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TECHNICAL PACKAGE FOR
PURIFICATION OF BEDAQUILINE AND FORMATION OF FUMARATE SALT
(MBR-M4ALL-BDQ-2)

Prepared By	Checked By	Reviewed By
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Designation: Research Scientist	Designation: Lead Scientist	Designation: Group Lead

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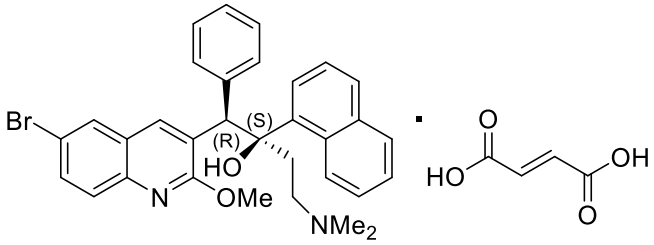
Contents

INTRODUCTION

Bedaquiline, a diarylquinoline (DARQs), inhibits mycobacterial adenosine triphosphate (ATP) synthase and is licensed to treat drug-resistant tuberculosis.

The description of bedaquiline is shown below:

Project Code	CR592
Product Name:	Bedaquiline fumarate
MBR Number:	MBR-M4ALL-BDQ-2
IUPAC Name	(1*R*,2*S*)-1-(6-bromo-2-methoxyquinolin-3-yl)-4-(dimethylamino)-2-naphthalen-1-yl-1-phenylbutan-2-ol;(*E*)-but-2-enedioic acid
Synonyms	(1*R*,2*S*)-1-(6-Bromo-2-methoxy-3-quinolyl)-4-dimethylamino-2-(1-naphthyl)-1-phenyl-butan-2-ol fumarate
CAS Number	845533-86-0
Chemical Formula	$C_{36}H_{35}BrN_2O_6$
Molecular Weight	671.6
Structure	
Physical Appearance	White solid
Solubility	Acetone

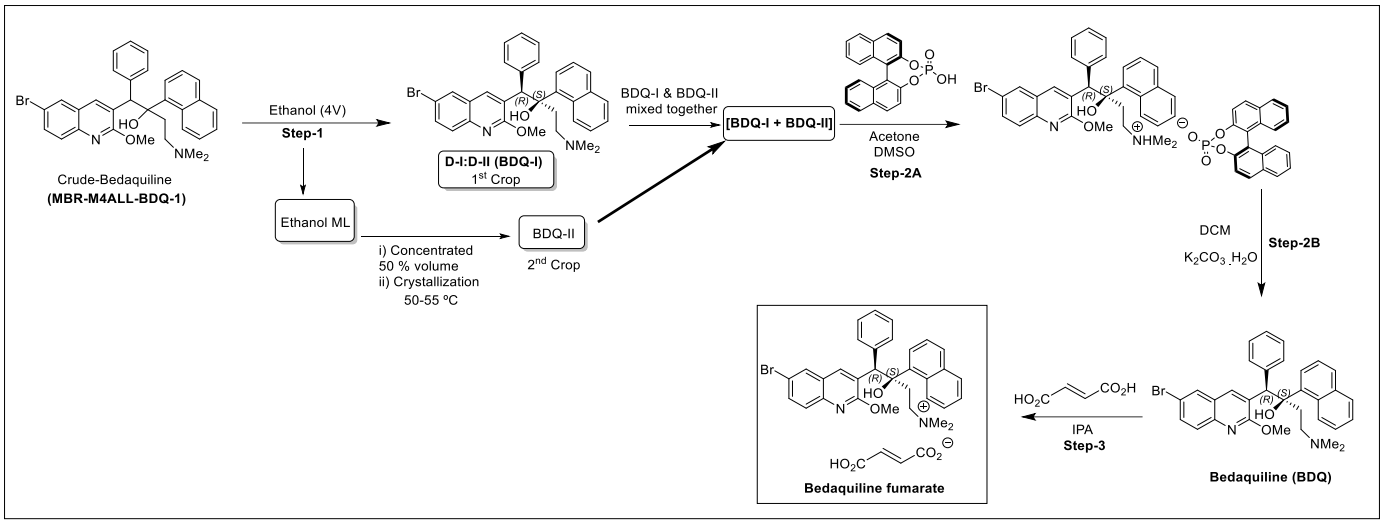
OBJECTIVE AND SCOPE

Bedaquiline fumarate is prepared by reacting equimolar amounts of bedaquiline and fumaric acid. It is used in combination therapy for treating pulmonary multi-drug resistant tuberculosis by inhibiting ATP synthase, an enzyme essential for the replication of the mycobacteria.

