# HPLC and Spectrophotometric Analysis of Injectables Advertised as GC376 or GS-441524 Found to Contain EIDD-2801 as the Main Active Ingredient

Presented by the FIP Warriors® Research Team



# The Following is a Summary of the Data Collected During an Investigation by FIP Warriors® between February -July 2023

#### The summary includes:

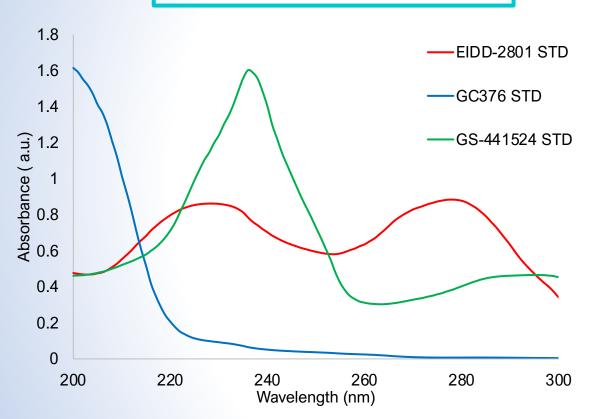
- UV and pH measurements performed by the FIP Warriors® research team
- HPLC and UV measurements performed by Expert Synthesis Solutions
- GC-MS performed by Toxicology Associates for FIP Warriors® and FIP Global CATS with disclaimer of improper testing
- Brief reports of incidents associated with the use of 2 of the tested brands
- Resources and contacts

NAME/DESCRIPTION	IMAGE	ADVERTISED DRUG AND CONCENTRATION	ACTUAL DRUG FOUND AND CONCENTRATION*	MEASURED PH	
GC376 2ml vial clear cap (sent by Nicole Jacque)		GC376 53mg/ml	EIDD-2801 50mg/ml (HPLC and UV)	6.53-7.08 (multiple vials tested)	* C O
SUYI BIO (same as Hebei Ruiyao Biotech)		GS-441524 20mg/ml	EIDD-2801 40mg/ml (HPLC and UV)	5.19	concentration
PethealthFIP Gold and pink cap (pethealthfip.com)		GS-441524 20mg/ml (pink) and 30mg/ml (gold)	EIDD-2801 70mg/ml (pink) and 60mg/ml (gold) (UV)	5.70 (pink ) 5.89 (gold)	<u>~</u> .
Cat rehabilitation nutrition supplement- appetizer (sold with syringe)	猫传染性腹膜炎 Feline Infectious peritonitis (Fipu)  传腹清**  Cat rehabilitation nutrition supplement  Veterinary over-the-counter  Appetizer  Appetizer	GS-441524 20mg/ml	EIDD-2801 70mg/ml (UV)	6.87	estimated
<b>Chariot</b> (distributed from North Carolina)		GS-441524 20mg/ml	EIDD-2801 35mg/ml (UV)	6.34	from the
5 and 10ml purple cap vials sold by "Susan" (XUN HE- connected to Ruiyao Biotech)	Susan +86 199481	GS-441524 20mg/ml (later seller declared to use "anti-freeze" which led to the death of 2 cats due to kidney failure)	EIDD-2801 65-70mg/ml (UV)	5.51 (5ml) 5.59 (10ml)	ne UV spectra
Labeled 1,2- dihydroxypropane 10ml vial	Tanzan Ta	GS-441524 20mg/ml	EIDD-2801 70mg/ml (UV)	4.80	tra

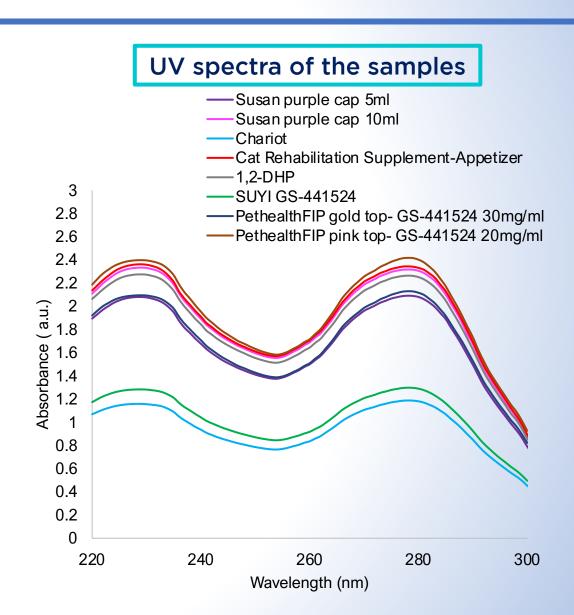
# **NOTES**

- The tested brands of GS-441524 were advertised as "painless", "water-based" GS-441524 at pH 6-7
- In China, the current cost of GC376 API is **5-10x the cost** of GS-441524 and about **100x the cost** of EIDD-2801. **Therefore, it is not possible to purchase vials of GC376 at a cost comparable to or lower than GS-441524.**
- The pH was measured with a Thermo Scientific Orion Star A111 pH meter, calibrated with 3 buffers. The correct measurement of the pH was further verified using a control buffer at pH 1.68 purchased from Fisher Scientific.
- The UV spectra were recorded with a spectrophotometer Spectramax M5 and compared to a standard reference purchased from Medkoo Biosciences.
- Expert Synthesis Solution (ESS) performed the HPLC analysis and replicated the UV spectrophotometric analysis confirming the results.

