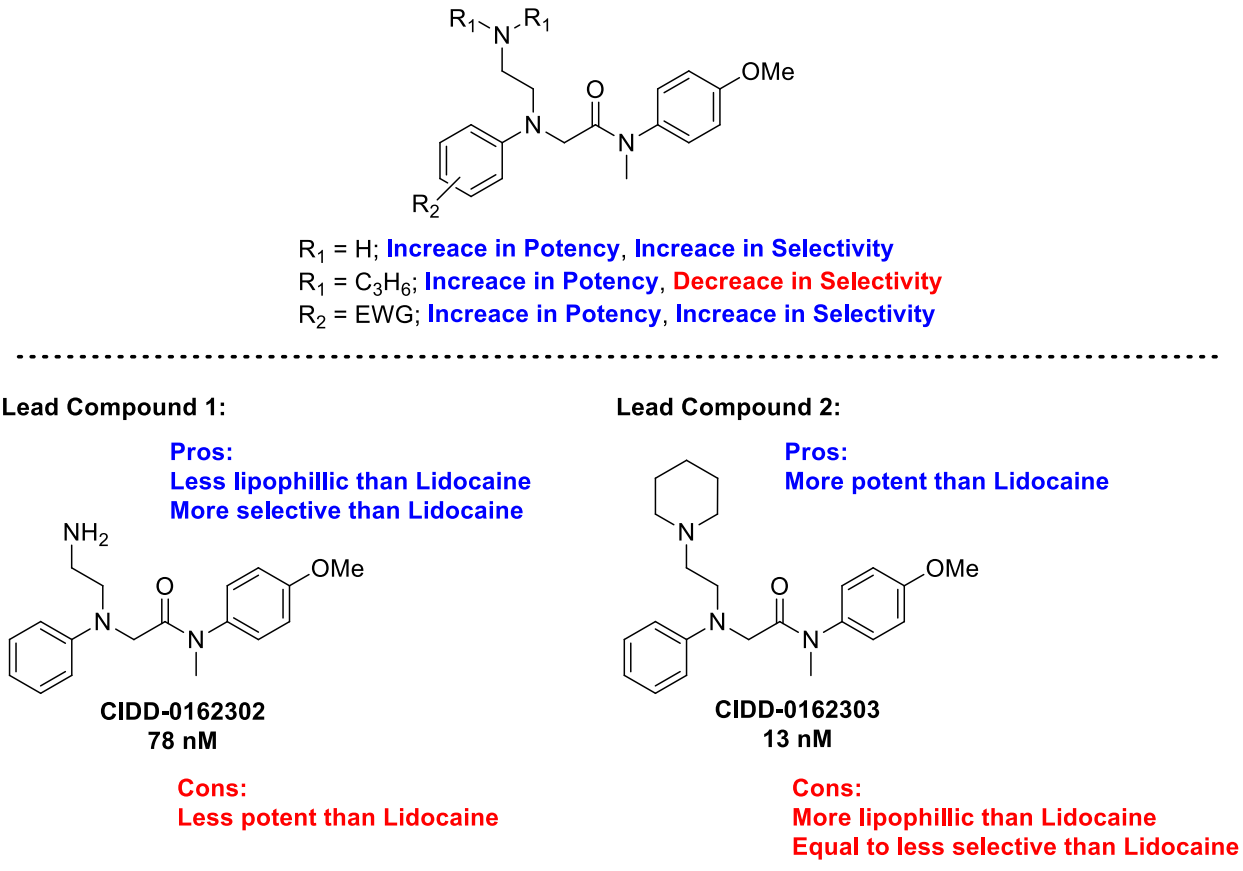


Figure 18. Summary of CIDD SAR and pros and cons of lead candidate: CIDD-0162302 and CIDD-0162303.

Though SwRI was able to provide PTC Innovations with novel promising topical anesthetic compounds with potencies already comparable to the current standard of care, lidocaine, this is still a relatively early point in the drug development process. The *in vitro* assay suggests that most compounds bind $Na_V1.7$ independent of conformation, but whether this actually translates to the desired phenotypic response of anesthesia is unknown. Additionally, while $Na_V1.5$ was used as an off target in the screen, the effect of these compounds on other sodium channels or other off-target effects has not been evaluated and the anesthetic effect of a likely allosteric modulator is difficult to predict. As such, SwRI stresses that CIDD-0162302 and CIDD-0162303 are lead compounds: promising compounds that may serve as starting points of optimization for the development of a new drug candidate. SwRI recommends that the first step in advancing these compounds would be to synthesize enough of CIDD-0162303 to test in an animal model to validate anesthetic activity. Once this has been confirmed, there is ample room for improvement that can be explored for these compounds: only half of the molecule was explored with respect to structure-activity relationships. This unexplored portion of the molecule will be vital to further hone activity, toxicity, and pharmokinetics to produce the desired drug candidate.

REFERENCES

1 a) de Lera Ruiz, M.; Kraus, R. L. J. Med. Chem. 2015, 58, 7093-7118; b) Swain, N. A.; Batchelor, D.; Beaudoin, S.; Bechle, B. M.; Bradley, P. A.; Brown, A. D.; Brown, B.; Butcher, K. J.; Butt, R. P.; Chapman, M. L.; Denton, S.; Ellis, D.; Galan, S. R. G.; Gaulier, S. M.; Greener, B. S.; de Groot, M. J.; Glossop, M. S.; Gurrell, I. K.; Hannam, J.; Johnson, M. S.; Lin, Z.; Markworth, C. J.; Marron, B. E.; Millan, D. S.; Nakagawa, S.; Pike, A.; Printzenhoff, D.; Rawson, D. J.; Ransley, S. J.; Reister, S. M.; Sasaki, K.; Storer, R. I.; Stupple, P. A.; West, C. W. J. Med. Chem. 2017, 60, 7029-7042.

ATTACHMENTS

CENTER FOR INNOVATIVE DRUG DISCOVERY

utcidd.org

To:

Charles River Laboratories

Attn: Denise Cinalli

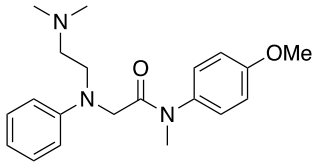
14656 Neo Parkway Cleveland,

OH 44128 USA

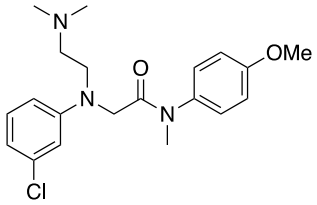
Tel: (216) 584-0501

Compound Information:

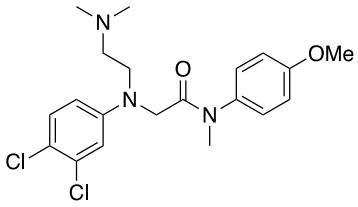
CIDD-0162290
Lot#: SM2021-127-61
FW: 341.46
Amount: 6.2mg
Structure:



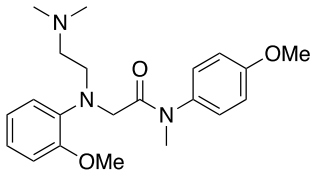
CIDD-0162291
Lot#: SM2021-127-55
FW: 375.90
Amount: 8.8mg
Structure:



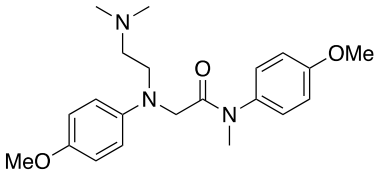
CIDD-0162292
Lot#: SM2021-127-73
FW: 410.34
Amount: 6.7mg
Structure:



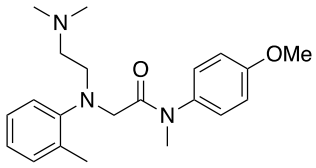
CIDD-0162293
Lot#: SM2021-127-74
FW: 371.48
Amount: 6.6mg
Structure:



CIDD-0162294
Lot#: SM2021-127-91
FW: 371.48
Amount: 8.1mg
Structure:

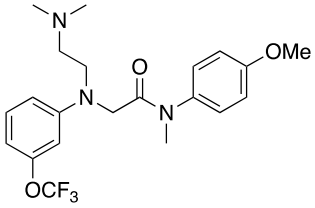


CIDD-0162295
Lot#: SM2021-127-75
FW: 355.48
Amount: 7.0mg
Structure:

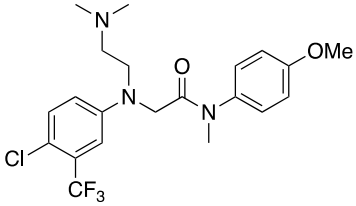


CENTER FOR INNOVATIVE DRUG DISCOVERY
utcidd.org

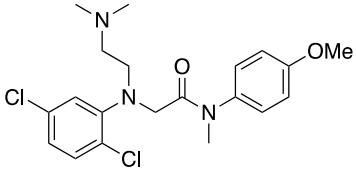
CIDD-0162296
Lot#: SM2021-127-83
FW: 425.45
Amount: 6.5mg
Structure:



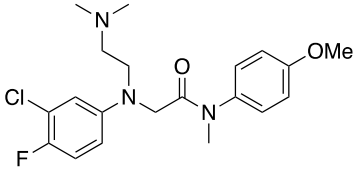
CIDD-0162297
Lot#: SM2021-127-63
FW: 443.90
Amount: 6.8mg
Structure:



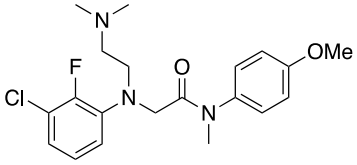
CIDD-0162298
Lot#: SM2021-127-76
FW: 410.34
Amount: 8.5mg
Structure:



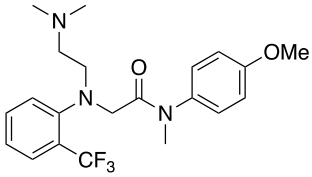
CIDD-0162299
Lot#: SM2021-127-65
FW: 393.89
Amount: 6.8mg
Structure:



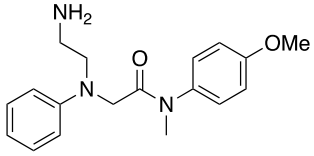
CIDD-0162300
Lot#: SM2021-127-77
FW: 393.89
Amount: 6.4mg
Structure:



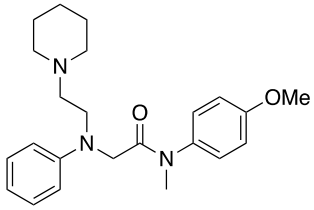
CIDD-0162301
Lot#: SM2021-127-78
FW: 409.45
Amount: 4.1mg
Structure:



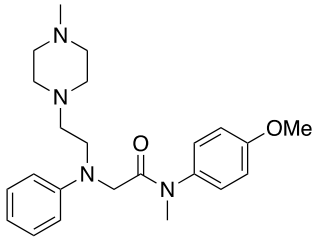
CIDD-0162302
Lot#: SM2021-127-86
FW: 313.40
Amount: 8.8mg
Structure:



CIDD-0162303
Lot#: SM2021-127-84
FW: 381.52
Amount: 4.9mg
Structure:



CIDD-0162304
Lot#: SM2021-127-87
FW: 396.54
Amount: 8.2mg
Structure:



WE MAKE LIVES BETTER
UT HEALTH SCIENCE CENTER SAN ANTONIO

High-Throughput Screening Facility
7703 Floyd Curl Drive • San Antonio, TX 78229
T 210.567.3796 • F 210.567.6595

UTSA The University of Texas at San Antonio™

Medicinal Chemistry and Synthesis Core Facility
One UTSA Circle • San Antonio, TX 78249
T 210.458.8676 • F 210.458.7428

SM2020-117-017

Sample Name:
SM2021-127-61
Data Collected on:
400MR.McHardy.Lab-vnmrs400
Archive directory:

Sample directory:

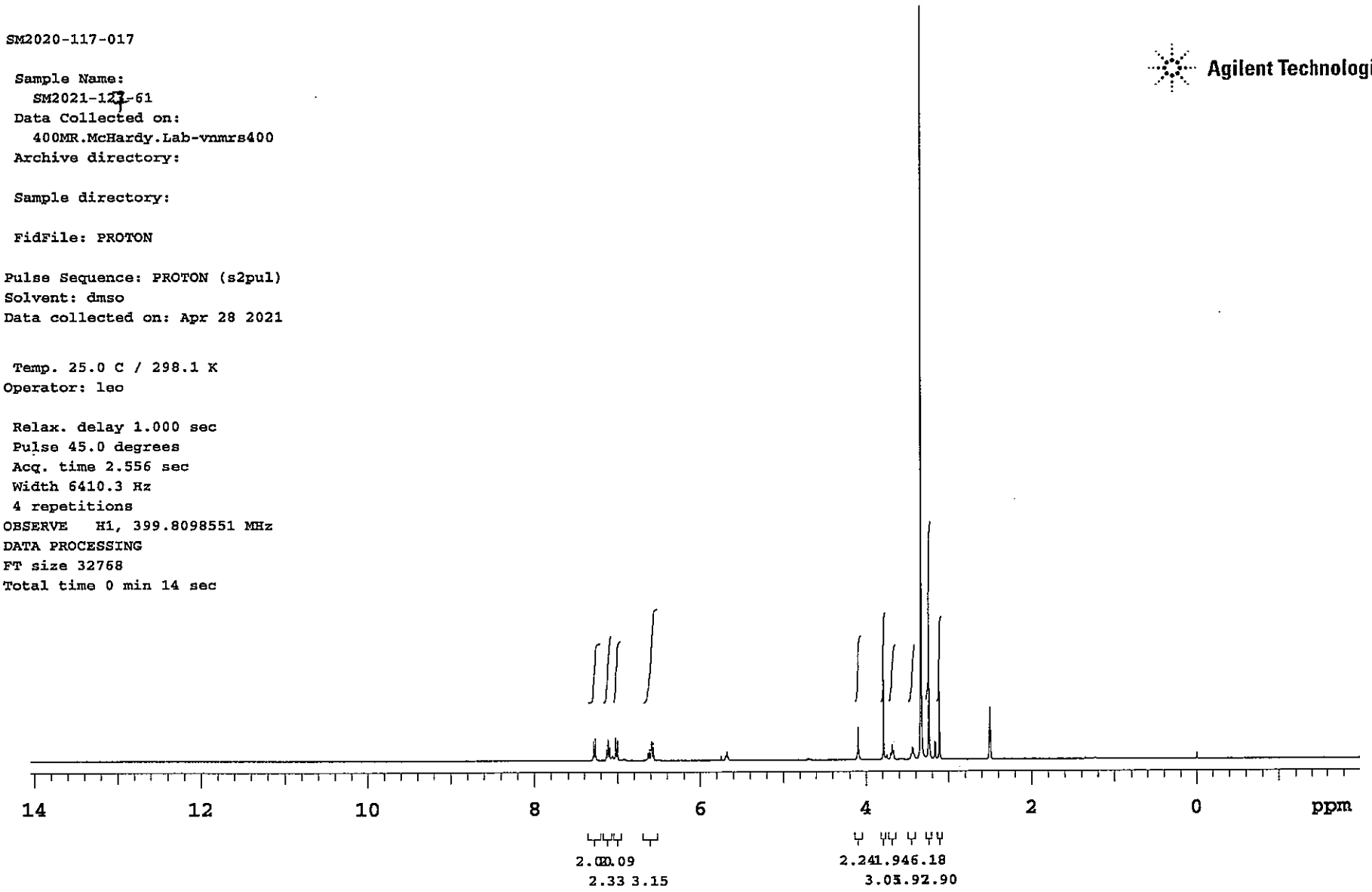
FidFile: PROTON

Pulse Sequence: PROTON (s2pul)
Solvent: dmso
Data collected on: Apr 28 2021

Temp. 25.0 C / 298.1 K
Operator: leo

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 2.556 sec
Width 6410.3 Hz
4 repetitions
OBSERVE H1, 399.8098551 MHz
DATA PROCESSING
FT size 32768
Total time 0 min 14 sec

Agilent Technologies

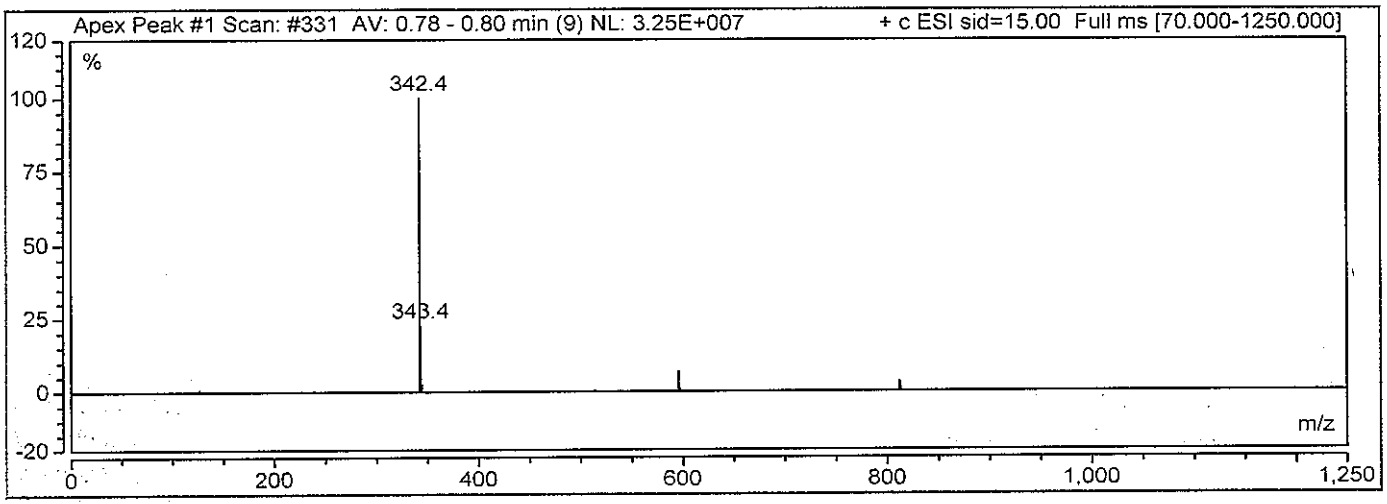


Peak Analysis

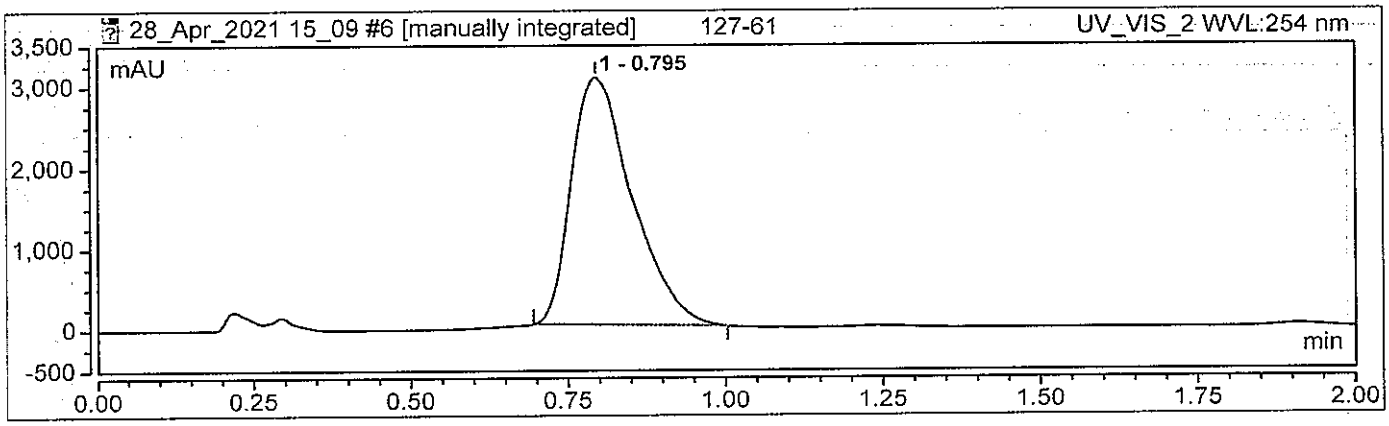
Injection Details

Injection Name:	**127-61**	*Run Time (min):*	**2.00**
Vial Number:	**R:A6**	*Injection Volume:*	**5.00**
Injection Type:	**Unknown**		
Calibration Level:			
Instrument Method:	**1.8uM_column_Tidwell_1.5_min_run4_agilent_zorbax1.8uM, 2.1x50**		
Processing Method:	**McHardy Mass Check**	*Dilution Factor:*	**1.0000**
Injection Date/Time:	**28/Apr/21 15:23**	*Sample Weight:*	**1.0000**