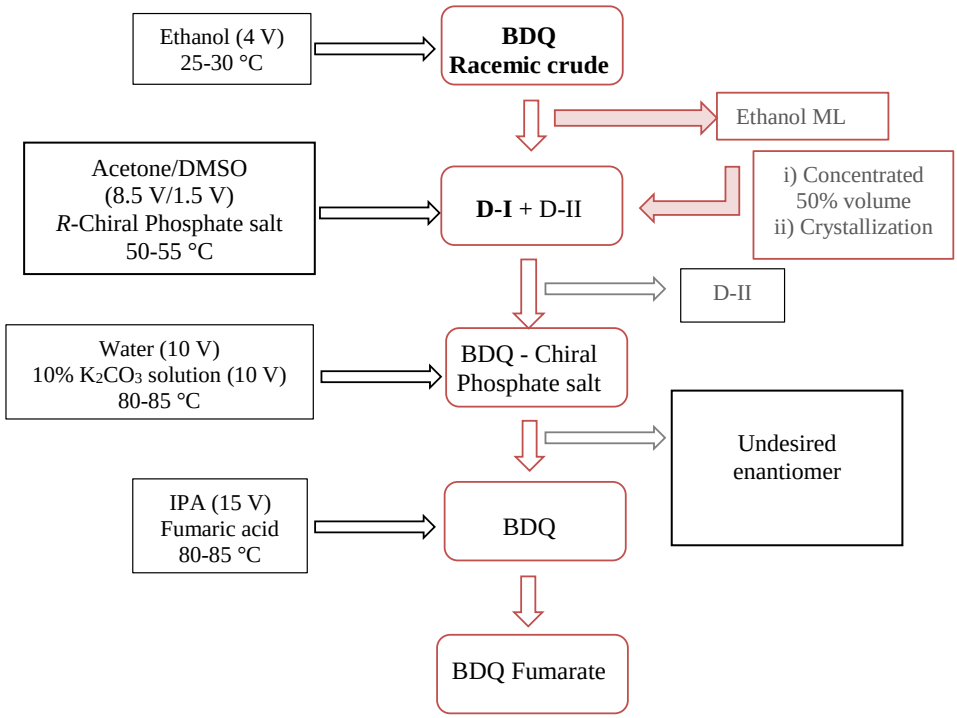
This batch record covers the multistep purification of the crude bedaquiline obtained from the assembly reaction covered in **MBR-M4ALL-BDQ-1** and the formation of its fumarate salt (final API).

Approach for preparation and isolation of Bedaquiline Fumarate

The mixture of isomers obtained at the end of the previous batch record was advanced directly to purification following the scheme below to yield Bedaquiline Fumarate as the final API.



Pictorial Presentation of Bedaquiline Isolation



List of Raw Materials Used for the Synthesis of Bedaquiline Fumarate

i. Crude - Bedaquiline (**MBR-M4ALL-BDQ-1**)
ii. (*R*)-(−)-1,1′-Binaphthyl-2,2′-diyl hydrogen phosphate
iii. Fumaric acid
iv. Potassium carbonate (K_2CO_3)
v. Ethanol
vi. DMSO
vii. Acetone
viii. Isopropyl Alcohol (IPA)
ix. Water (tap)
x. Dichloromethane (DCM)
xi. Sodium sulphate (Na_2SO_4)

List of Raw Material and Certificate of Analysis (COA)

Table No. 1:

S. No.	Raw Material Name	Source	COA	Remarks
1.	Crude bedaquiline	**MBR-M4ALL-BDQ-1**		
2.	(*R*)-(−)-1,1′-Binaphthyl-2,2′-diyl hydrogen phosphate	BLD Pharma		
3.	Fumaric acid	Alfa Aesar		
4.	Potassium carbonate (K_2CO_3)	Spectrochem		
5.	Ethanol	CSC		
6.	DMSO	Rankem		
7.	Acetone	Rankem		
8.	Isopropyl alcohol (IPA)	Rankem		
9.	DCM	Standard Reagent	DCM	
10.	Sodium sulfate (Na_2SO_4)	Finar Chemical		
11.	Raw water			