#### UV spectra of the standards



CONCLUSION: The spectra of the samples match the one of EIDD-2801 standard reference. HPLC is needed to confirm if the samples contain EIDD-2801 or EIDD-1931.



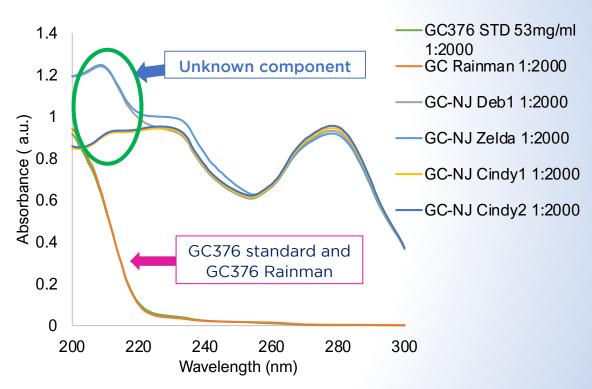


Rainman GC376



New "GC376"

UV spectra of a standard of GC376 compared to Rainman GC and the new vials advertised as GC376



Earlier this year, it was noted that the GC376 vials distributed had changed without any notice. Therefore, multiple samples from different parents have been collected and tested.

All the samples tested showed the presence of EIDD and possibly other components at the UV.

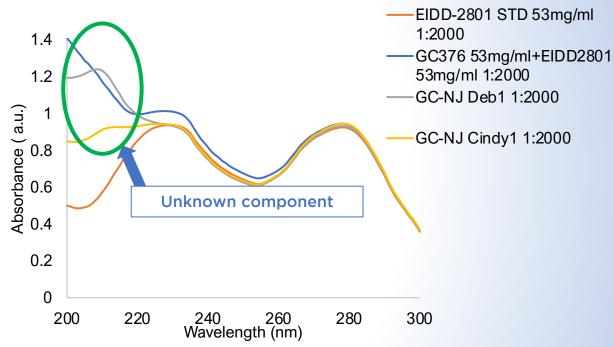


Rainman GC376



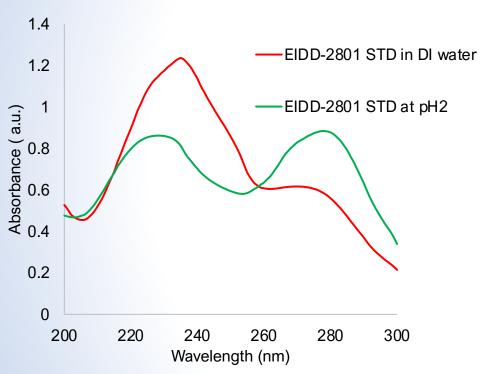
New "GC376"

# UV spectra of a standard of EIDD-2801 compared to the new vials advertised as GC376

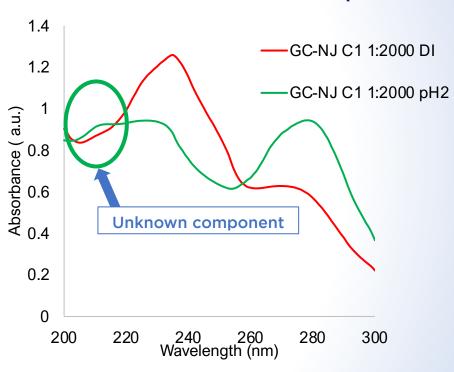


- The "new GC" in clear cap vials was compared to a standard of EIDD-2801 at 53mg/ml showing a perfect overlap of the region between 230nm and 300nm. That indicates the possible presence of EIDD-2801 at similar concentration
- We compared the spectra to a solution combining GC376 and EIDD-2801 but there is no overlap in the lower region
- The samples were sent to ESS for HPLC analysis, **confirming the presence of EIDD-2801. GC376 was not detected**





# UV spectra of the new "GC376" at different pH



The spectrum of EIDD-2801 is well documented in literature\* and shows a different profile depending on the pH. The new vials distributed as substitution to GC376 from Rainman showed a UV spectrum similar to EIDD-2801 at both the tested pH. A third peak below 220nm appears at pH 2 which may be due to an excipient or another drug.