Mass Spectrum



Chromatogram



Table

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1		0.795	342.499	3051.994	100.00	100.00
Total:			**342.499**	**3051.994**	**100.00**	**100.00**

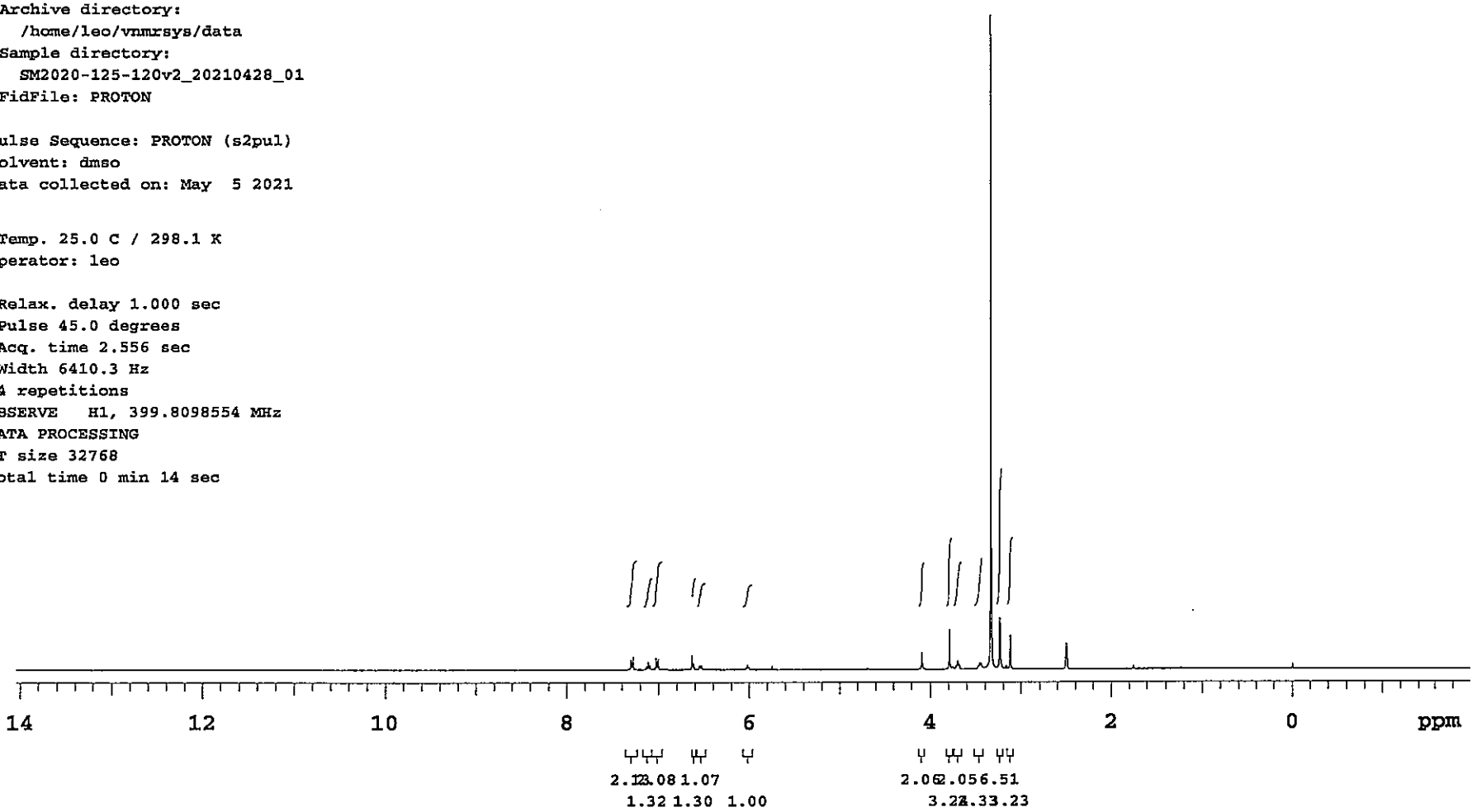
Agilent Technologies

Sample Name:
SM2021-127-55
Data Collected on:
400MR.McHardy.Lab-vnmrs400
Archive directory:
/home/leo/vnmrsys/data
Sample directory:
SM2020-125-120v2_20210428_01
FidFile: PROTON

Pulse Sequence: PROTON (s2pul)
Solvent: dmso
Data collected on: May 5 2021

Temp. 25.0 C / 298.1 K
Operator: leo

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 2.556 sec
Width 6410.3 Hz
4 repetitions
OBSERVE H1, 399.8098554 MHz
DATA PROCESSING
FT size 32768
Total time 0 min 14 sec

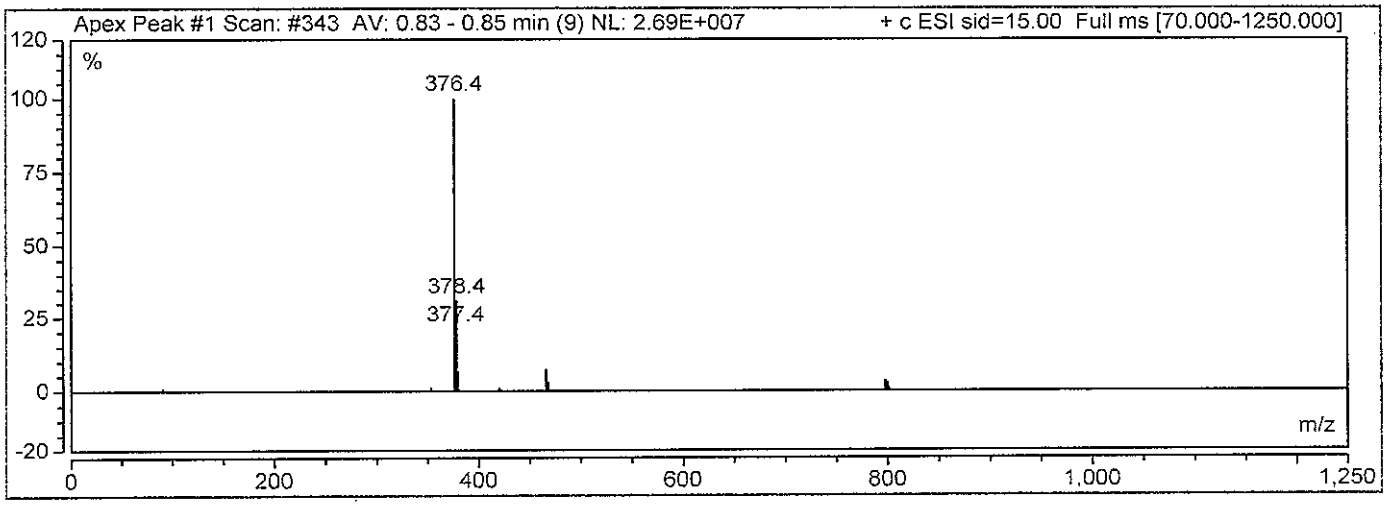


Peak Analysis

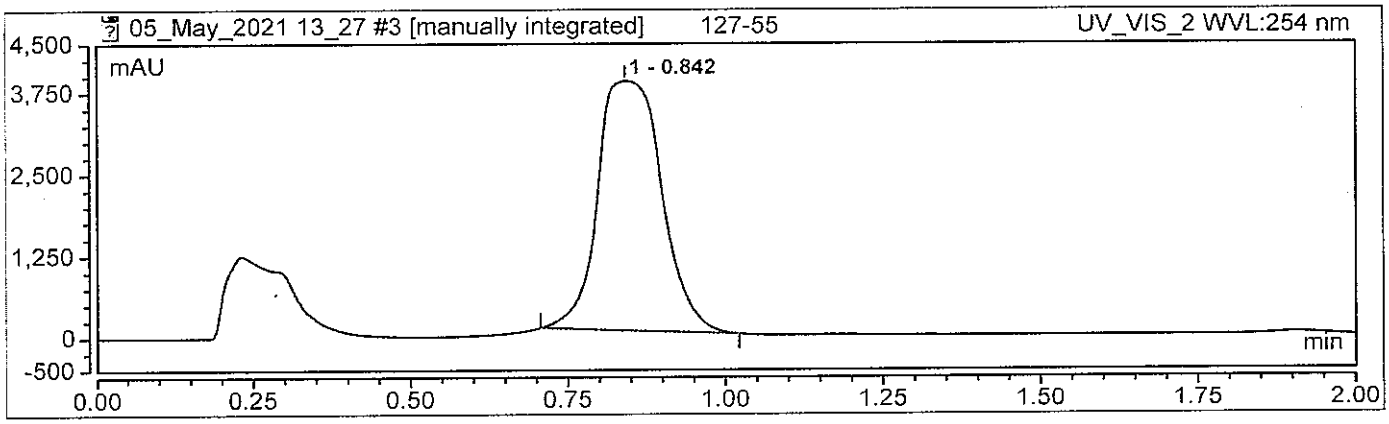
Injection Details

Injection Name:	**127-55**	*Run Time (min):*	**2.00**
Vial Number:	**R:A3**	*Injection Volume:*	**10.00**
Injection Type:	**Unknown**		
Calibration Level:			
Instrument Method:	**1.8uM_column_Tidwell_1.5_min_run4_agilent_zorbax1.8uM, 2.1x50**		
Processing Method:	**McHardy Mass Check**	*Dilution Factor:*	**1.0000**
Injection Date/Time:	**05/May/21 13:33**	*Sample Weight:*	**1.0000**

Mass Spectrum



Chromatogram



Table

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1		0.842	450.896	3821.372	100.00	100.00
Total:			**450.896**	**3821.372**	**100.00**	**100.00**

SM2020-117-017

Sample Name:
SM2021-127-73
Data Collected on:
400MR.McHardy.Lab-vnmrs400
Archive directory:

Sample directory:

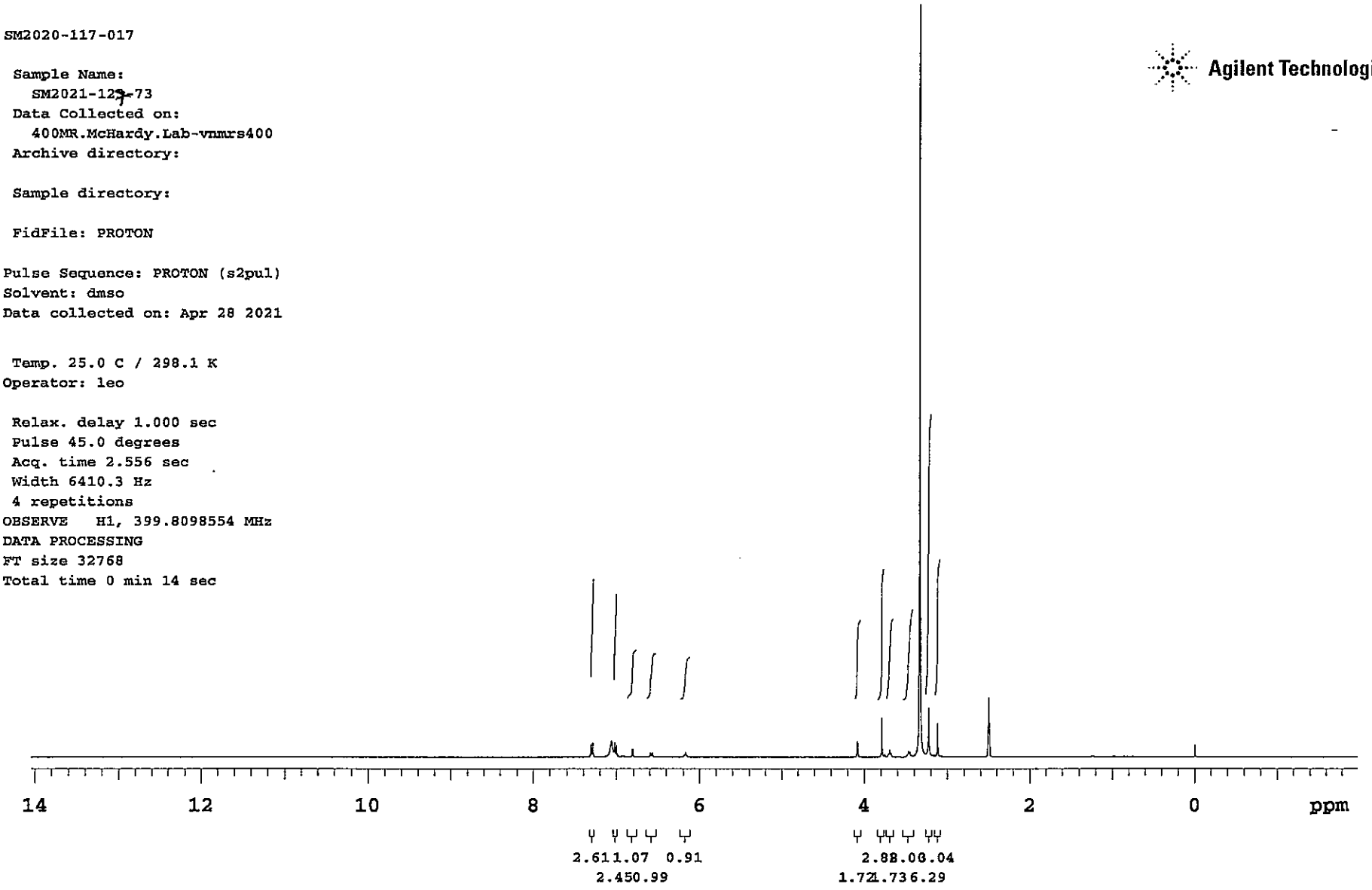
FidFile: PROTON

Pulse Sequence: PROTON (s2pul)
Solvent: dmso
Data collected on: Apr 28 2021

Temp. 25.0 C / 298.1 K
Operator: leo

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 2.556 sec
Width 6410.3 Hz
4 repetitions
OBSERVE H1, 399.8098554 MHz
DATA PROCESSING
FT size 32768
Total time 0 min 14 sec

Agilent Technologies

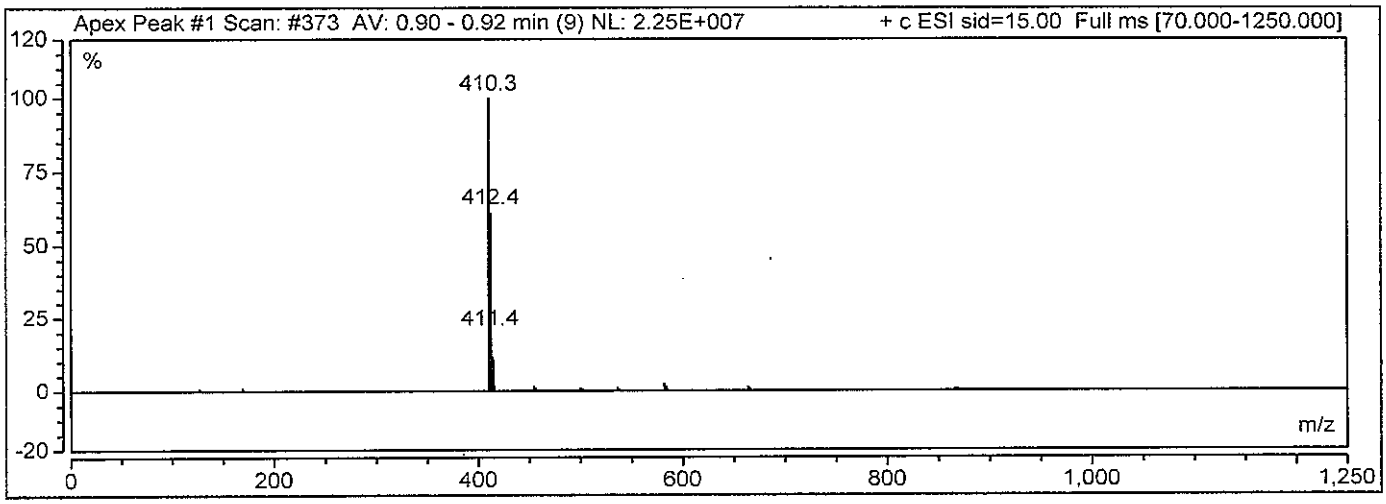


Peak Analysis

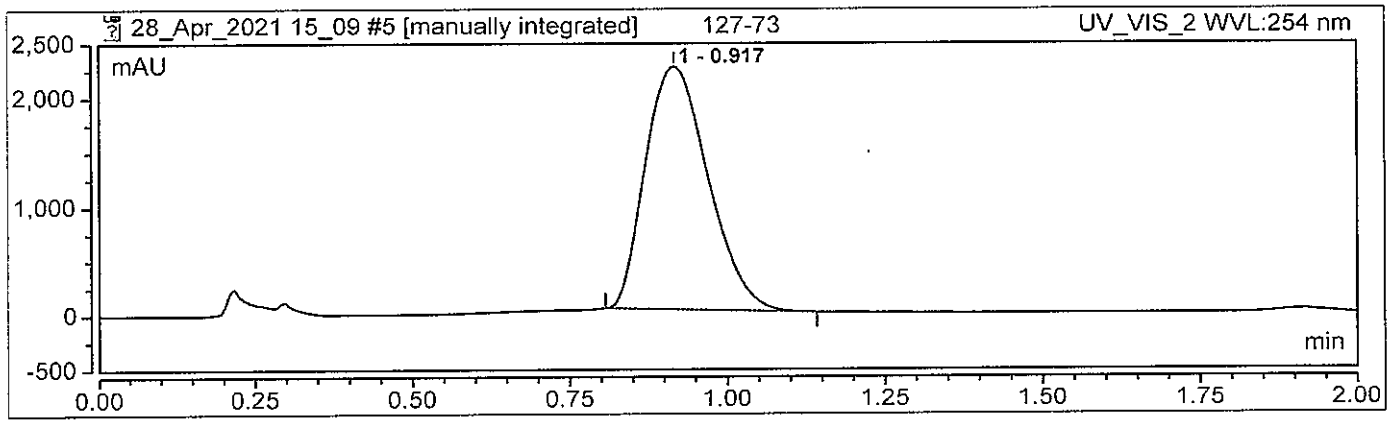
Injection Details

Injection Name:	**127-73**	*Run Time (min):*	**2.00**
Vial Number:	**R:A5**	*Injection Volume:*	**5.00**
Injection Type:	**Unknown**		
Calibration Level:			
Instrument Method:	**1.8uM_column_Tidwell_1.5_min_run4_agilent_zorbax1.8uM, 2.1x50**		
Processing Method:	**McHardy Mass Check**	*Dilution Factor:*	**1.0000**
Injection Date/Time:	**28/Apr/21 15:20**	*Sample Weight:*	**1.0000**