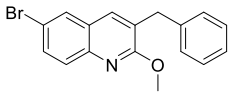
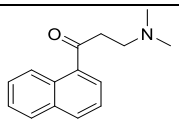
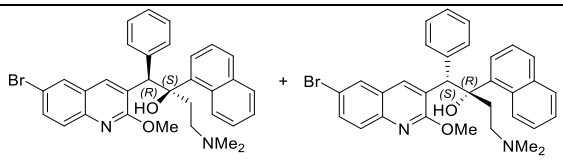
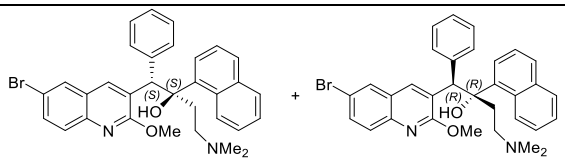
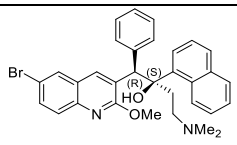
ABBREVIATION LIST

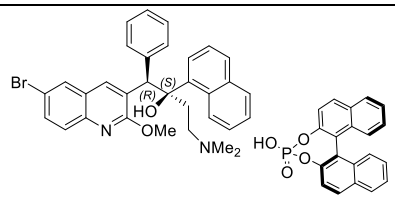
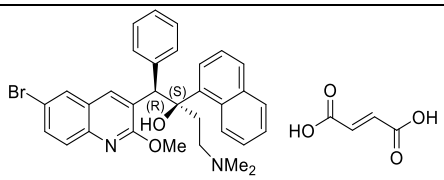
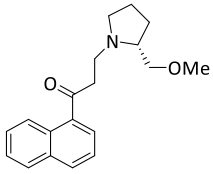
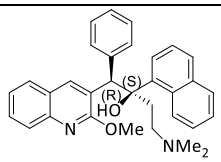
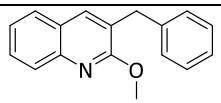
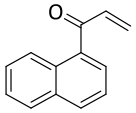
Table No. 2:

S. No.	Abbreviation	Full Form
1.	D-I	Desired diastereomers
2.	D-II	Undesired diastereomers
3.	BDQ	Bedaquiline
4.	DCM	Dichloromethane
5.	HDPE	High Density Polyethylene
6.	*R*-Chiral phosphoric acid	(*R*)-(−)-1,1′-Binaphthyl-2,2′-diyl hydrogen phosphate
7.	MOC	Material of construction
8.	RBF	Round-bottom flask
9.	MLR	Mother liquor
10.	Related Substance	Analysis by HPLC purity

NAME AND STRUCTURE IDENTIFICATION

Table No. 3:

S. No.	Name	Identification structure
1.	KSM-I	
2.	KSM-II	
3.	D-I	
4.	D-II	
5.	BDQ	

6.	BDQ phosphoric acid salt	
7.	BDQ fumarate	
8.	Imp-1	
9.	Imp-2	
10.	Imp-3	
11.	Enone	

EQUIPMENT LIST

Table No. 4:

S. No.	Equipment Name	Equipment ID.	MOC	Capacity	Remarks
1.	Reactor	RBF-7	All glass	3L 4-neck RBF	
2.	Reactor	RBF-9	All glass	2L 1-neck RBF	
3.	Reactor	RBF-10	All glass	3L 4-neck RBF	
4.	Reactor	RBF-11	All glass	2L 1-neck RBF	
5.	Reactor	RBF-12	All glass	5L 3-neck RBF	
6.	Reactor	RBF-13	All glass	3L 4-neck RBF	
7.	Reactor	RBF-14	All glass	2L 1-neck RBF	

8.	Reactor	RBF-15	All glass	3L 4-neck RBF	
9.	Reactor	RBF-16	All glass	3L 4-neck RBF	
10.	Reactor	RBF-17	All glass	2L 1-neck RBF	
11.	Reactor	RBF-18	All glass	3L 4-neck RBF	
12.	Reactor	RBF-19	All glass	2L 1-neck RBF	
13.	Buchner Funnel	FLT-1	Porcelain	3L	
14.	Buchner Funnel	FLT-2	Porcelain	2L	
15.	Buchner Funnel	FLT-3	Porcelain	2L	
16.	Buchner Funnel	FLT-4	Porcelain	3 L	
17.	Buchner Funnel	FLT-5	Porcelain	3 L	
18.	Addition funnel	Addition funnel-1	All glass	2 L	
19.	Addition funnel	Addition funnel-2	All glass	2 L	
20.	Addition funnel	Addition funnel-3	All glass	2 L	
21.	Separating funnel	Sep funnel-1	All glass	5 L	
22.	HDPE Container	Total containers-12	Polyethylene	-	
23.	Rotavapor	Rotavapor-1	All glass	2L	