## **HPLC** and UV Measurements Performed by Expert Synthesis Solutions



FINAL REPORT

Document#: FR-ESS070823 Revision: 1

Date: 07/08/23

# REPORT FOR HPLC METHOD DEVELOPMENT FOR THE IDENTIFICATION OF EIDD-2801, EIDD-1931, GC376, AND GS-441524 IN CAPSULES AND INIECTABLE FINISHED PRODUCTS

Revision history: R01. 07/08/23 - The document was generated

Prepared by:

Viorica Rusu, QA/QC Manager

Table 1. List of Raw Materials and Finished Products

Raw Material (Standard) Name	Lot#	Manufacturer	Quantity	Date received
EIDD-1931 (99%)	T20D09P22	MedKoo Biosciences	100 mg	
EIDD-2801 (99%)	C22R02B02	MedKoo Biosciences	100 mg	ctandarde
GC376 Sodium	C9R03B20	MedKoo Biosciences	100 mg	standards
GS-441524	C21R07B12	MedKoo Biosciences	135 mg	
Finished Product	Composition			
EIDD-2801 capsules	50mg EIDD-2801	Not available		
EIDD-1931 capsules	50mg EIDD-1831	Not available		-
GS-441524 capsules	20mg GS-441524	Not available		
GC-NJ	GC-376/EIDD-2801/EIDD- 1931, 53 mg/mL	Not available	5 vials x 2mL	06/29/2023
GS-SUYI	GS-441524/EIDD- 2801/EIDD-1931, 20-40mg/mL	Not available	1 vial x 5mL	
GC-RAINMAN	GC376 (PEG400 Ethanol Water ) 53mg/mL	Not available	1 vial x 4mL	
GS-441524 Injectable (batch 4-12-23)	GS-441524 (PEG400, prop gly, water, ethanol) 20mg/mL	Not available	1 vial x10mL	
Placebo (to GS-441524 Injectable)	(PEG400, prop gly, water, ethanol)	Not available	1 vial x10mL	
GC-TC *	Not Available	Not available	1 vial x2mL	07/06/23

\*GC-TC was shipped directly from Canada to ESS for testing

The vial was donated by an owner whose cat had passed while utilizing the 2ml vials of "GC376"

The cat exhibited low WBC (more details will be discussed later)

## HPLC and UV Measurements Performed by Expert Synthesis Solutions



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FINAL REPORT

Document#: FR-ESS070823 Revision: 1 Date: 07/08/23

High-performance liquid chromatography (HPLC) methods were developed to identify the active ingredients in finished products listed in Section 2.

The HPLC (equipped with DAD) technique involved the separation (elution at different retention times) of EIDD-2801, EIDD-1931, GC376, and GS-441524 in Raw Material (RM) solutions, monitoring the UV spectra as additional means of identification of these compounds, and compare them with main active ingredient in the samples prepared from injectable and capsule finished product listed in Section 2. The chromatographic analysis was performed using Zorbax SB-C18 4.6x250mm 5µm column, UV detection at 220nm with full UV spectrum acquisition. The mobile phase A was 100% 20mM KH2PO4 buffer with pH7.5, and Mobile phase B was 100% Acetonitrile with a flow rate 1.0ml/min and the gradient ratios as per chromatographic conditions in Section 4. The individual Raw Materials were analyzed to identify their unique retention times (RT) and UV spectra. Finished Product sample solutions were analyzed and the RT and UV spectra of main peaks were compared to those of individual Raw Materials. The summary of the identification is presented in Table 2. Additionally, spiked with EIDD-2801 samples were prepared for GC-NJ, GS-SUYI and GC-TC injectable Finished Product and analyzed to confirm the presence of EIDD-2801 as main active ingredient in the unspiked samples.

Table 2. Summary of API identification in Finished Product

T 11 1D 1	API				
Finished Product	EIDD-1931	EIDD-2801	GC376 Sodium	GS-441524	
EIDD-2801 capsules		v			
EIDD-1931 capsules	V				
GS-441524 capsules				V	
GC-NJ		V			
GS-SUYI		V			
GC-RAINMAN			V*		
GS-441524 Injectable				v	
(batch 4-12-23)					
Placebo (to GS-441524				ND	
Injectable)					
GC-TC		V			

\*NOTE 2: Some stability issues of GC376 in solution was observed (Lab Book (LB) reference: VR-4, p.140, 148, 150, 151)

#### 5.3 ACTIVE INGREDIENT EIDD-2801











In the RM solution a major peak eluting at RT 9.682 min was observed (INJ ID: SIG11418). Similar major peak with matching RTs and same UV spectrum profile were observed in the following Finished Products:

-GC-NJ (INJ ID: SIG11420), confirmed by spiked sample (INJ ID: SIG11444);

-CS-SUYI (INJ ID: SIG11422), confirmed by spiked sample (INJ ID: SIG11446, SIG11448);