Mass Spectrum



Chromatogram



Table

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1		0.917	258.901	2237.188	100.00	100.00
Total:			**258.901**	**2237.188**	**100.00**	**100.00**

Chromeleon (c) Dionex
Version 7.2.10.23925

SM2020-117-017

Sample Name:
SM2021-123-74
Data Collected on:
400MR.McHardy.Lab-vnmrs400
Archive directory:

Sample directory:

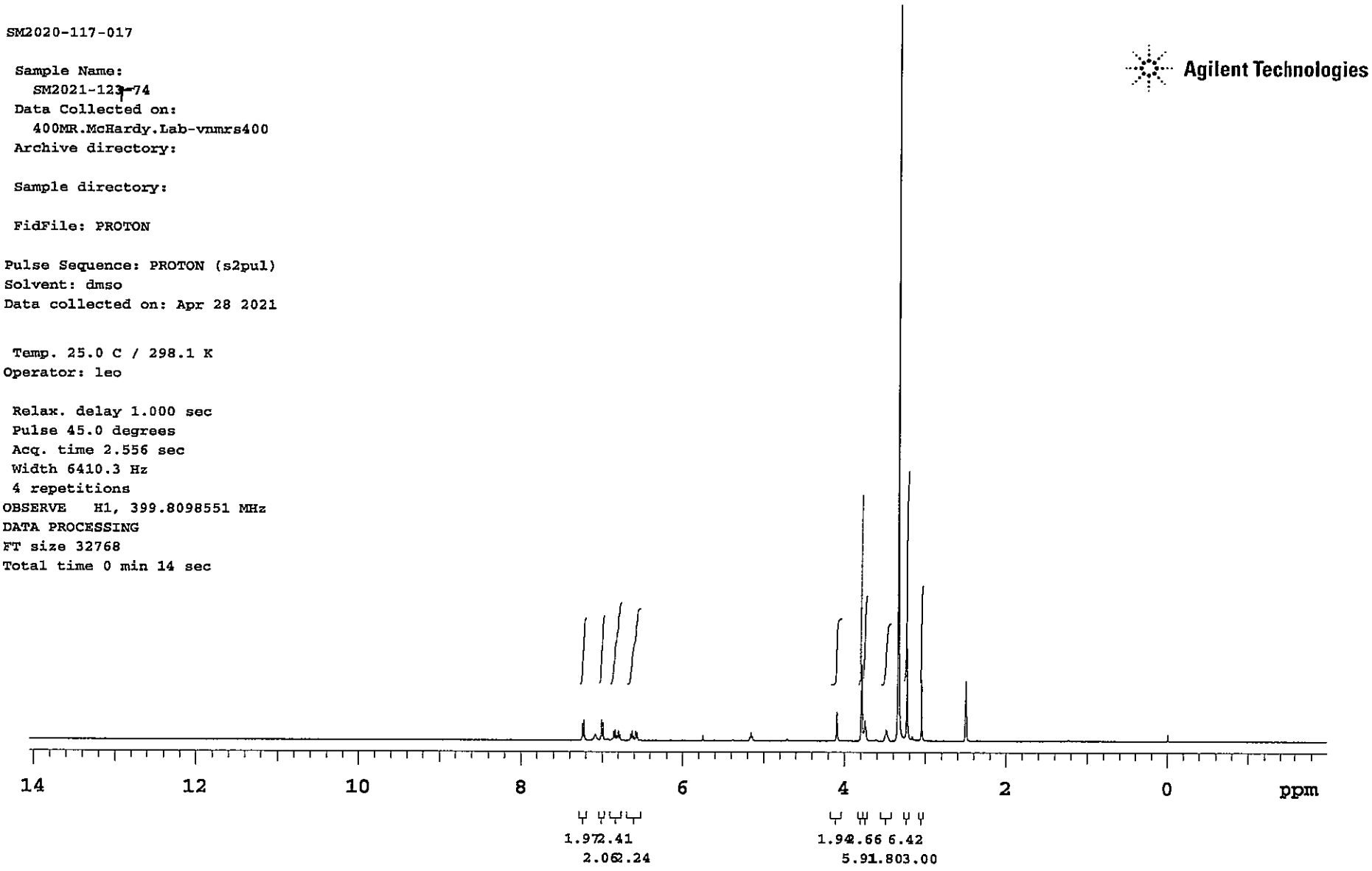
FidFile: PROTON

Pulse Sequence: PROTON (s2pul)
Solvent: dmso
Data collected on: Apr 28 2021

Temp. 25.0 C / 298.1 K
Operator: leo

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 2.556 sec
Width 6410.3 Hz
4 repetitions
OBSERVE H1, 399.8098551 MHz
DATA PROCESSING
FT size 32768
Total time 0 min 14 sec

Agilent Technologies

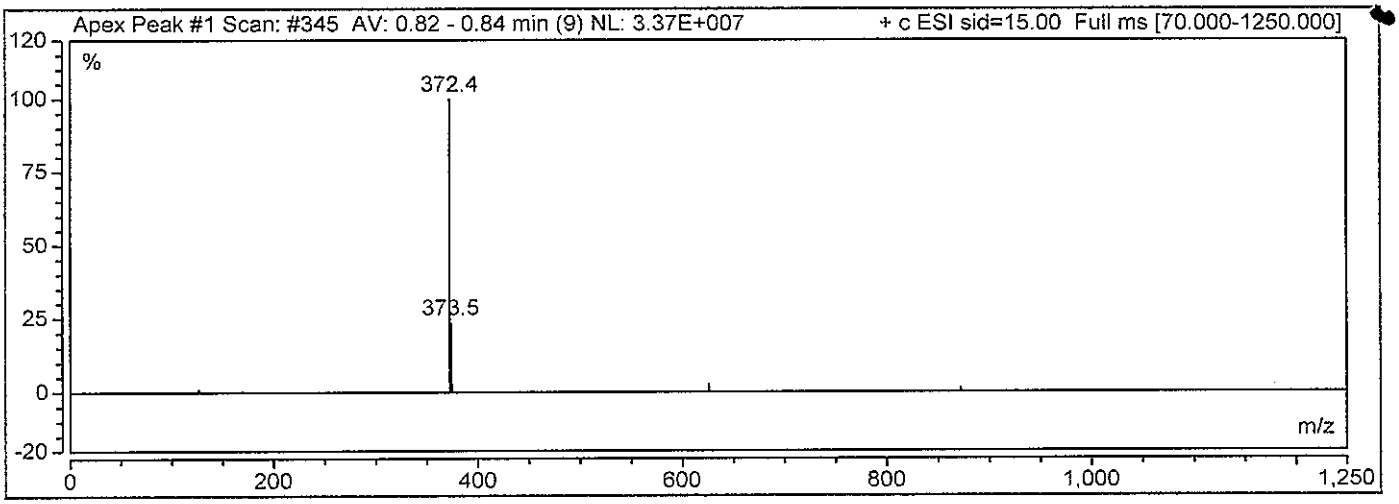


Peak Analysis

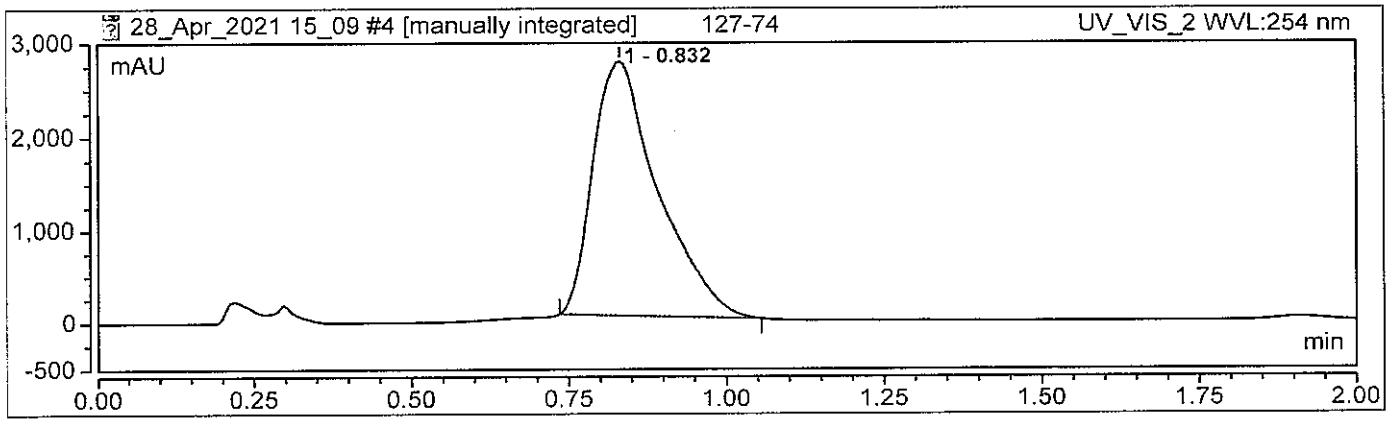
Injection Details

Injection Name:	**127-74**	*Run Time (min):*	**2.00**
Vial Number:	**R:A4**	*Injection Volume:*	**5.00**
Injection Type:	**Unknown**		
Calibration Level:			
Instrument Method:	**1.8uM_column_Tidwell_1.5_min_run4_agilent_zorbax1.8uM, 2.1x50**		
Processing Method:	**McHardy Mass Check**	*Dilution Factor:*	**1.0000**
Injection Date/Time:	**28/Apr/21 15:18**	*Sample Weight:*	**1.0000**

Mass Spectrum



Chromatogram



Table

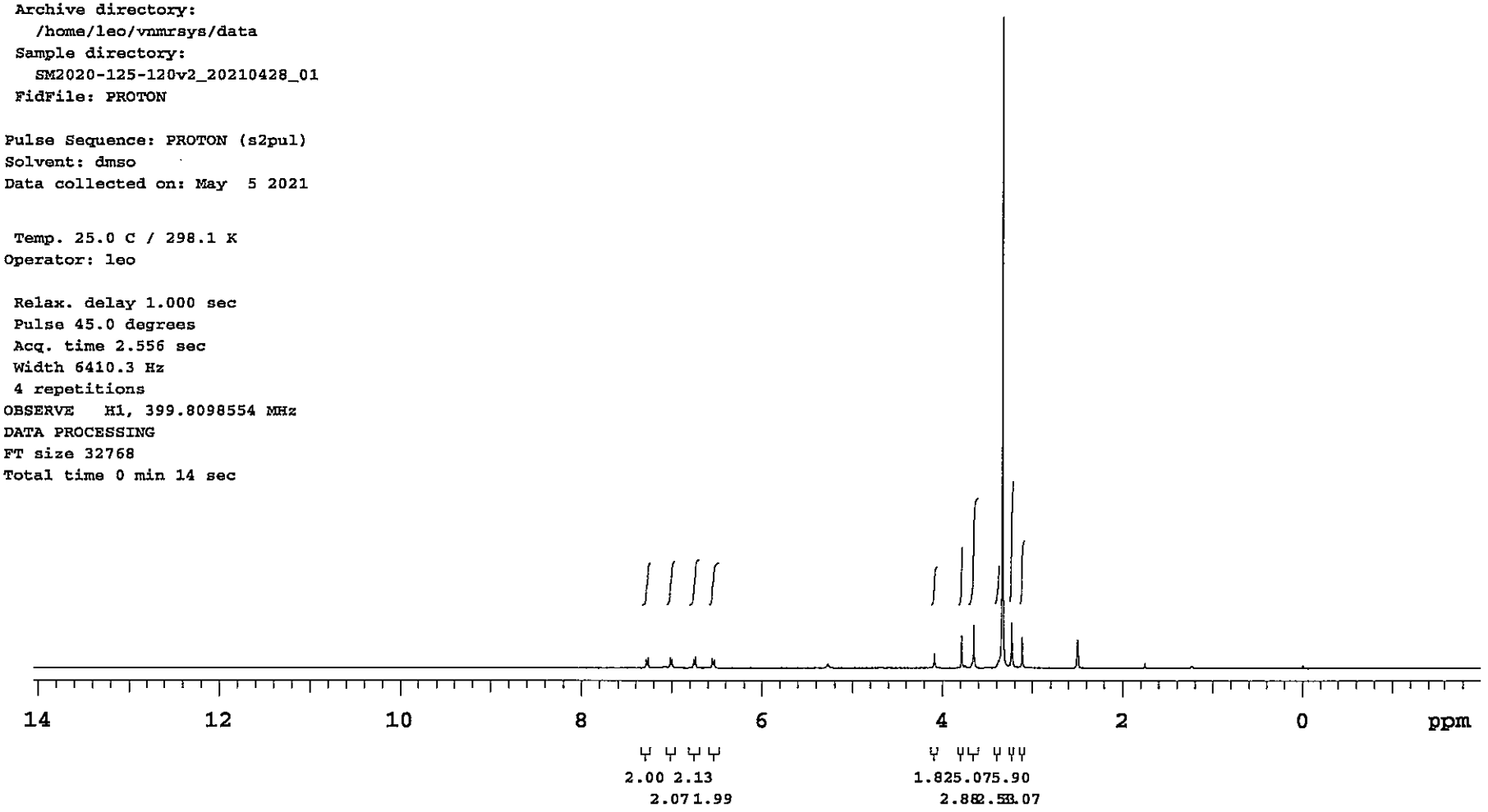
No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1		0.832	327.318	2724.303	100.00	100.00
Total:			**327.318**	**2724.303**	**100.00**	**100.00**

Sample Name:
SM2021-127-91
Data Collected on:
400MR.McHardy.Lab-vnmrs400
Archive directory:
/home/leo/vnmrsys/data
Sample directory:
SM2020-125-120v2_20210428_01
FidFile: PROTON

Pulse Sequence: PROTON (s2pul)
Solvent: dmso
Data collected on: May 5 2021

Temp. 25.0 C / 298.1 K
Operator: leo

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 2.556 sec
Width 6410.3 Hz
4 repetitions
OBSERVE H1, 399.8098554 MHz
DATA PROCESSING
FT size 32768
Total time 0 min 14 sec

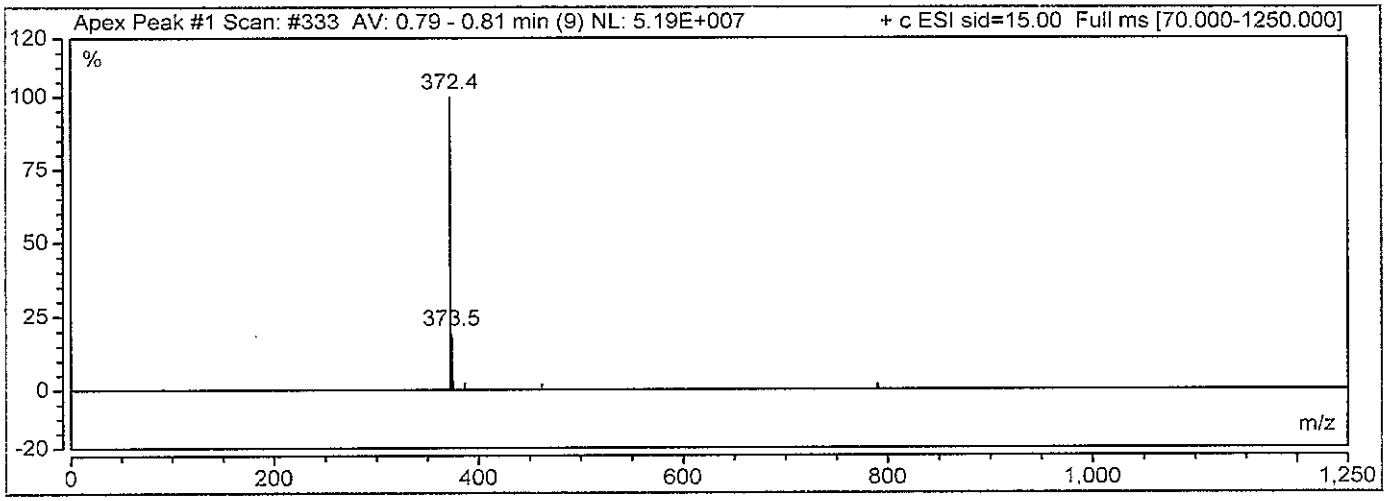


Peak Analysis

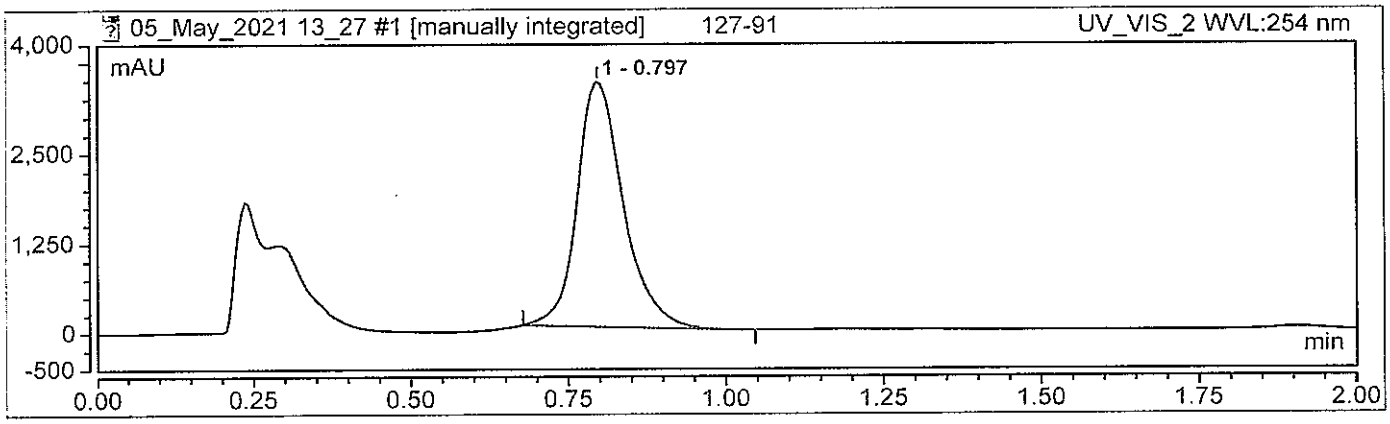
Injection Details

Injection Name:	**127-91**	*Run Time (min):*	**2.00**
Vial Number:	**R:A1**	*Injection Volume:*	**10.00**
Injection Type:	**Unknown**		
Calibration Level:			
Instrument Method:	**1.8uM_column_Tidwell_1.5_min_run4_agilent_zorbax1.8uM, 2.1x50**		
Processing Method:	**McHardy Mass Check**	*Dilution Factor:*	**1.0000**
Injection Date/Time:	**05/May/21 13:27**	*Sample Weight:*	**1.0000**