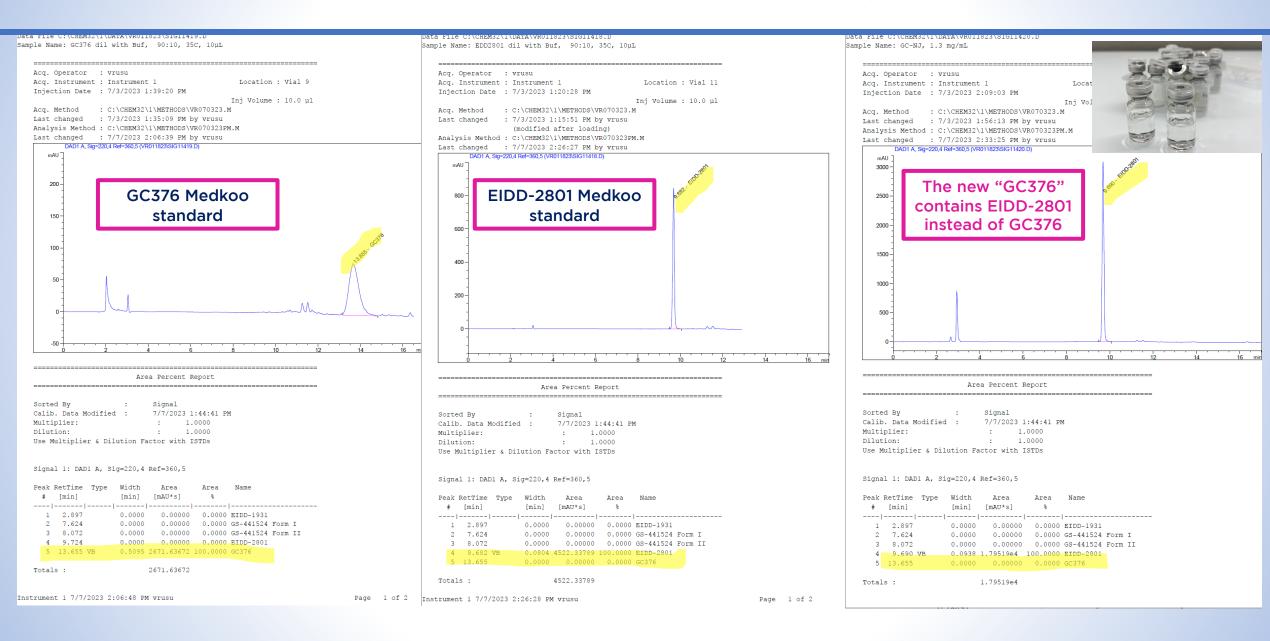
-GC-TC (INJ ID: SIG11457, SIG11460), confirmed by spiked sample (INJ ID: SIG11458, SIG11459);

-EIDD-2801 white capsules (INJ ID: SIG11427) (no need for spiked sample preparation as tis product doesn't contradict with the active ingredient identification claim).

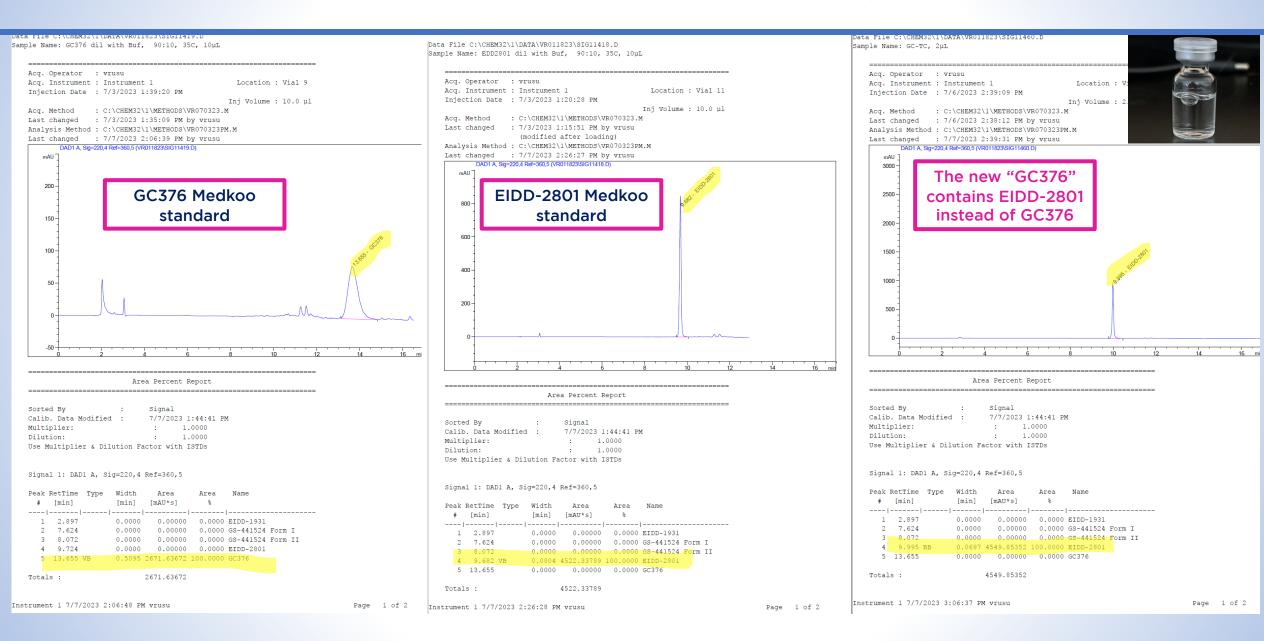
It is concluded that these Finished Products contain EIDD-2801 as active ingredient.

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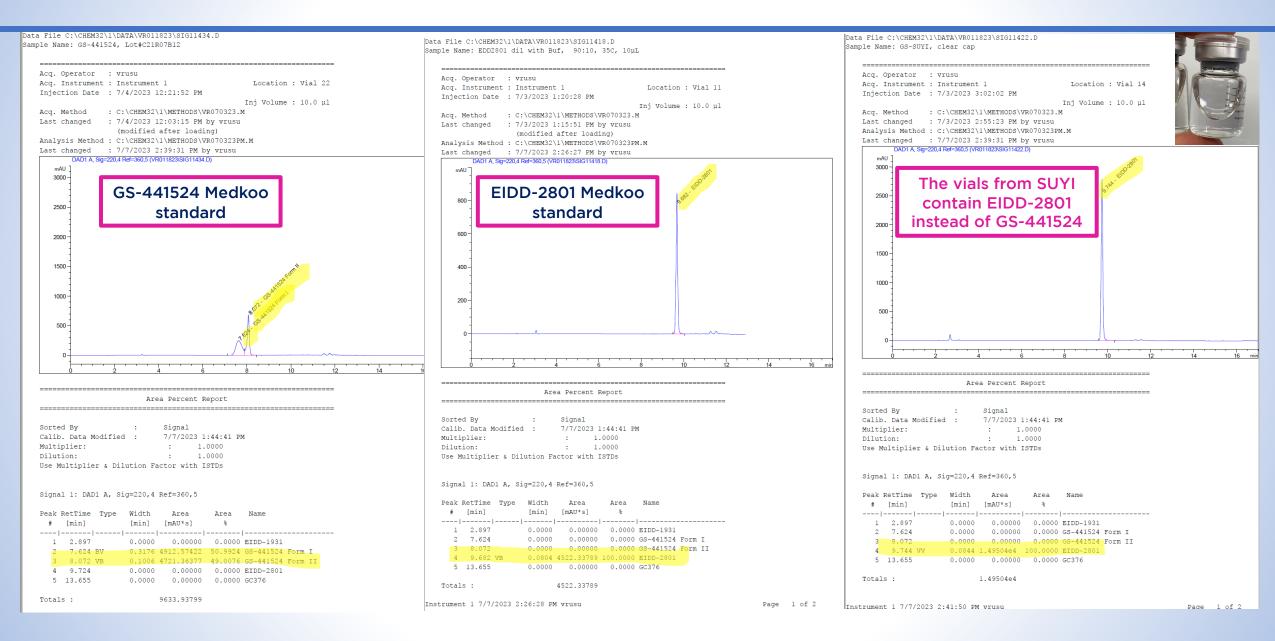
# **HPLC Measurements Performed by Expert Synthesis Solutions**



## **HPLC Measurements Performed by Expert Synthesis Solutions**



## **HPLC Measurements Performed by Expert Synthesis Solutions**



#### **UV** measurements performed by Expert Synthesis Solutions

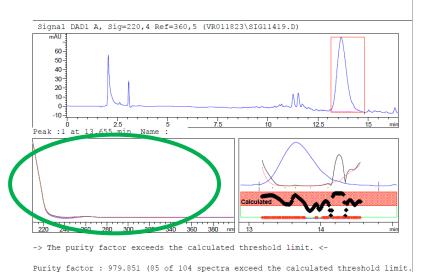
#### GC376 Medkoo standard

**EIDD-2801** Medkoo standard

The new "GC376" contains EIDD-2801 instead of GC376



Data File C:\CHEM32\1\DATA\VR011823\SIG11419.D Sample Name: GC376 dil with Buf, 90:10, 35C, 10uL



: 996.886 (Calculated with 85 of 104 spectra)

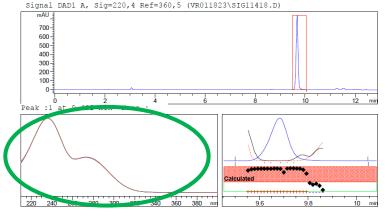
\*\*\* End of Report \*\*\*

: 5 (Selection automatic, 5)

Noise Threshol: 0.147 (12 spectra, St.Dev 0.0529 + 3 \* 0.0313)

: Peak start and end spectra (integrated) (13.143 / 14.809)

Data File C:\CHEM32\1\DATA\VR011823\SIG11418.D Sample Name: EDD2801 dil with Buf, 90:10, 35C, 10µL



-> The purity factor exceeds the calculated threshold limit. <-

Purity factor: 998.840 (19 of 24 spectra exceed the calculated threshold limit.) : 999.835 (Calculated with 19 of 24 spectra)

: Peak start and end spectra (integrated) (9.500 / 10.046) Reference

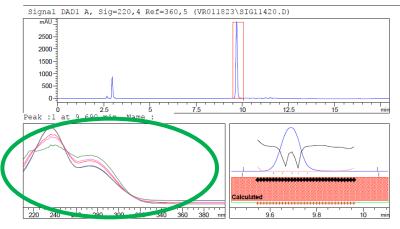
: 5 (Selection automatic, 5)

Noise Threshol: 0.110 (12 spectra, St.Dev 0.0541 + 3 \* 0.0186)

: Spectral absorbances > 1000 mAU (see help for more information)

\*\*\* End of Report \*\*\*

Data File C:\CHEM32\1\DATA\VR011823\SIG11420.D Sample Name: GC-NJ, 1.3 mg/mL



-> The purity factor exceeds the calculated threshold limit. <-

Purity factor: 964.163 (32 of 32 spectra exceed the calculated threshold limit.) : 999.949 (Calculated with 32 of 32 spectra)

: Peak start and end spectra (integrated) (9.481 / 10.067)

: 5 (Selection automatic, 5)

Noise Threshol: 0.037 (12 spectra, St.Dev 0.0211 + 3 \* 0.0054)

: Spectral absorbances > 1000 mAU (see help for more information)

\*\*\* End of Report \*\*\*

ESS recorded the UV spectra of the samples (green circle), confirming the data reported by the FIP Warriors® research team.