Mass Spectrum



Chromatogram



Table

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1		0.797	287.976	3390.070	100.00	100.00
Total:			**287.976**	**3390.070**	**100.00**	**100.00**

Chromeleon (c) Dionex
Version 7.2.10.23925

SM2020-117-017

Sample Name:
SM2021-123-75
Data Collected on:
400MR.McHardy.Lab-vnmrs400
Archive directory:

Sample directory:

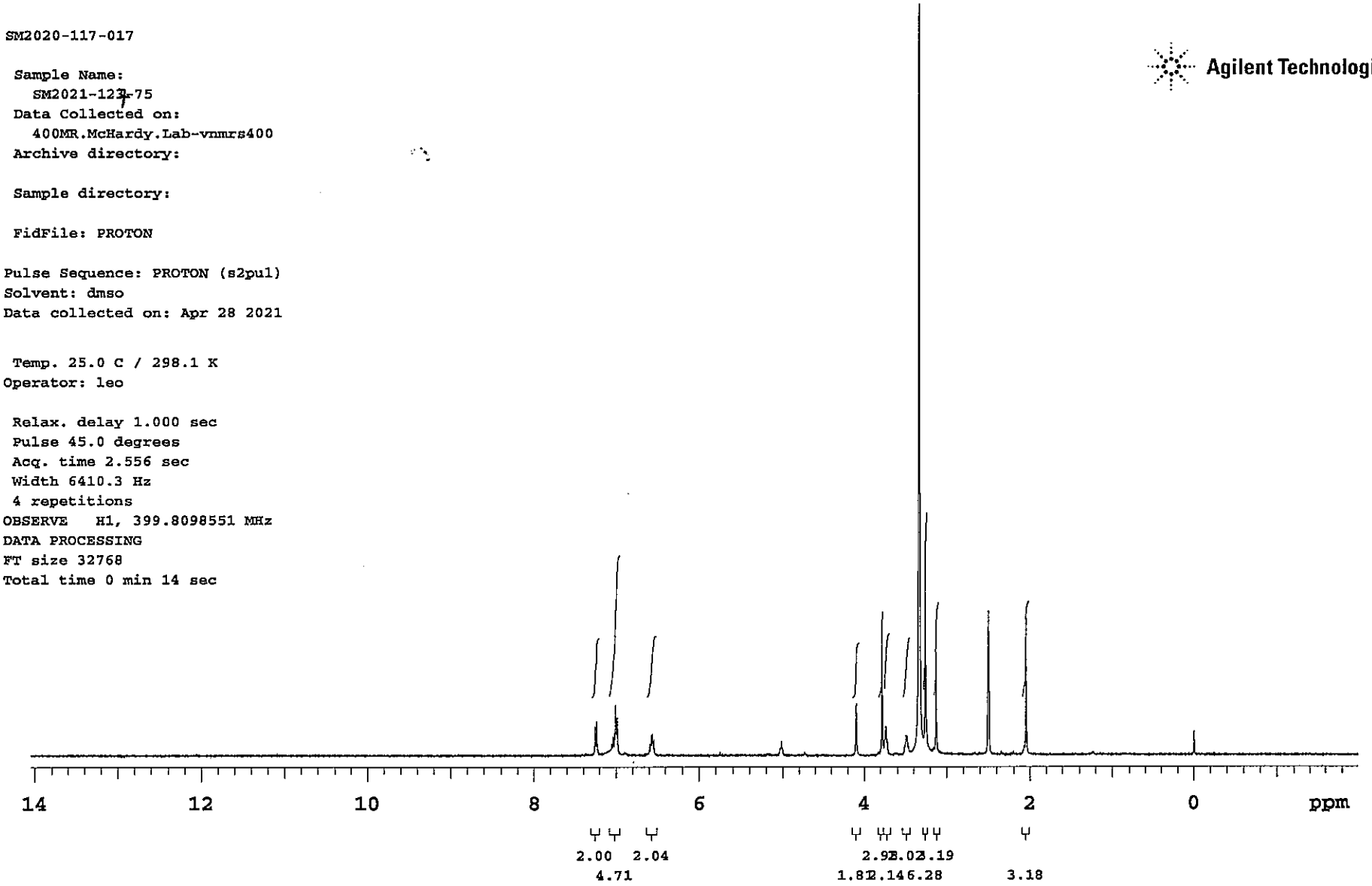
FidFile: PROTON

Pulse Sequence: PROTON (s2pul)
Solvent: dmso
Data collected on: Apr 28 2021

Temp. 25.0 C / 298.1 K
Operator: leo

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 2.556 sec
Width 6410.3 Hz
4 repetitions
OBSERVE H1, 399.8098551 MHz
DATA PROCESSING
FT size 32768
Total time 0 min 14 sec

Agilent Technologies

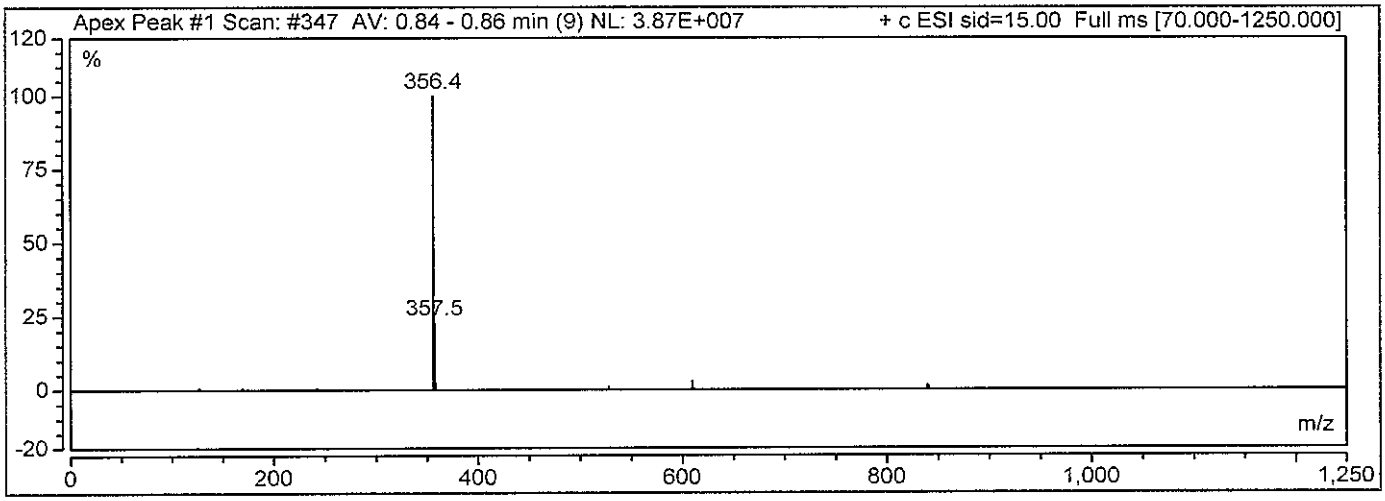


Peak Analysis

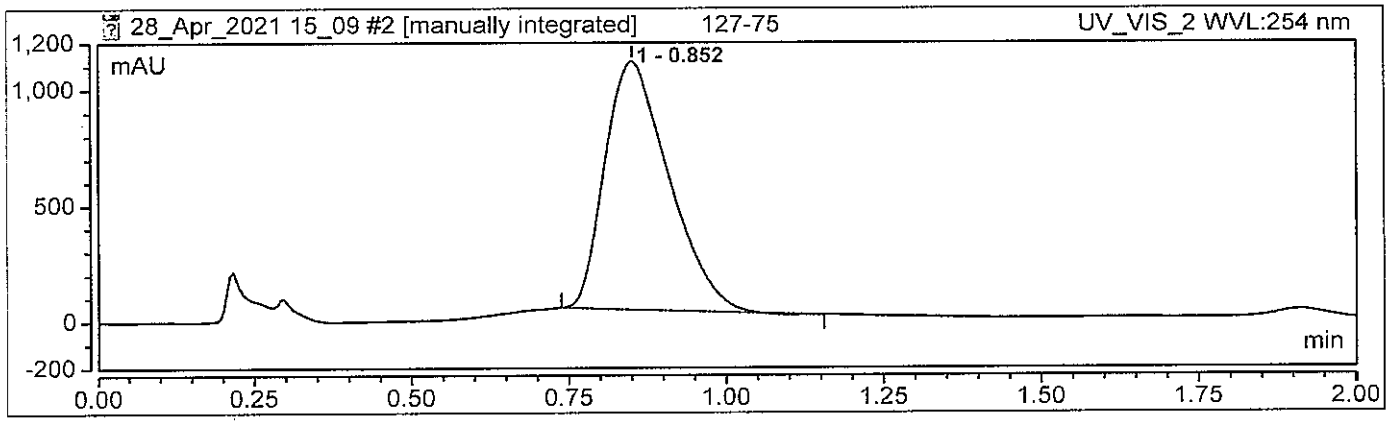
Injection Details

Injection Name:	**127-75**	*Run Time (min):*	**2.00**
Vial Number:	**R:A2**	*Injection Volume:*	**5.00**
Injection Type:	**Unknown**		
Calibration Level:			
Instrument Method:	**1.8uM_column_Tidwell_1.5_min_run4_agilent_zorbax1.8uM, 2.1x50**		
Processing Method:	**McHardy Mass Check**	*Dilution Factor:*	**1.0000**
Injection Date/Time:	**28/Apr/21 15:12**	*Sample Weight:*	**1.0000**

Mass Spectrum



Chromatogram



Table

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1		0.852	126.014	1070.704	100.00	100.00
Total:			**126.014**	**1070.704**	**100.00**	**100.00**

SM2020-117-017

Sample Name:
SM2021-123-83
Data Collected on:
400MR.McHardy.Lab-vnmrs400
Archive directory:

Sample directory:

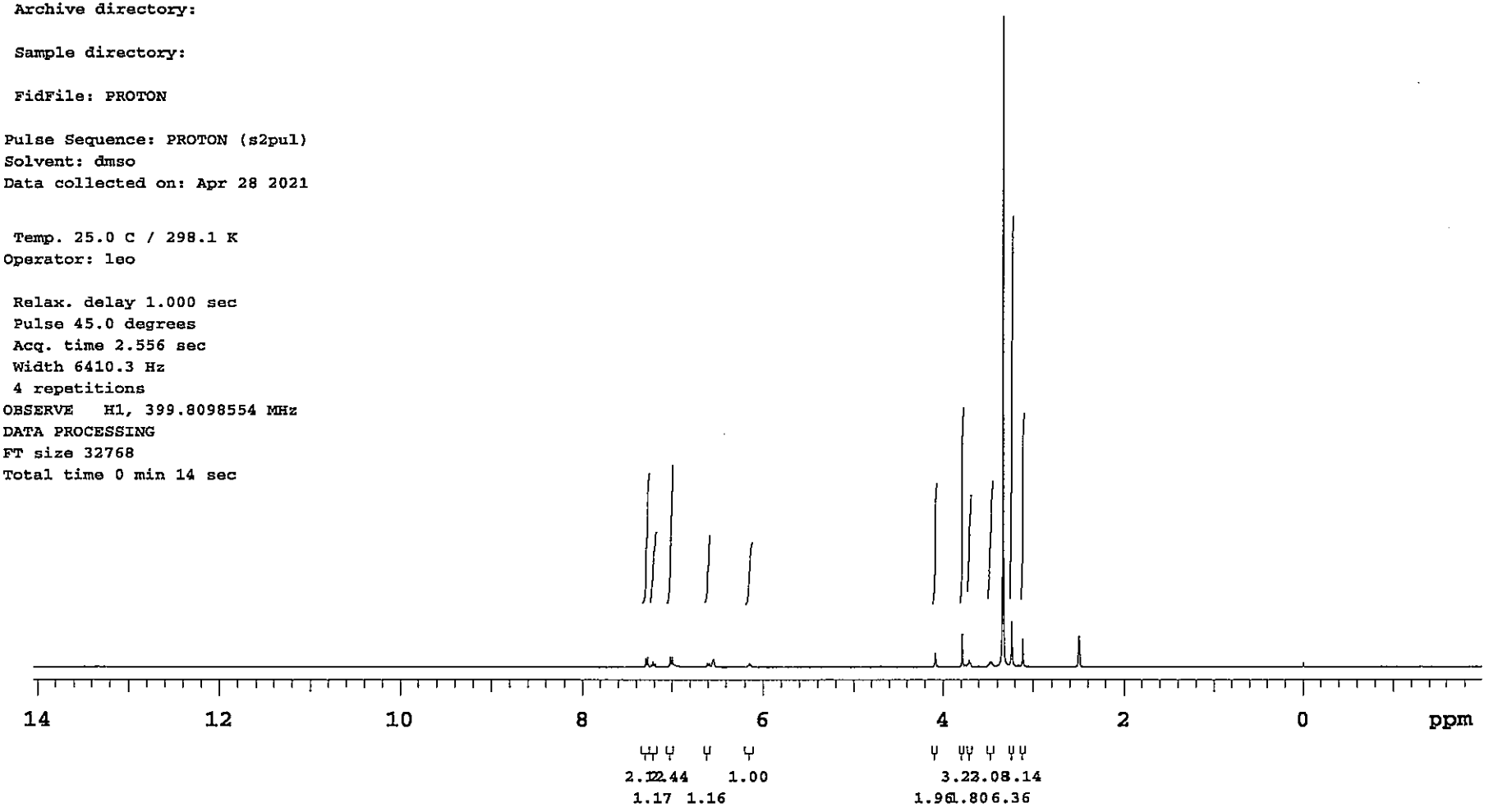
FidFile: PROTON

Pulse Sequence: PROTON (s2pul)
Solvent: dmso
Data collected on: Apr 28 2021

Temp. 25.0 C / 298.1 K
Operator: leo

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 2.556 sec
Width 6410.3 Hz
4 repetitions
OBSERVE H1, 399.8098554 MHz
DATA PROCESSING
FT size 32768
Total time 0 min 14 sec

Agilent Technologies

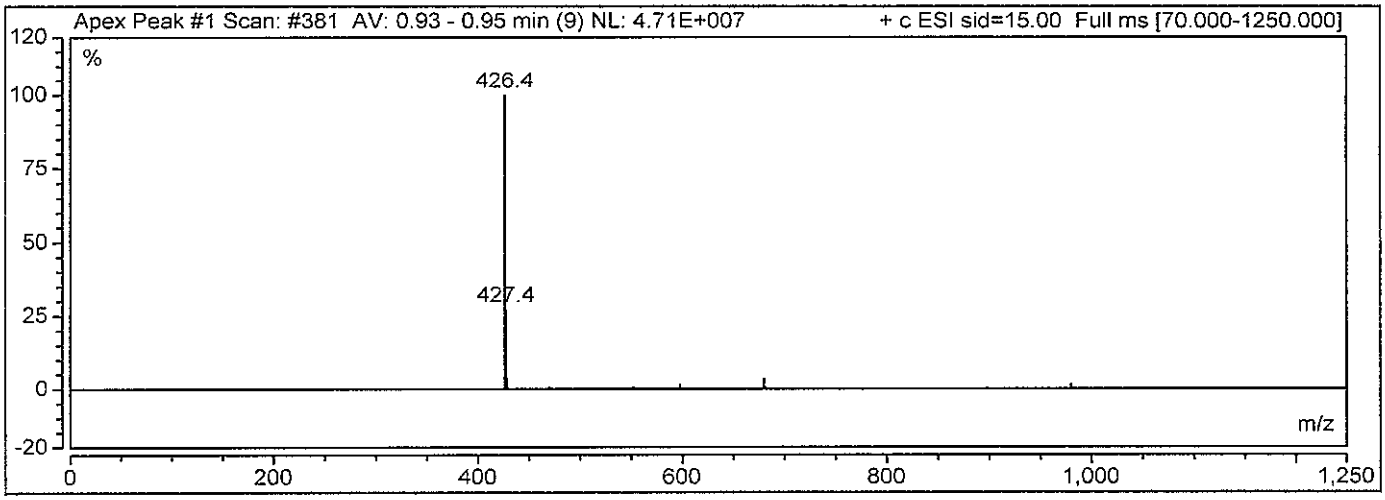


Peak Analysis

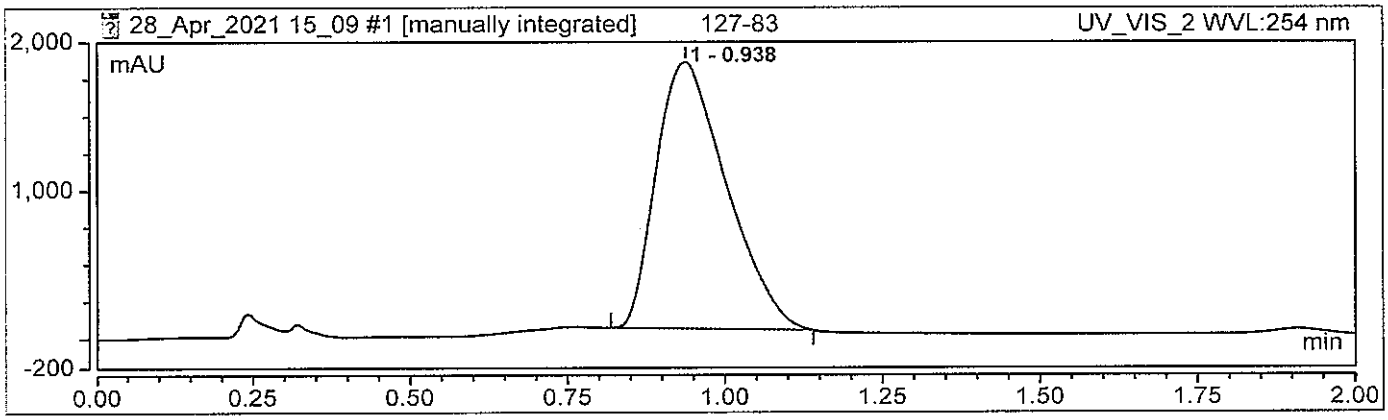
Injection Details

Injection Name:	**127-83**	*Run Time (min):*	**2.00**
Vial Number:	**R:A1**	*Injection Volume:*	**5.00**
Injection Type:	**Unknown**		
Calibration Level:			
Instrument Method:	**1.8uM_column_Tidwell_1.5_min_run4_agilent_zorbax1.8uM, 2.1x50**		
Processing Method:	**McHardy Mass Check**	*Dilution Factor:*	**1.0000**
Injection Date/Time:	**28/Apr/21 15:10**	*Sample Weight:*	**1.0000**