## **UV Measurements Performed by Expert Synthesis Solutions**

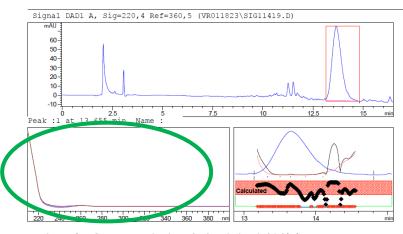
#### GC376 Medkoo standard

#### EIDD-2801 Medkoo standard

The vial of "GC376" from a parent in Canada contains EIDD-2801 instead of GC376



Data File C:\CHEM32\1\DATA\VR011823\SIG11419.D Sample Name: GC376 dil with Buf, 90:10, 35C, 10µL



-> The purity factor exceeds the calculated threshold limit. <-

Purity factor: 979.851 (85 of 104 spectra exceed the calculated threshold limit. Threshold: 996.886 (Calculated with 85 of 104 spectra)

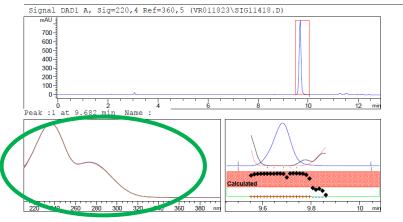
Reference : Peak start and end spectra (integrated) (13.143 / 14.809)

Spectra : 5 (Selection automatic, 5)

Noise Threshol: 0.147 (12 spectra, St.Dev 0.0529 + 3 \* 0.0313)

\*\*\* End of Report \*\*\*

Data File C:\CHEM32\1\DATA\VR011823\SIG11418.D Sample Name: EDD2801 dil with Buf, 90:10, 35C, 10µL



-> The purity factor exceeds the calculated threshold limit. <-

Purity factor: 998.840 (19 of 24 spectra exceed the calculated threshold limit.)

Threshold : 999.835 (Calculated with 19 of 24 spectra)

Reference : Peak start and end spectra (integrated) (9.500 / 10.046)

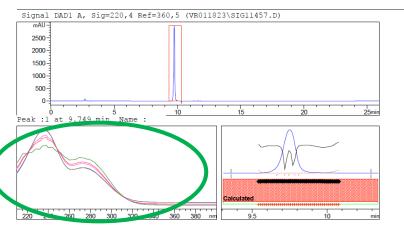
Spectra : 5 (Selection automatic, 5)

Noise Threshol: 0.110 (12 spectra, St.Dev 0.0541 + 3 \* 0.0186)

rning : Spectral absorbances > 1000 mAU (see help for more information)

\*\*\* End of Report \*\*\*

Data File C:\CHEM32\1\DATA\VR011823\SIG11457.D Sample Name: GR-TC



-> The purity factor exceeds the calculated threshold limit. <-

Purity factor: 973.445 (41 of 41 spectra exceed the calculated threshold limit.)

Threshold : 999.965 (Calculated with 41 of 41 spectra)

Reference : Peak start and end spectra (integrated) (9.359 / 10.292)

Spectra : 5 (Selection automatic, 5)

Noise Threshol: 0.031 (12 spectra, St.Dev 0.0219 + 3 \* 0.0031)

Warning : Spectral absorbances > 1000 mAU (see help for more information)

\*\*\* End of Report \*\*\*

ESS recorded the UV spectra of the samples (green circle), confirming the data reported by the FIP Warriors® research team.

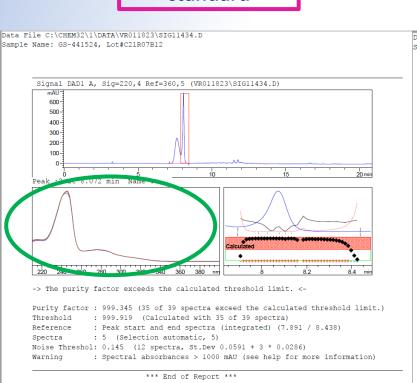
## **UV Measurements Performed by Expert Synthesis Solutions**

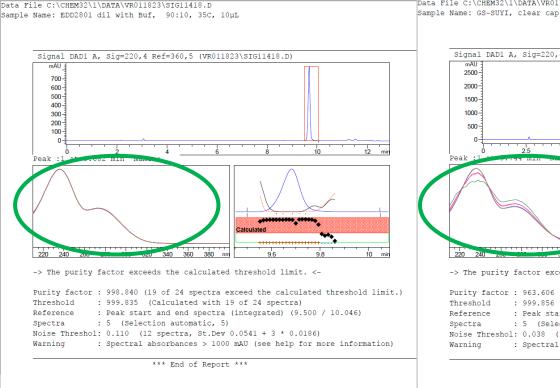
GC376 Medkoo standard

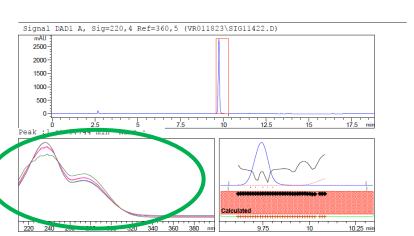
#### **EIDD-2801** Medkoo standard

The vials from SUYI contain EIDD-2801 instead of GS-441524









-> The purity factor exceeds the calculated threshold limit. <-

Purity factor: 963.606 (35 of 35 spectra exceed the calculated threshold limit.)

: 999.856 (Calculated with 35 of 35 spectra)

: Peak start and end spectra (integrated) (9.563 / 10.310)

: 5 (Selection automatic, 5)

Noise Threshol: 0.038 (12 spectra, St.Dev 0.0214 + 3 \* 0.0057)

: Spectral absorbances > 1000 mAU (see help for more information)

\*\*\* End of Report \*\*\*

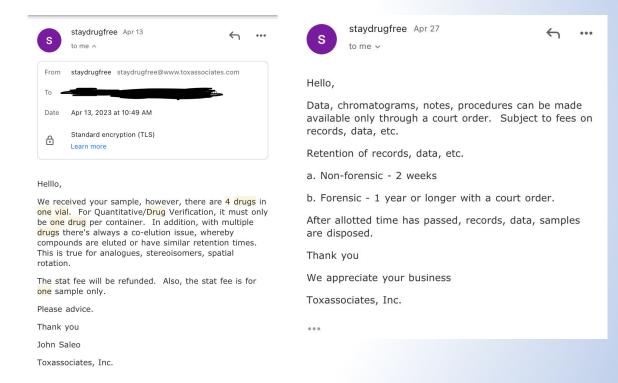
ESS recorded the UV spectra of the samples (green circle), confirming the data reported by the FIP Warriors® research team.

## **GC-MS** Measurements Performed by Toxicology Associates

Before the samples were sent to ESS for HPLC analysis both <u>FIP Warriors®</u> and <u>FIP Global CATS</u> used Toxicology Associates as external lab for testing. However, further tests proved that the results obtained by that lab were not reliable.

#### **RED FLAGS:**

- They do not request reference standards to compare the results
- They did not request placebos to make sure that there was no interference (when determining the purity)
- Tests are under \$300 for any drug (only the standard may cost \$500 or more)
- They cannot test multiple drugs in the same sample therefore they cannot verify which drug is in the sample- a drug is found and the name provided by the client is assigned as drug name
- They do not provide the raw data as any reliable testing company should
- When 4 vials of the same batch were sent for testing 1 drug per vial they found all the drugs requested at similar purity.



Because we believed that the results from Toxicology Associates were not reliable, the reports of testing for the 2ml "GC376" vials were not divulgated by FIP Warriors® in the previous report.

Results are shown in the next slides for information purposes only.

#### **GC-MS** Measurements Performed by Toxicology Associates

DISCLAIMER: the reports on this slide are not reliable and are being shown for information purposes only.

ALL THE REQUESTED DRUGS WERE PRESENT ACCORDING TO TOXICOLOGY ASSOCIATES. HOWEVER, ONLY EIDD-2801 CAN BE SEEN IN THE HPLC.

Four vials of the new "GC376" were sent for testing to Toxicology Associates in April 2023. Because the company was unable to test 4 drugs in one vial, 4 vials were sent to be tested individually for each drug requested.





## **GC-MS** Measurements Performed by Toxicology Associates

# DISCLAIMER: the reports on this slide are not reliable and are being shown for information purposes only.

#### GC376

Date Obtained from Vendor: April 2023

Advertised Strength: 53 mg/ml

Sold As: 2 mL vial

Notes:



Concentration: Not tested

Purity: 97.65% (+/- 15%)

pH: Not tested

Testing Lab: Toxicology Associates

Testing Method: GCMS
Date Tested: 5/22/2023

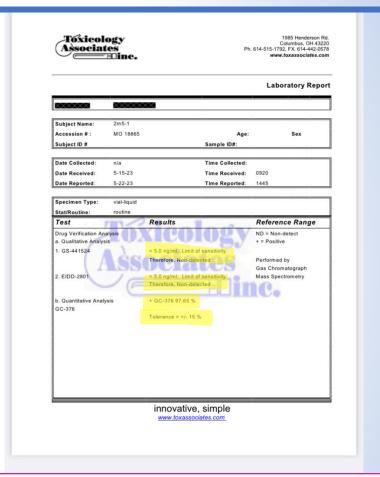
Other Testing Results/Notes:

This sample was also tested for the presence of GS-441524 and Molnupiravir (EIDD-2801). None was detected.