Mass Spectrum



Chromatogram



Table

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1		0.938	233.225	1801.625	100.00	100.00
Total:			**233.225**	**1801.625**	**100.00**	**100.00**

SM2020-117-017

Sample Name:
SM2021-123-63
Data Collected on:
400MR.McHardy.Lab-vnmrs400
Archive directory:

Sample directory:

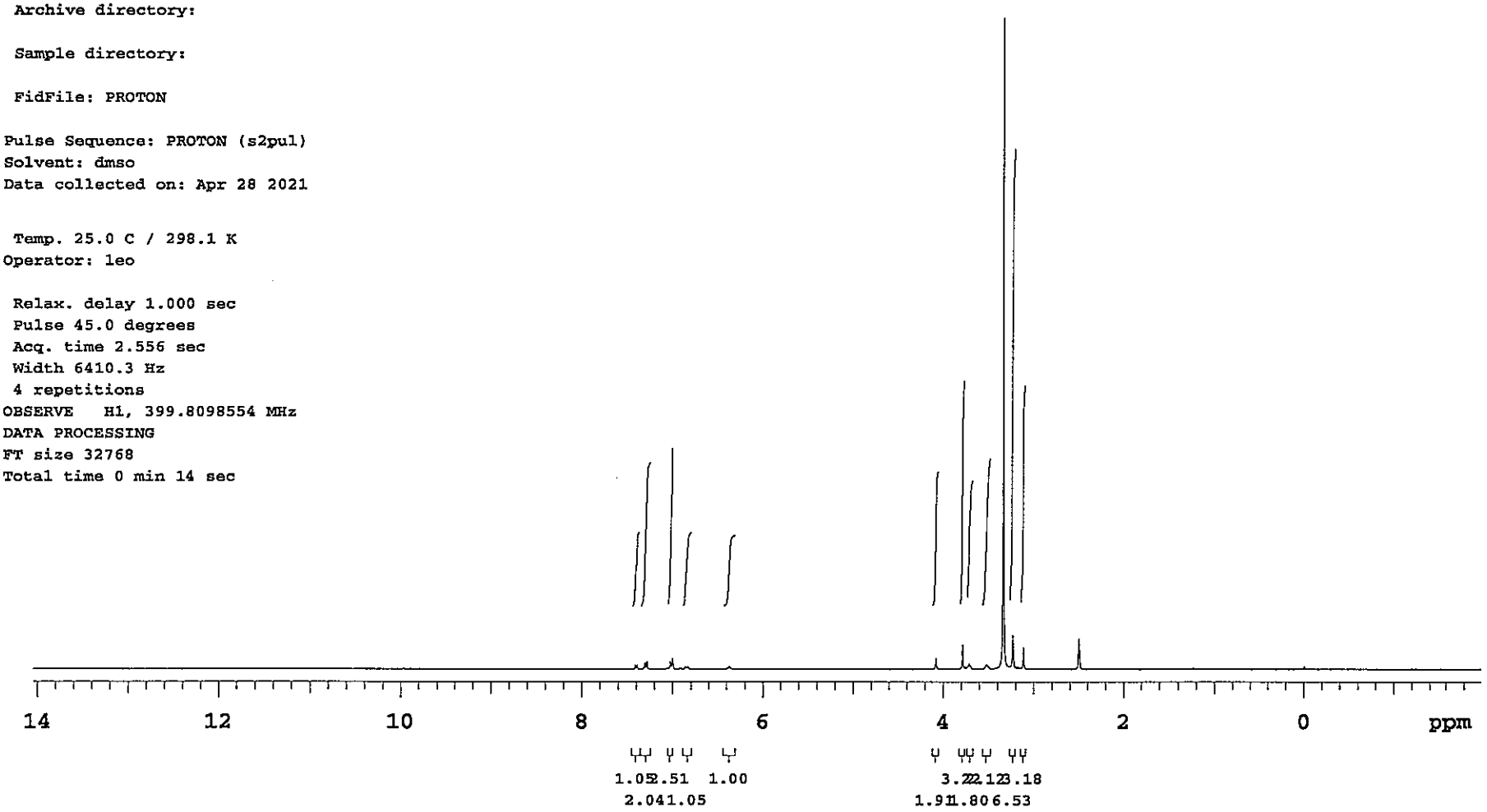
FidFile: PROTON

Pulse Sequence: PROTON (s2pul)
Solvent: dmso
Data collected on: Apr 28 2021

Temp. 25.0 C / 298.1 K
Operator: leo

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 2.556 sec
Width 6410.3 Hz
4 repetitions
OBSERVE H1, 399.8098554 MHz
DATA PROCESSING
FT size 32768
Total time 0 min 14 sec

Agilent Technologies

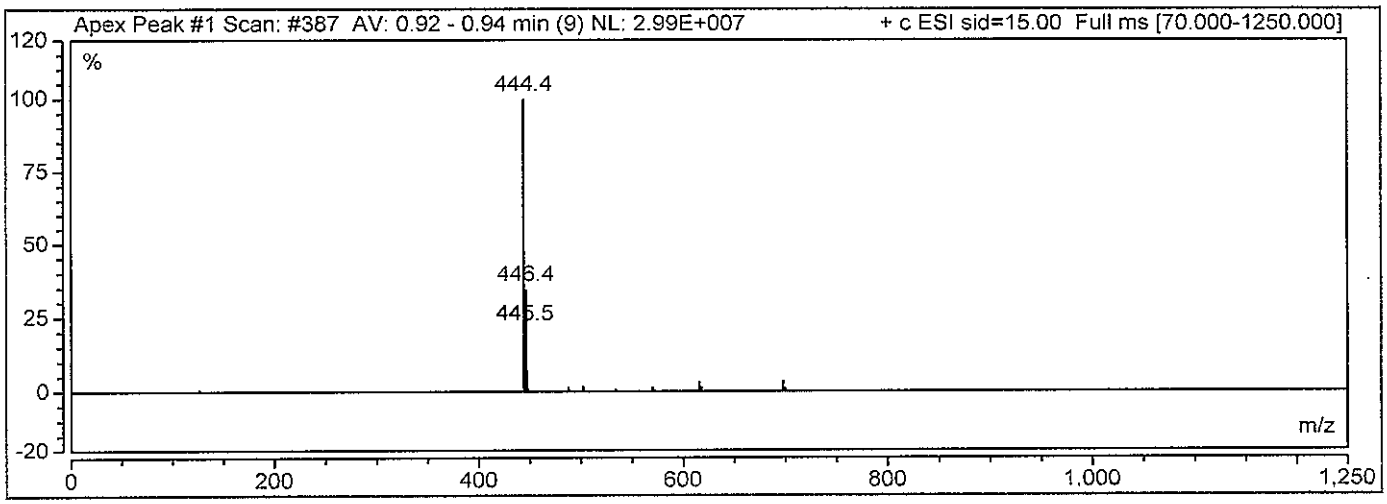


Peak Analysis

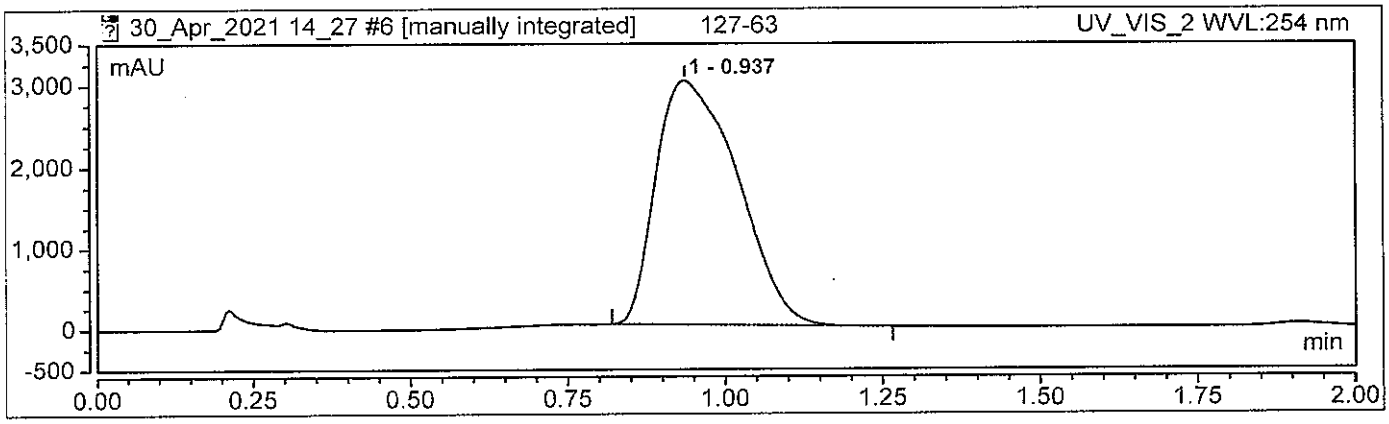
Injection Details

Injection Name:	**127-63**	*Run Time (min):*	**2.00**
Vial Number:	**R:A6**	*Injection Volume:*	**5.00**
Injection Type:	**Unknown**		
Calibration Level:			
Instrument Method:	**1.8uM_column_Tidwell_1.5_min_run4_agilent_zorbax1.8uM, 2.1x50**		
Processing Method:	**McHardy Mass Check**	*Dilution Factor:*	**1.0000**
Injection Date/Time:	**30/Apr/21 14:41**	*Sample Weight:*	**1.0000**

Mass Spectrum



Chromatogram



Table

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1		0.937	452.016	2997.541	100.00	100.00
Total:			**452.016**	**2997.541**	**100.00**	**100.00**

SM2020-117-017

Sample Name:
SM2021-127-76
Data Collected on:
400MR.McHardy.Lab-vnmrs400
Archive directory:

Sample directory:

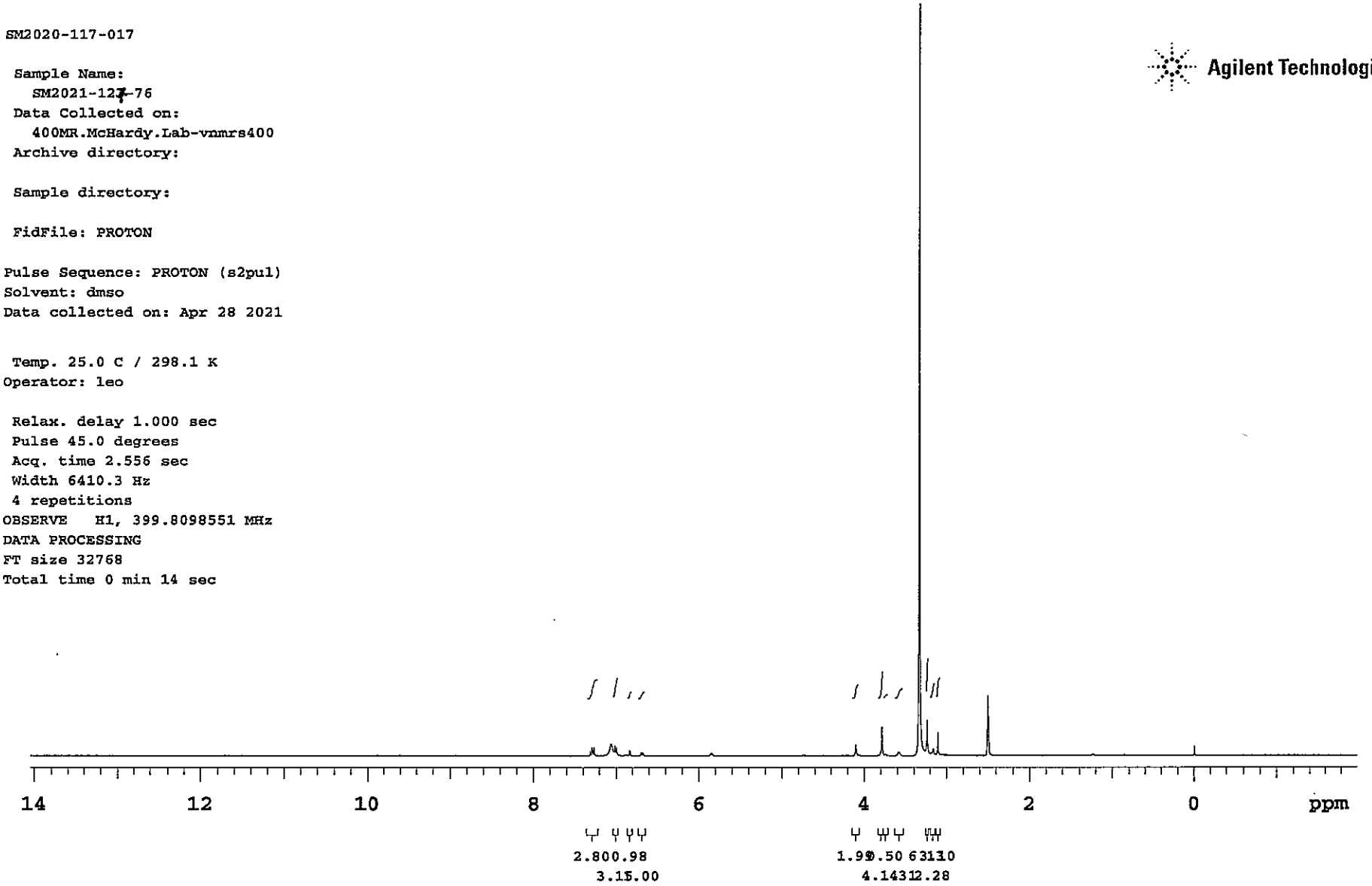
FidFile: PROTON

Pulse Sequence: PROTON (s2pul)
Solvent: dmso
Data collected on: Apr 28 2021

Temp. 25.0 C / 298.1 K
Operator: leo

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 2.556 sec
Width 6410.3 Hz
4 repetitions
OBSERVE H1, 399.8098551 MHz
DATA PROCESSING
FT size 32768
Total time 0 min 14 sec

Agilent Technologies

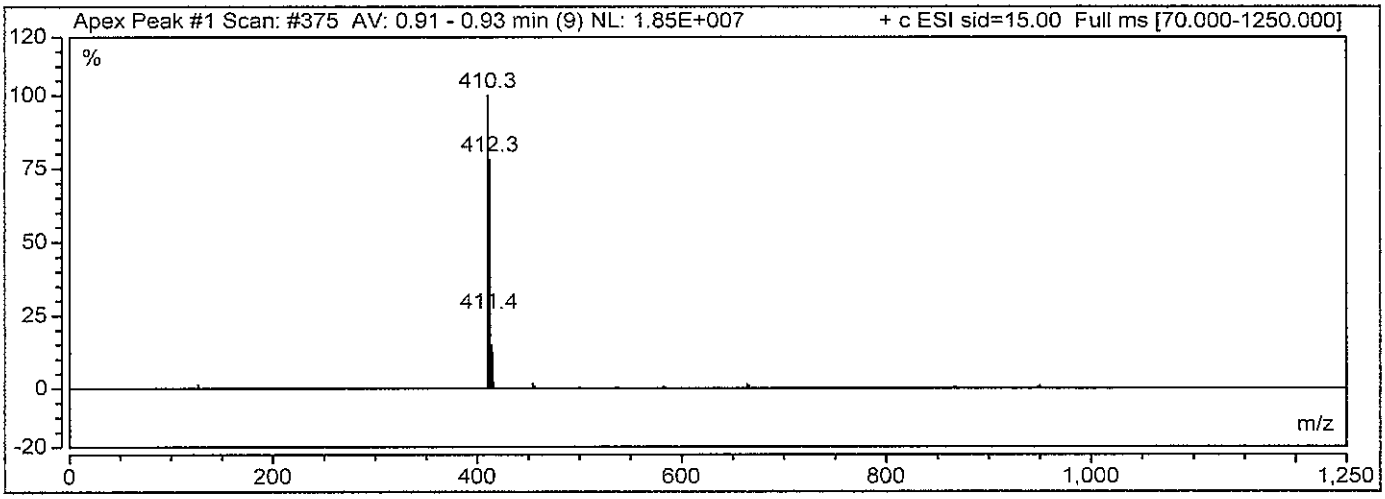


Peak Analysis

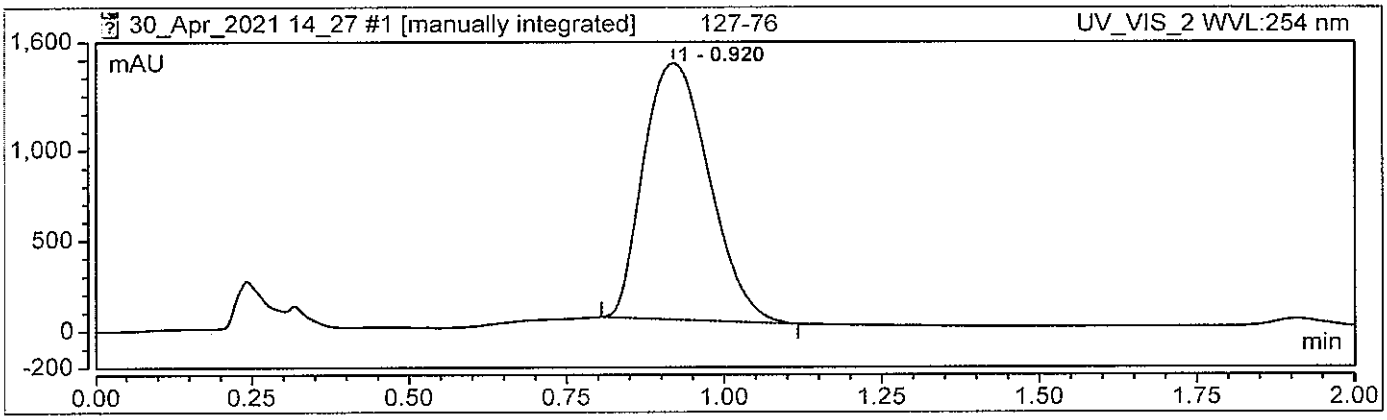
Injection Details

Injection Name:	**127-76**	*Run Time (min):*	**2.00**
Vial Number:	**R:A1**	*Injection Volume:*	**5.00**
Injection Type:	**Unknown**		
Calibration Level:			
Instrument Method:	**1.8uM_column_Tidwell_1.5_min_run4_agilent_zorbax1.8uM, 2.1x50**		
Processing Method:	**McHardy Mass Check**	*Dilution Factor:*	**1.0000**
Injection Date/Time:	**30/Apr/21 14:28**	*Sample Weight:*	**1.0000**