FROM FIPSAFE.ORG



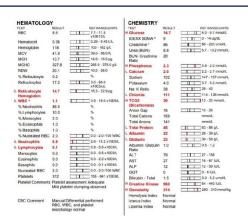
SAMPLE SENT BY FIP GLOBAL CATS

#### Reported Incidents with the New "GC376"



The vial of "GC376" from a parent in Canada contains EIDD-2801 instead of GC376 <u>Cat #1</u> in Canada was initially treated with EIDD and Spark oral GS. Oral GS was interrupted but because the cat experienced seizures, oral treatment with GS was resumed. The WBC count was normal on CBC. Two days after the bloodwork, EIDD was stopped and substituted with injections of the new "GC376" at 20mg/kg BID. The cat experienced seizures again after a week and the dosage of both drugs was increased to 60mg/kg oral GS and 50mg/kg BID "GC376". The WBC count was low and the vet suspected that the treatment was the cause. The cat passed away of cardiac arrest. The parent donated the vial that was sent to ESS for HPLC testing, proving that the main active ingredient was EIDD-2801.

Cat #2 in Canada exhibited an initial improvement on these vials at 20mg/kg BID and oral GS-441524. The dosage was increased to 40mg/kg BID due to decline after switching to another brand of real GC376. Treatment with the new "GC376" was resumed after few days. The dosage of "GC376" was raised again (60-70mg/kg BID). The cat exhibited droopy ears, hair loss, weight loss and low WBC count. The brand of GS was changed but the cat continued to decline and the ears did not go back to normal as it is seen when stopping EIDD within 2-3 weeks. The cat was humanely euthanized after a month.









March 16<sup>th</sup> 2023 (the ears continued to fold)

<u>Cat #3</u> in USA received a combination of oral GS-441524 and the new "GC376" at 20mg/kg BID for 2.5 months then 30mg/kg BID for 2 weeks.

The cat showed hair loss under the chin and whiskers loss which resolved in observation. Unfortunately, the cat relapsed with weight loss, depression and AKI. Cat n. 3 is currently undergoing treatment for the third time.









Whiskers grew back after coming off EIDD-2801 ("new GC376")

#### Reported incidents with SUYI



The vials from SUYI contain EIDD-2801 instead of GS-441524

Cat #1 in Korea showed droopy ears after 2 months of administration at 1pill/kg (pills were labeled as 40mg or 60mg- the actual amount of the drug has not been tested yet). Owner reported that droopy ears is a common side effect noted with SUYI in Korea and that it was reported by many parents. Owner reduced the dosage and the ears returned normal within days.





Cat #1 on SUYI pills

Cat #2 in Hungary exhibited droopy ears and low WBC/Neu. The admin following the parent reported that the cat showed droopy ears on day 70 of relapse treatment at 17mg/kg of SUYI injectable sold as 20mg/ml GS-441524 (40mg/ml EIDD tested in lab). Therefore the estimated dosage of EIDD was about 34mg/kg. Few days later bloodwork showed low WBC, neutrophils, lymphocytes and monocytes. The cat was put on antibiotics to prevent infections and switched to another brand of GS-441524 to continue treatment. Ears went back to normal and WBC increased after 2-3 weeks.

#### Cat #2 CBC

Fehérvérsejtszám (WBC)		2.3	G/L	6.0 - 15.0
Neutrophil abs. (Advia 120)		0.8	G/L	3.1 - 12.5
Lymphocyta abs. (Advia 120)	-	1.40	G/L	1.50 - 7.50
Monocyta abs. (Advia 120)	-	0.00	G/L	0.15 - 1.10
Eosinophil abs. (Advia 120)		0.10	G/L	0.06 - 2.21
Basophil abs. (Advia 120)		0.00	G/L	- 0.08
Larna Unetained Calle also (Advis190)		0.00	G/I	- 0.26



Cat #2 on SUYI injections



Cat #2 3 weeks after interruption of SUYI injections

# CONCLUSIONS

#### The following vials were found to contain **EIDD-2801** instead of the advertised drugs:

- "GC376" 2ml vial
- SUYI injectable (pills will be tested soon)
- Chariot
- PetHealthFIP
- Cat rehabilitation nutrition supplement- appetizer (sold with syringe)
- Purple Cap vials by Susan (XUN HE-connected to Ruiyao Biotech)
- 10ml vial labeled as 1,2-Dihydroxypropane

<u>Expert Synthesis Solution (ESS)</u> performed the HPLC analysis and the UV spectrophotometric analysis confirming the results previously divulgated by FIP Warriors®

Brands advertising their products as <u>"water-based"/"painless"/high pH</u>GS-441524 should be <u>avoided</u>

Sellers are warned that products will be periodically tested and that EIDD-2801 should not be used instead of GS-441524, which remains the safest drug for treatment of FIP

#### **Resources & Contacts**

Please join **FIP Warriors® Research and Data** to stay up to date with tests and the research: https://www.facebook.com/groups/fipwarriorsresearch



If you used or are using one of the brands mentioned in this report please contact your admin or the research team at FIPwarriors.data@gmail.com

#### DO NOT STOP TREATMENT WITHOUT DISCUSSING WITH YOUR VETERINARIAN AND YOUR ADMIN

some vials containing EIDD-2801 instead of GS-441524 can be recognized by the color (clear vs light yellow tinge) and the smell ("disinfectant" vs ethanol).