Mass Spectrum



Chromatogram



Table

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1		0.920	171.304	1417.787	100.00	100.00
Total:			**171.304**	**1417.787**	**100.00**	**100.00**

SM2020-117-017

Sample Name:
SM2021-123-65
Data Collected on:
400MR.McHardy.Lab-vnmrs400
Archive directory:

Sample directory:

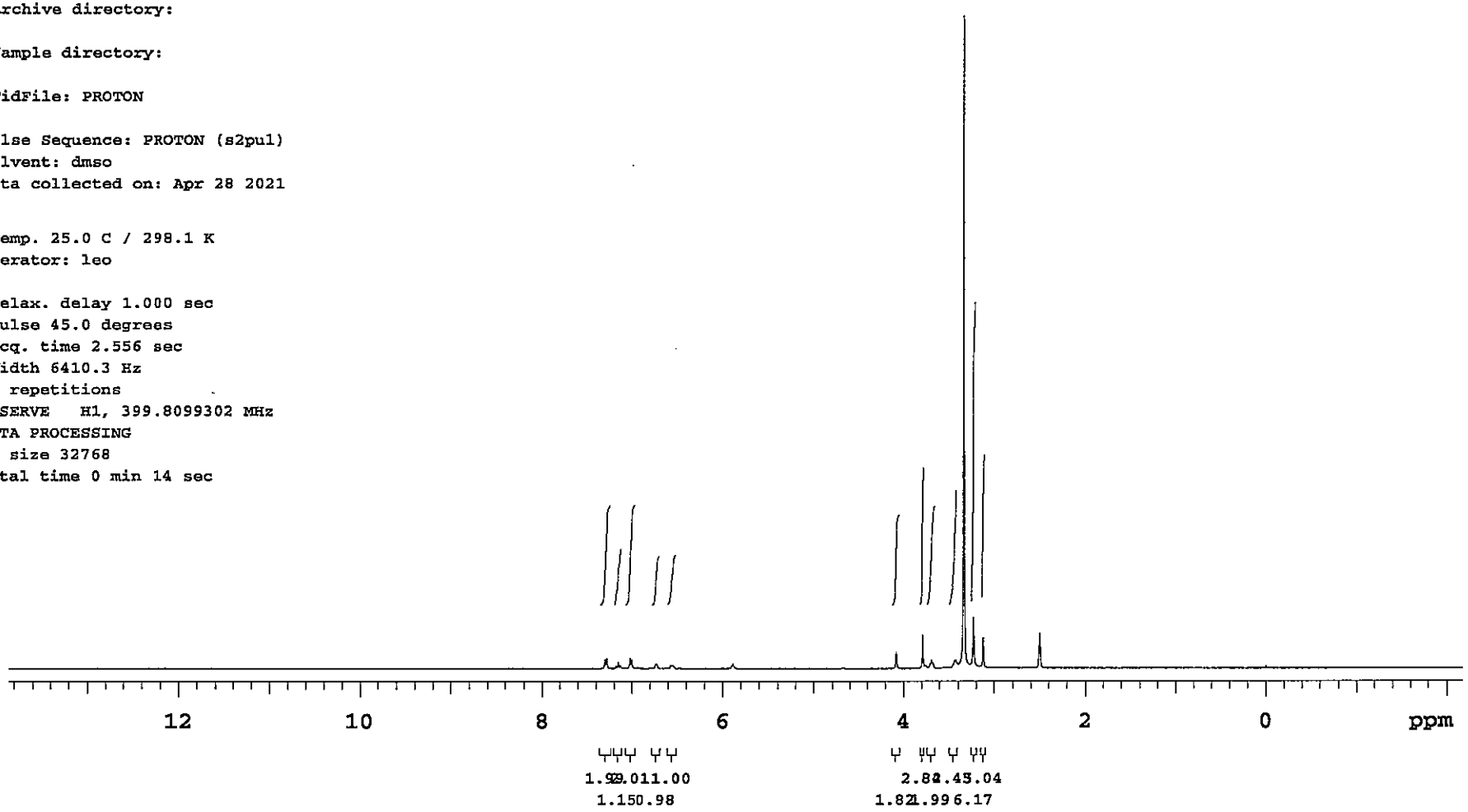
FidFile: PROTON

Pulse Sequence: PROTON (s2pul)
Solvent: dmso
Data collected on: Apr 28 2021

Temp. 25.0 C / 298.1 K
Operator: leo

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 2.556 sec
Width 6410.3 Hz
4 repetitions
OBSERVE H1, 399.8099302 MHz
DATA PROCESSING
FT size 32768
Total time 0 min 14 sec

Agilent Technologies

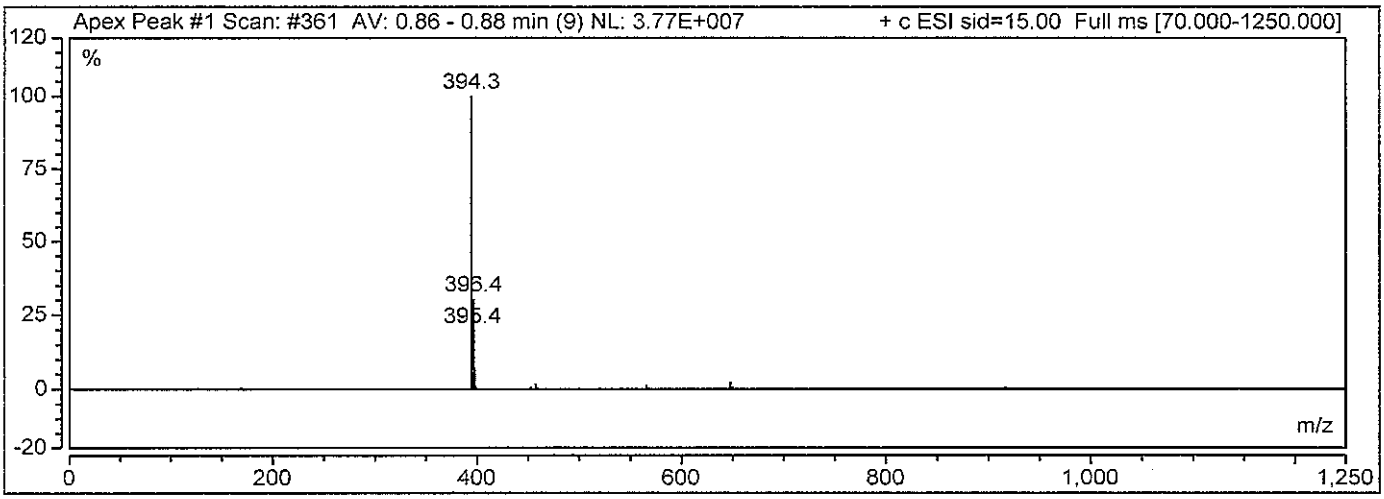


Peak Analysis

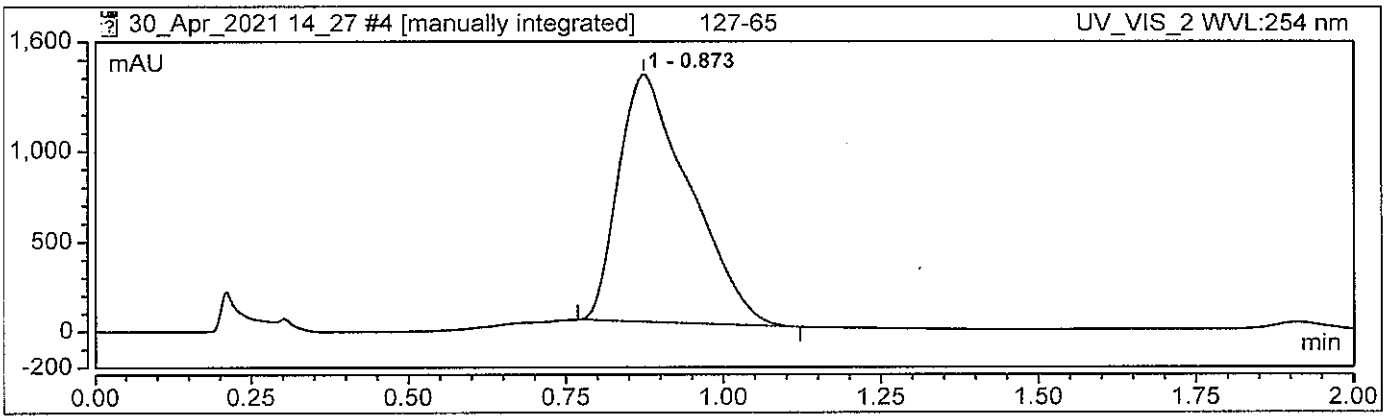
Injection Details

Injection Name:	**127-65**	*Run Time (min):*	**2.00**
Vial Number:	**R:A4**	*Injection Volume:*	**5.00**
Injection Type:	**Unknown**		
Calibration Level:			
Instrument Method:	**1.8uM_column_Tidwell_1.5_min_run4_agilent_zorbax1.8uM, 2.1x50**		
Processing Method:	**McHardy Mass Check**	*Dilution Factor:*	**1.0000**
Injection Date/Time:	**30/Apr/21 14:36**	*Sample Weight:*	**1.0000**

Mass Spectrum



Chromatogram



Table

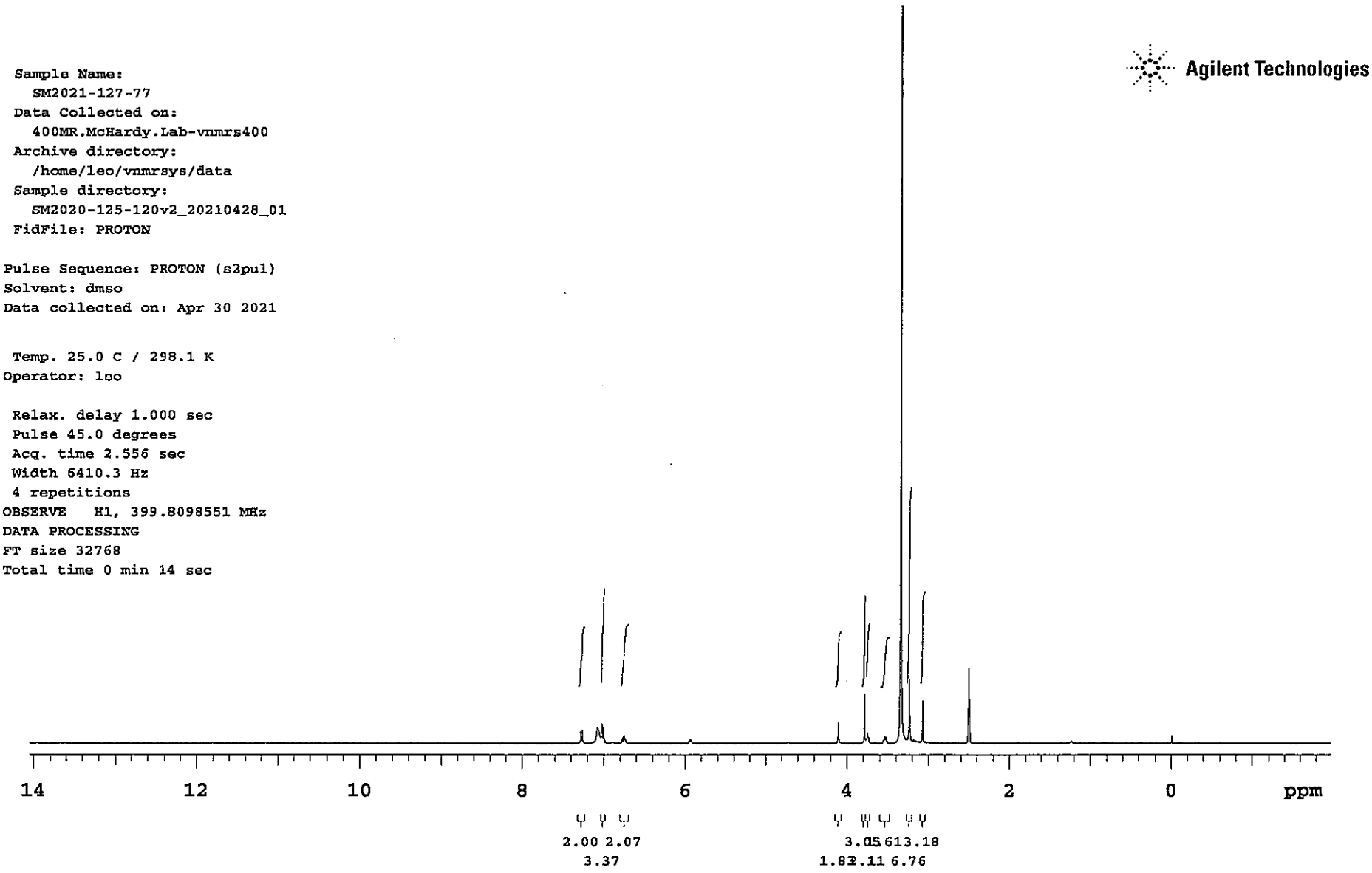
No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1		0.873	180.581	1366.429	100.00	100.00
Total:			**180.581**	**1366.429**	**100.00**	**100.00**

Sample Name:
SM2021-127-77
Data Collected on:
400MR.McHardy.Lab-vnmrs400
Archive directory:
/home/leo/vnmrsys/data
Sample directory:
SM2020-125-120v2_20210428_01
FidFile: PROTON

Pulse Sequence: PROTON (s2pul)
Solvent: dmso
Data collected on: Apr 30 2021

Temp. 25.0 C / 298.1 K
Operator: leo

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 2.556 sec
Width 6410.3 Hz
4 repetitions
OBSERVE H1, 399.8098551 MHz
DATA PROCESSING
FT size 32768
Total time 0 min 14 sec

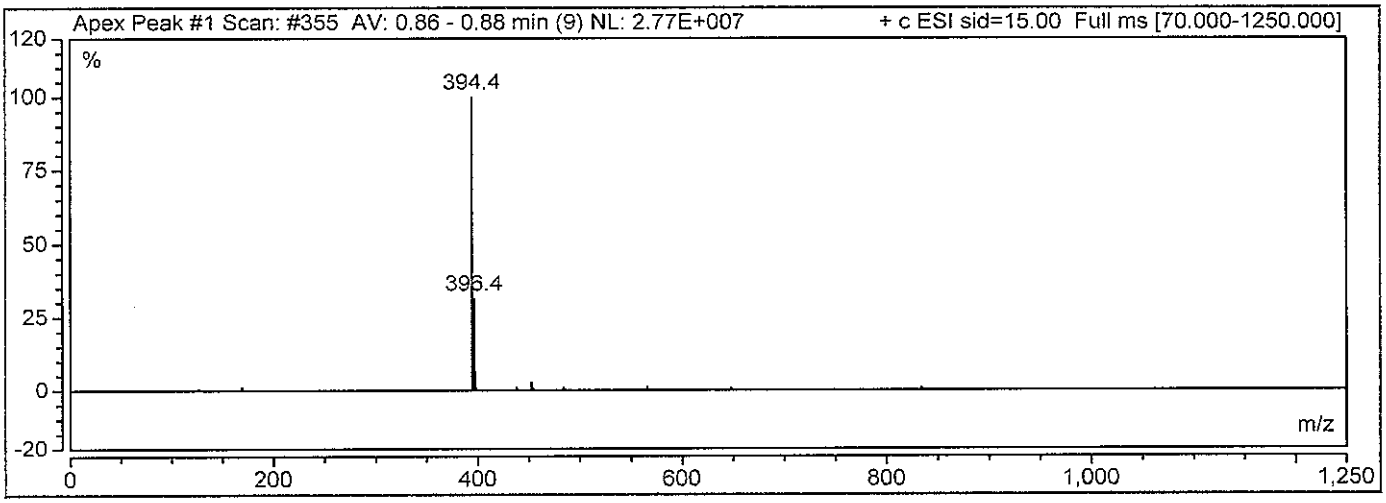


Peak Analysis

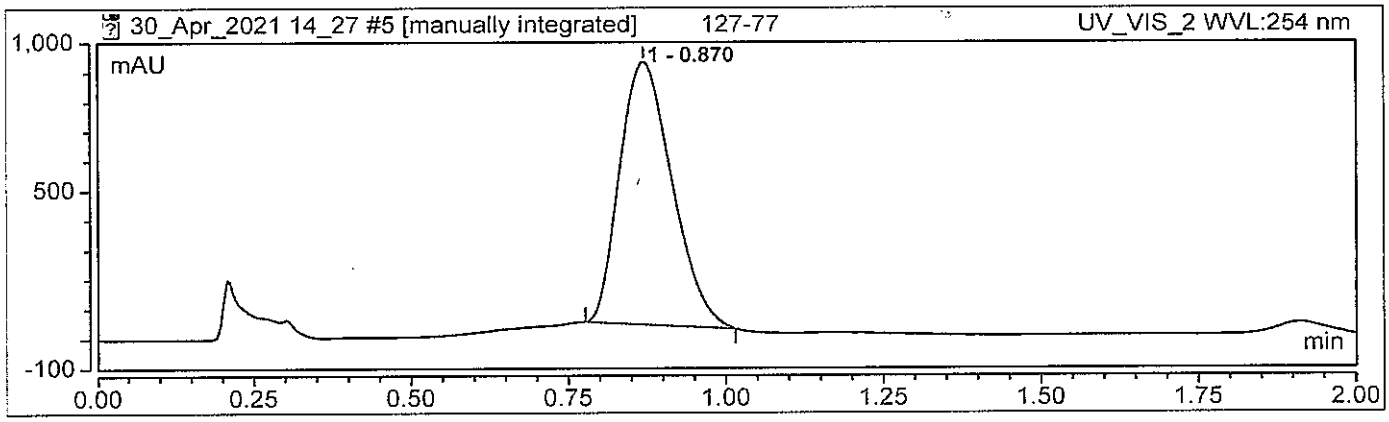
Injection Details

Injection Name:	**127-77**	*Run Time (min):*	**2.00**
Vial Number:	**R:A5**	*Injection Volume:*	**5.00**
Injection Type:	**Unknown**		
Calibration Level:			
Instrument Method:	**1.8uM_column_Tidwell_1.5_min_run4_agilent_zorbax1.8uM, 2.1x50**		
Processing Method:	**McHardy Mass Check**	*Dilution Factor:*	**1.0000**
Injection Date/Time:	**30/Apr/21 14:38**	*Sample Weight:*	**1.0000**

Mass Spectrum

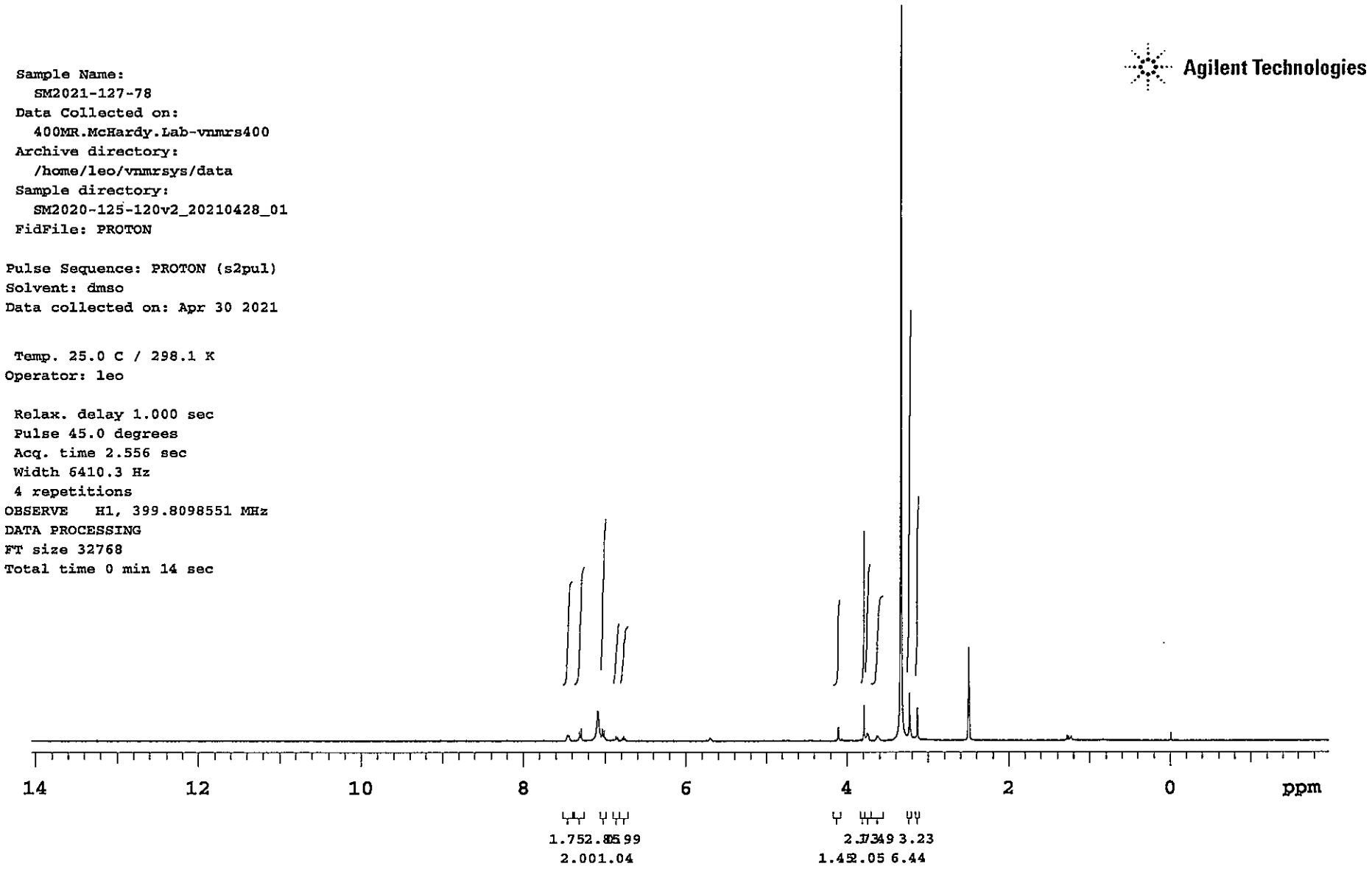


Chromatogram



Table

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1		0.870	85.506	886.478	100.00	100.00
Total:			**85.506**	**886.478**	**100.00**	**100.00**

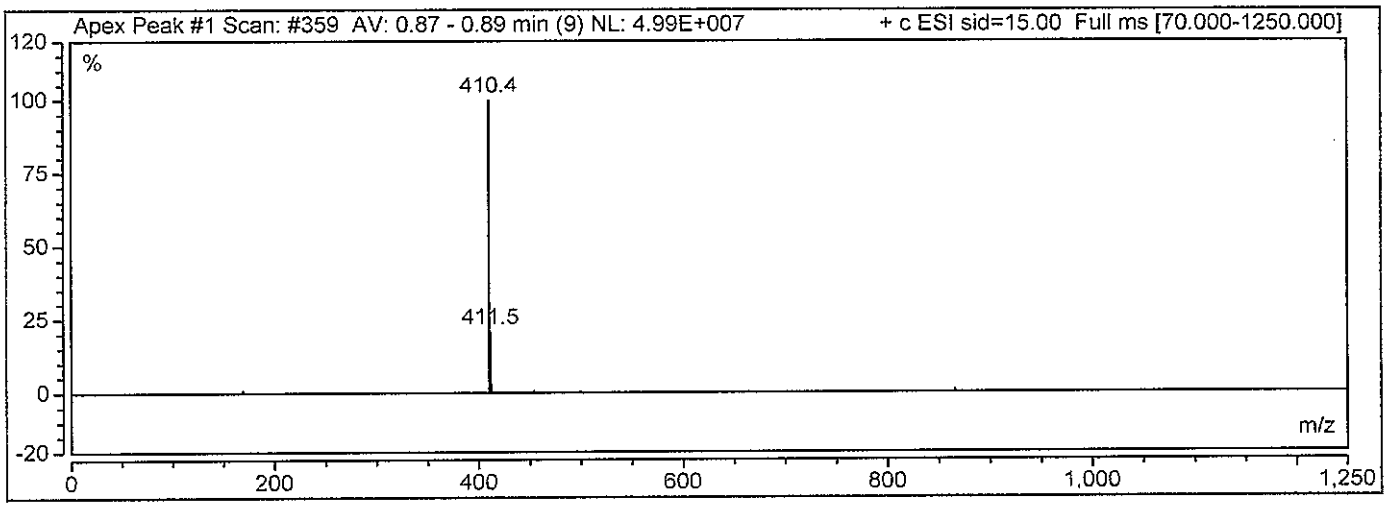

Agilent Technologies
Sample Name:
SM2021-127-78
Data Collected on:
400MR.McHardy.Lab-vnmrs400
Archive directory:
/home/leo/vnmrsys/data
Sample directory:
SM2020-125-120v2_20210428_01
FidFile: PROTON
Pulse Sequence: PROTON (s2pul)
Solvent: dmso
Data collected on: Apr 30 2021
Temp. 25.0 C / 298.1 K
Operator: leo
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 2.556 sec
Width 6410.3 Hz
4 repetitions
OBSERVE H1, 399.8098551 MHz
DATA PROCESSING
FT size 32768
Total time 0 min 14 sec
14
12
10
8
6
4
2
0
ppm
1.752.8599
2.001.04
2.7349 3.23
1.452.05 6.44

Peak Analysis

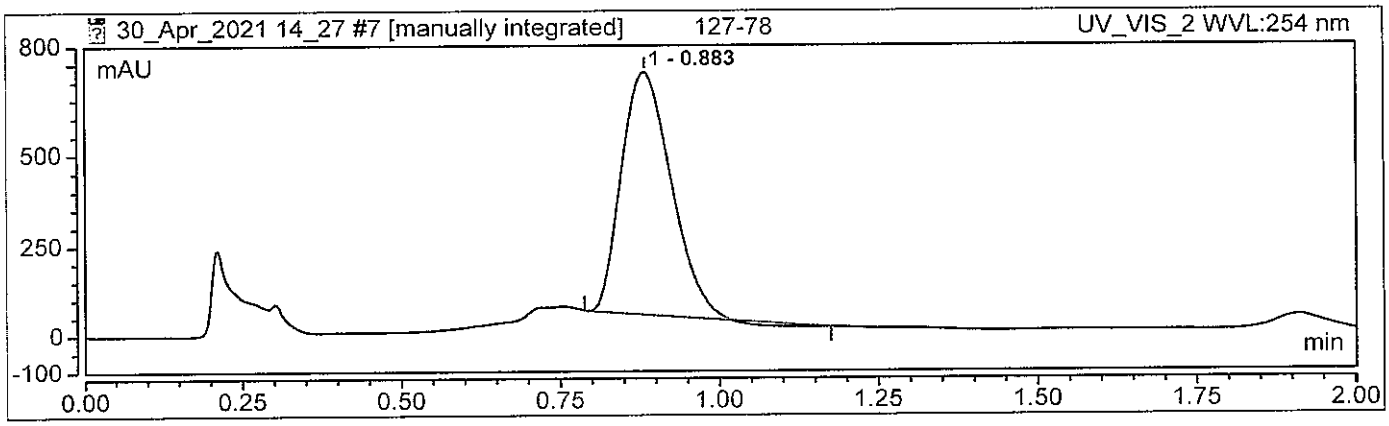
Injection Details

Injection Name:	**127-78**	*Run Time (min):*	**2.00**
Vial Number:	**R:A7**	*Injection Volume:*	**5.00**
Injection Type:	**Unknown**		
Calibration Level:			
Instrument Method:	**1.8uM_column_Tidwell_1.5_min_run4_agilent_zorbax1.8uM, 2.1x50**		
Processing Method:	**McHardy Mass Check**	*Dilution Factor:*	**1.0000**
Injection Date/Time:	**30/Apr/21 14:44**	*Sample Weight:*	**1.0000**

Mass Spectrum



Chromatogram



Table

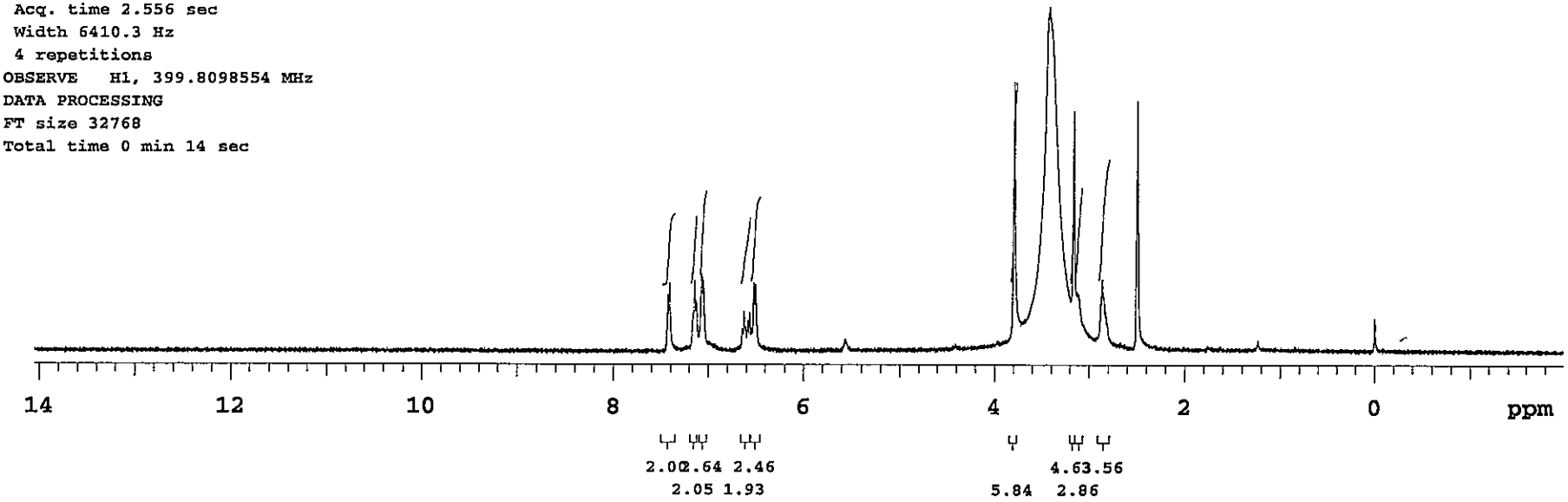
No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1		0.883	60.068	668.292	100.00	100.00
Total:			**60.068**	**668.292**	**100.00**	**100.00**

Sample Name:
SM2021-127-86
Data Collected on:
400MR.McHardy.Lab-vnmrs400
Archive directory:
/home/leo/vnmrsys/data
Sample directory:
SM2020-125-120v2_20210428_01
FidFile: PROTON

Pulse Sequence: PROTON (s2pul)
Solvent: dmso
Data collected on: May 5 2021

Temp. 25.0 C / 298.1 K
Operator: leo

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 2.556 sec
Width 6410.3 Hz
4 repetitions
OBSERVE H1, 399.8098554 MHz
DATA PROCESSING
FT size 32768
Total time 0 min 14 sec

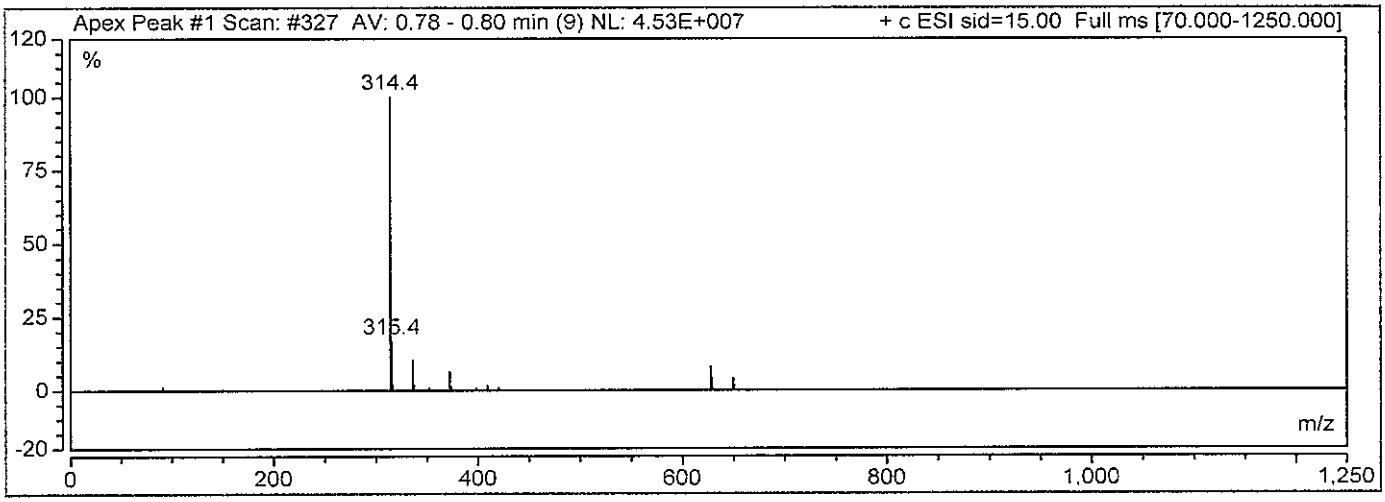


Peak Analysis

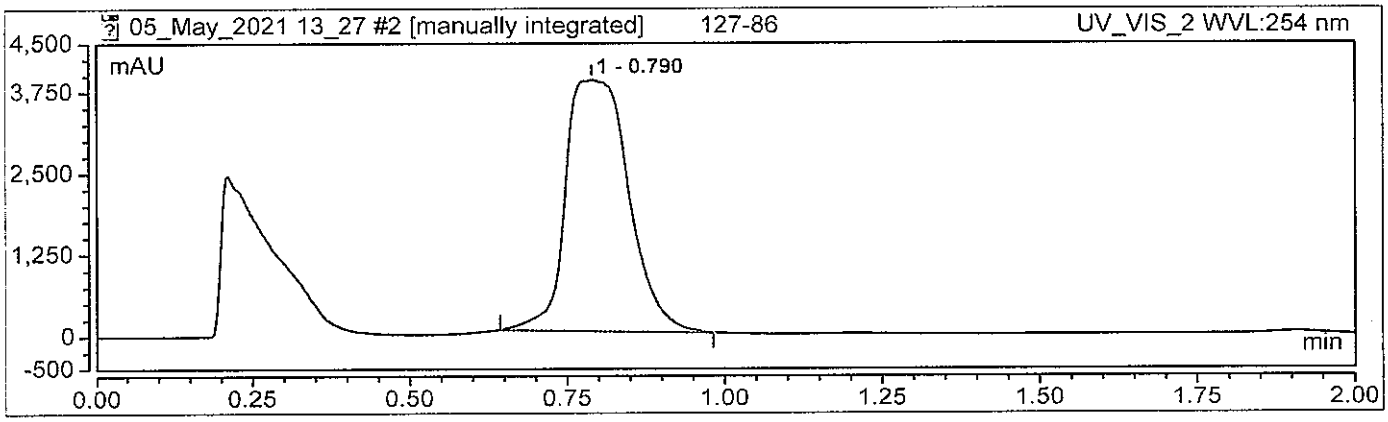
Injection Details

Injection Name:	**127-86**	*Run Time (min):*	**2.00**
Vial Number:	**R:A2**	*Injection Volume:*	**10.00**
Injection Type:	**Unknown**		
Calibration Level:			
Instrument Method:	**1.8uM_column_Tidwell_1.5_min_run4_agilent_zorbax1.8uM, 2.1x50**		
Processing Method:	**McHardy Mass Check**	*Dilution Factor:*	**1.0000**
Injection Date/Time:	**05/May/21 13:30**	*Sample Weight:*	**1.0000**

Mass Spectrum



Chromatogram



Table

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1		0.790	445.642	3854.108	100.00	100.00
Total:			**445.642**	**3854.108**	**100.00**	**100.00**

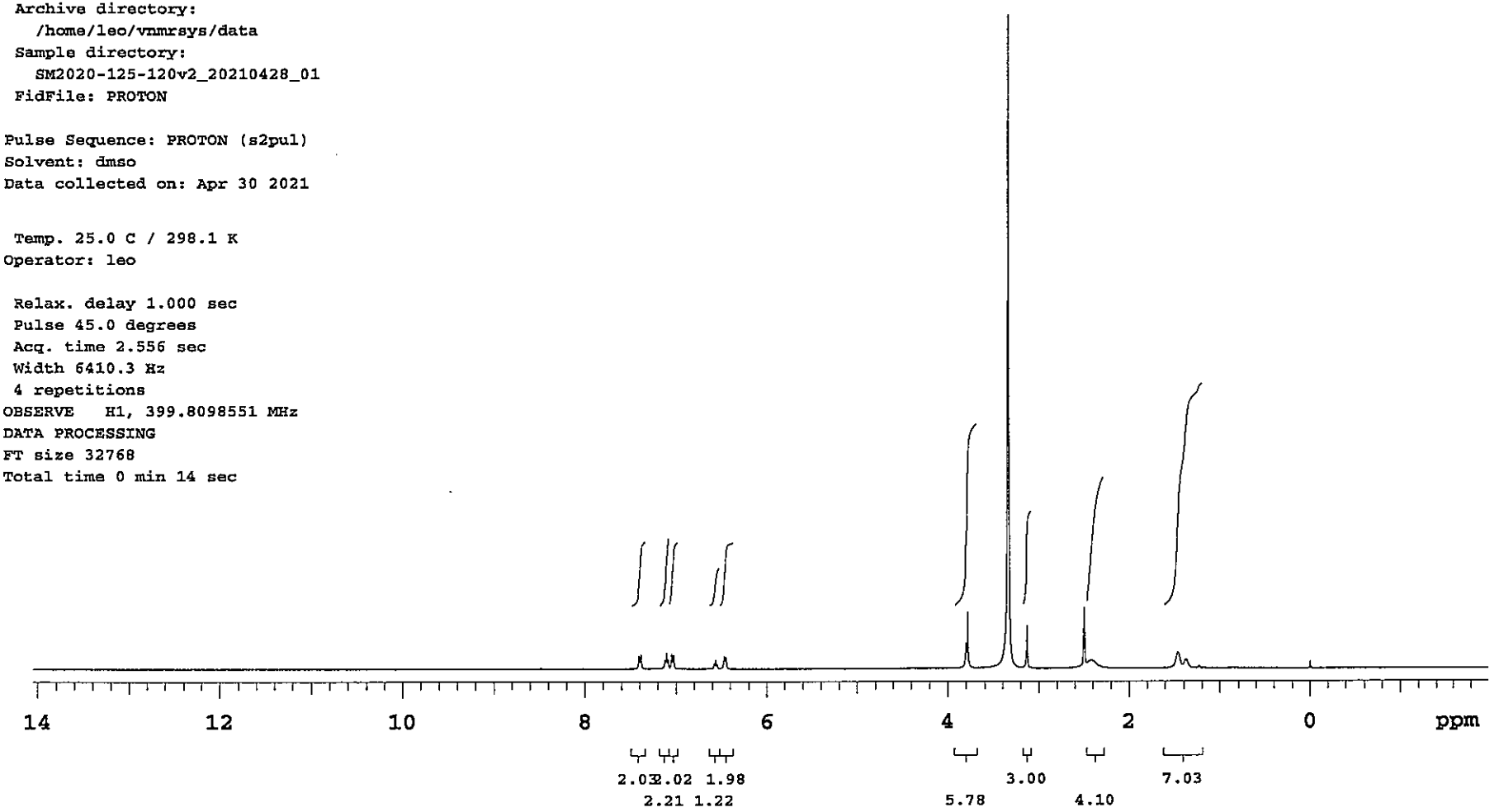
Agilent Technologies

Sample Name:
SM2021-127-84
Data Collected on:
400MR.McHardy.Lab-vnmrs400
Archive directory:
/home/leo/vnmrsys/data
Sample directory:
SM2020-125-120v2_20210428_01
FidFile: PROTON

Pulse Sequence: PROTON (s2pul)
Solvent: dmso
Data collected on: Apr 30 2021

Temp. 25.0 C / 298.1 K
Operator: leo

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 2.556 sec
Width 6410.3 Hz
4 repetitions
OBSERVE H1, 399.8098551 MHz
DATA PROCESSING
FT size 32768
Total time 0 min 14 sec

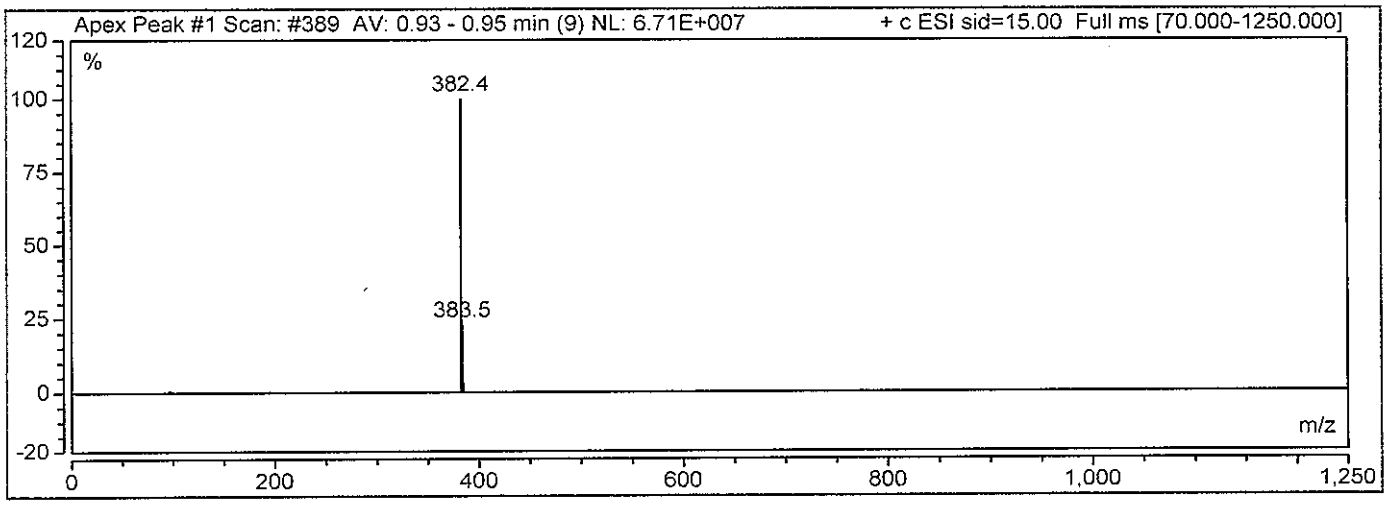


Peak Analysis

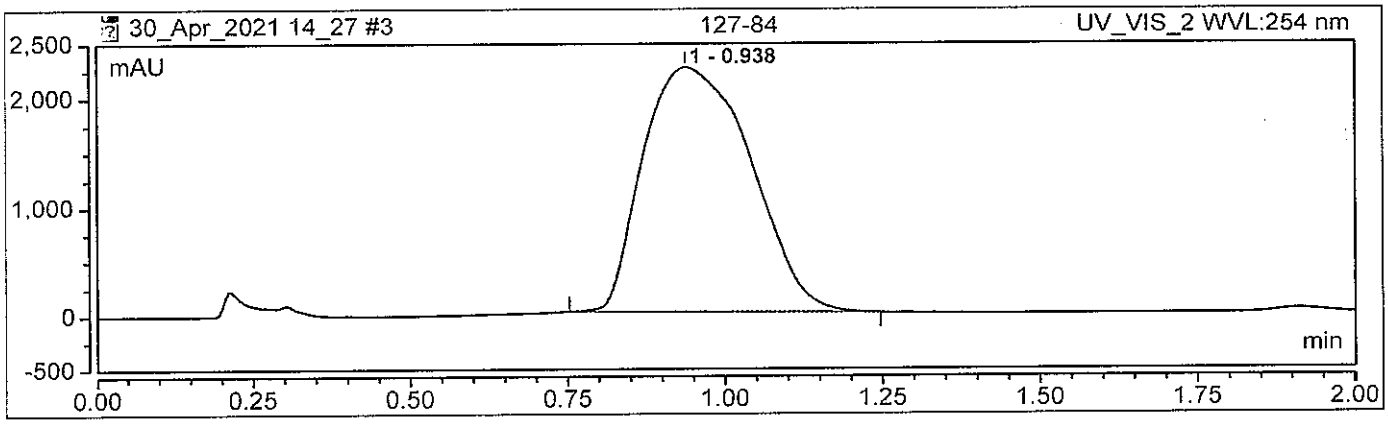
Injection Details

Injection Name:	**127-84**	*Run Time (min):*	**2.00**
Vial Number:	**R:A3**	*Injection Volume:*	**5.00**
Injection Type:	**Unknown**		
Calibration Level:			
Instrument Method:	**1.8uM_column_Tidwell_1.5_min_run4_agilent_zorbax1.8uM, 2.1x50**		
Processing Method:	**McHardy Mass Check**	*Dilution Factor:*	**1.0000**
Injection Date/Time:	**30/Apr/21 14:33**	*Sample Weight:*	**1.0000**

Mass Spectrum



Chromatogram



Table

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1		0.938	447.477	2252.141	100.00	100.00
Total:			**447.477**	**2252.141**	**100.00**	**100.00**

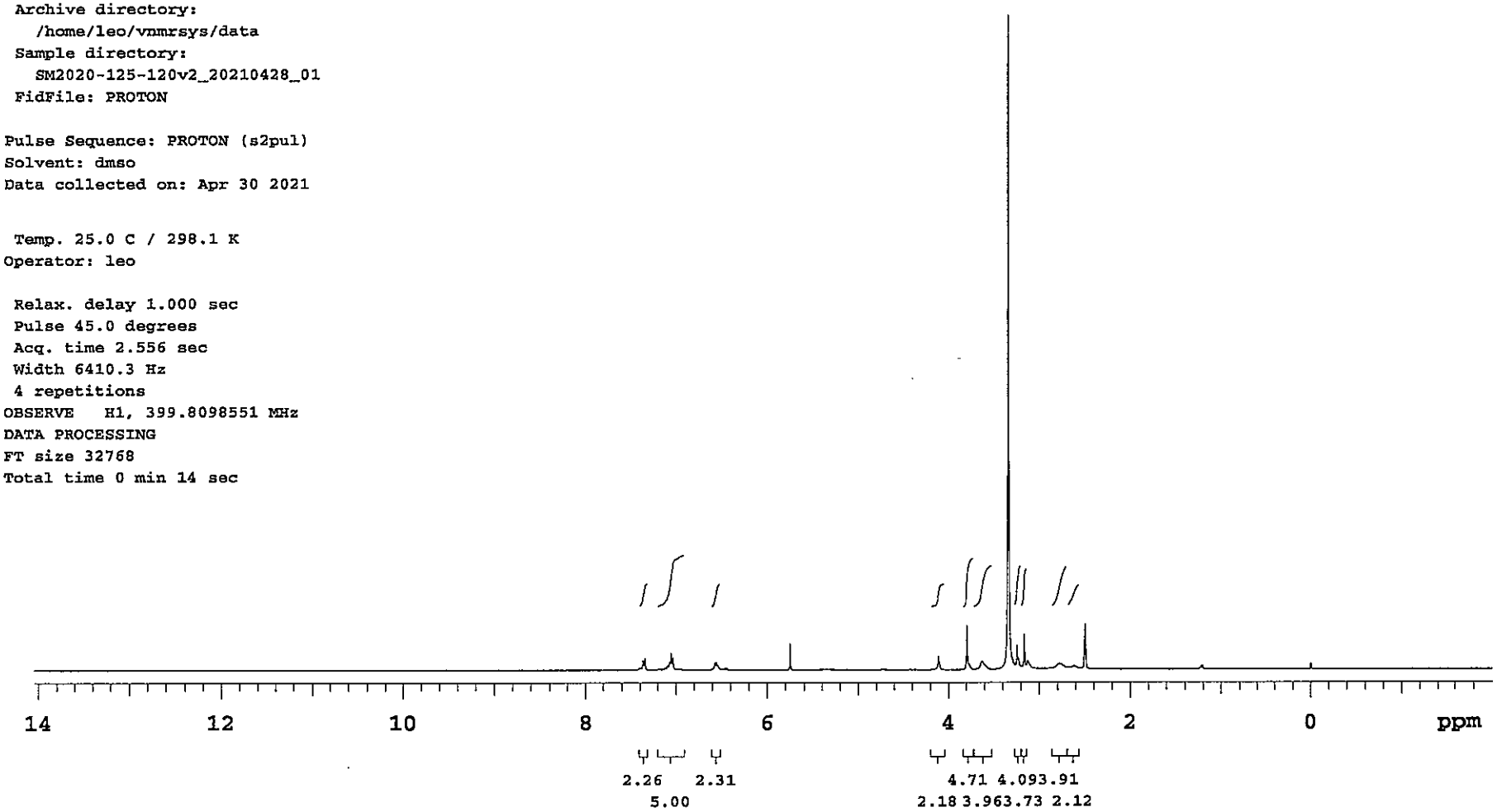
Agilent Technologies

Sample Name:
SM2021-127-87
Data Collected on:
400MR.McHardy.Lab-vnmrs400
Archive directory:
/home/leo/vnmrsys/data
Sample directory:
SM2020-125-120v2_20210428_01
FidFile: PROTON

Pulse Sequence: PROTON (s2pul)
Solvent: dmso
Data collected on: Apr 30 2021

Temp. 25.0 C / 298.1 K
Operator: leo

Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 2.556 sec
Width 6410.3 Hz
4 repetitions
OBSERVE H1, 399.8098551 MHz
DATA PROCESSING
FT size 32768
Total time 0 min 14 sec

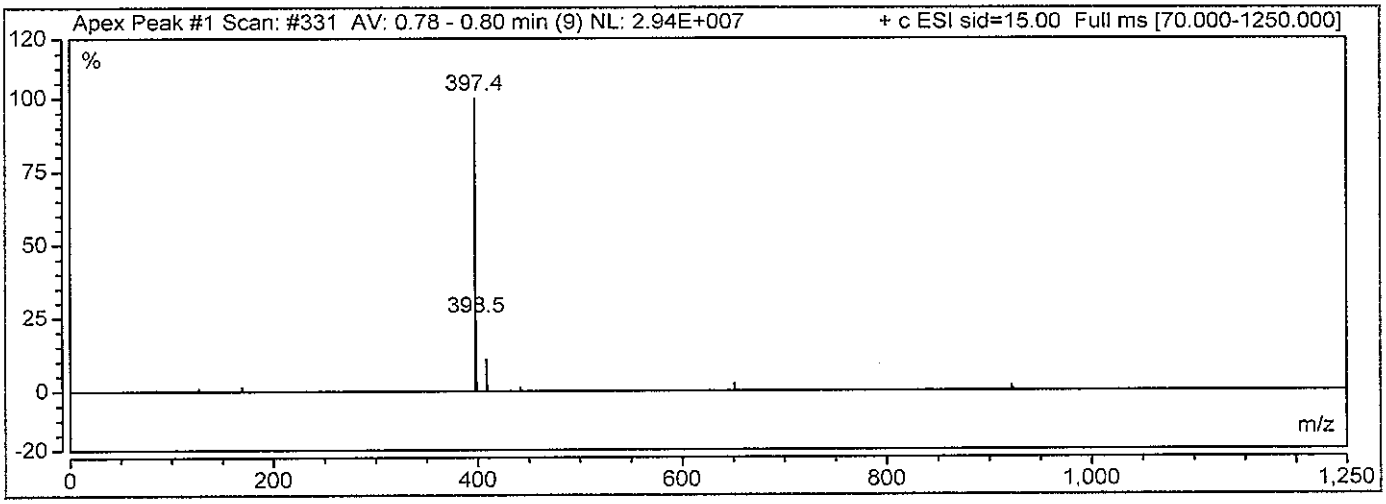


Peak Analysis

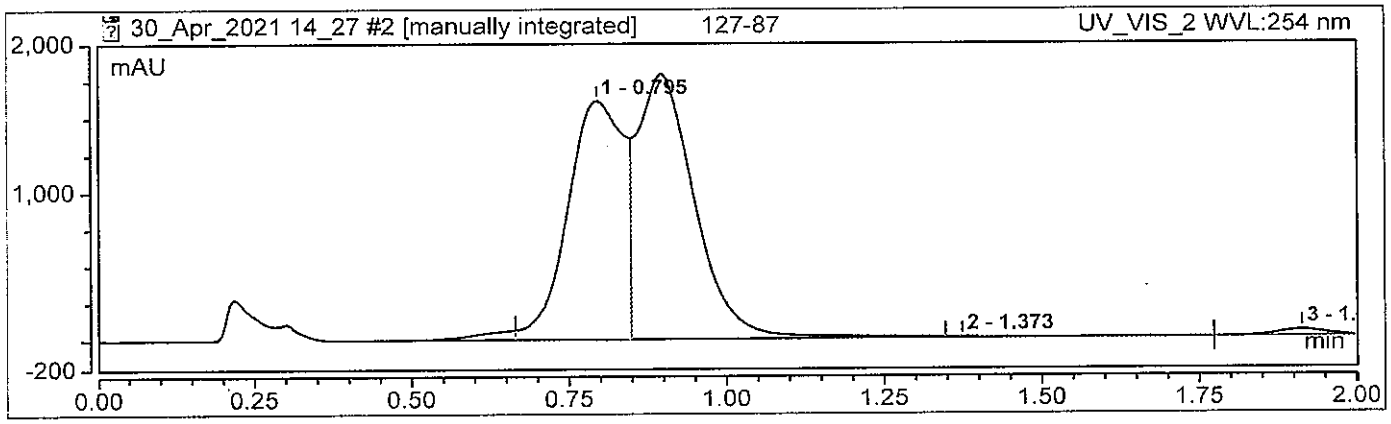
Injection Details

Injection Name:	**127-87**	*Run Time (min):*	**2.00**
Vial Number:	**R:A2**	*Injection Volume:*	**5.00**
Injection Type:	**Unknown**		
Calibration Level:			
Instrument Method:	**1.8uM_column_Tidwell_1.5_min_run4_agilent_zorbax1.8uM, 2.1x50**		
Processing Method:	**McHardy Mass Check**	*Dilution Factor:*	**1.0000**
Injection Date/Time:	**30/Apr/21 14:31**	*Sample Weight:*	**1.0000**

Mass Spectrum



Chromatogram



Table

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
1		0.795	165.541	1612.284	97.20	97.08
2		1.373	1.113	7.315	0.65	0.44
3		1.913	3.664	41.126	2.15	2.48
Total:			**170.317**	**1660.725**	**100.00**	**100.00**