PROCESS FOR THE PURIFICATION OF BEDAQUILINE AND ITS FUMARATE SALT FORMATION

*All the calculation has been done with respect to 75.0 g of **KSM-I** Batch Size*

Process Information:

i. Procedure-A (Ethanol) **- RBF-7 to RBF-11**
ii. Procedure-B (BDQ-(*R*)-(−)-1,1′-Binaphthyl-2,2′-diyl hydrogen phosphate salt) **- RBF-12 to RBF-17**
iii. Procedure-C (Bedaquiline fumarate) **- RBF-18 to RBF-19**

i. Procedure-A (Ethanol Purification) [RBF-7]

Actual batch size and quantity (Calculation on the basis of 75.0 g KSM-1):

S. No.	Reagent		Unit	Qty	Mol Wt.	mol	Mol Ratio / wt. times vol	Source
1	Crude (Bedaquiline) (Source-**MBR-M4ALL-BDQ-1)**		g	130.0	555.5	-	1.0 eq	Input Batch - 75.0 g
2	Ethanol	Lot-1	mL	300.0	-	-	4.0 V	-
		Lot-2		75.0			1.0 V	
		Lot-3		19.0			0.25 V	
3	1 % Seed (Bedaquiline)		g	0.75	-	-	-	Pure Bedaquiline (~99 %)

Process Information:

Table No. 5:

S. No.	Procedure	Required Qty (units)	Actual Qty (units)	Remarks	Sign
1.	Reactor **RBF-7** (3 L 4-neck flask) fitted with an internal temperature probe, overhead stirrer, and condenser with N_2 inlet.	130.0 g	130.0 g	CR592-20218-17-CR **MBR-M4ALL-BDQ-1** (Crude BDQ was in RBF-7) Crude BDQ was semi-solid in nature	
2.	Charge **Ethanol-Lot-1** through graduated measuring cylinder into the **RBF-7** at 25-30 °C. **Note:** Heterogeneous mixture.	300.0 mL	300.0 mL		
3.	Reaction mass was stirred (120-140 RPM) for 12-16 h at 25-30 °C. **Note:** heterogeneous solution.				
4.	Filter the reaction mass through Buchner funnel (**FLT-1**).			Filtrate kept in **HDPE-1**	
5.	Wash residue with **Ethanol-Lot-2** and let the solid dry while applying vacuum to the Buchner funnel for 1-2 h.	75.0 mL	75.0 mL	Vacuum 740 - 750 mmHg Collect the Filtrate in **HDPE-1**	

6.	Unload solid through solid funnel in **RBF-9** and dry under vacuum in rotavapor at 45-50 °C for 3-4 h.			1-neck RBF	
7.	Unload solid through funnel and store in dedicated **HDPE-2** (tared) and record the weight (**CROP-I**).	91.1 g	91.1 g	**LOT-1:** Desired diastereomer (**D-I**) Appearance: Off-white solid	
8.	Submit sample for purity analysis (Related Substance).	100 mg		Purity by HPLC & SFC for information only	
9.	Charge Filtrate-MLR (**HDPE-1)** into the **RBF-10** (3 L 4-neck flask) fitted with a vacuum line. **Note:** Homogeneous solution.	~ 400 mL	400 mL		
10.	Raise the oil bath temperature of **RBF-10** to 50-55 °C.	50-55 °C	50 °C	Digital thermometer	
11.	Apply the vacuum slowly and continue the heating of the oil bath at 50-55 °C for 4-5 h. **Note:** Distill the ethanol up to 70 % from reaction mixture.			Vacuum: 740-750 mmHg	
12.	Remove the vacuum and cool the reaction mixture to 25-30 °C. **Note:** Homogeneous solution.				
13.	Charge 1 % seed bedaquiline (~99.0 A%) into the **RBF-10**.	0.75 g	750 mg		
14.	Stir (100-120 RPM) the mixture at 25-30 °C for 12 h. **Note:** Heterogeneous solution.				
15.	Filter the reaction mass through Buchner funnel (**FLT-2**).			Collect the Filtrate in **HDPE-3**	
16.	Wash residue with **Ethanol-Lot- 3** and suck dry the solid for 1-2 h.	19.0 mL	19.0 mL	Collect the Filtrate in **HDPE-3**	
17.	Unload solid through funnel and transfer into **RBF-11** and dry under vacuum at 45-50 °C for 3-4 h.			1-neck RBF (**RBF-11**)	

				Vacuum: 740-750 mmHg	
18.	Unload solid through funnel and store in dedicated **HDPE-4** (tared) and record the weight.	0.85 g	850 mg	**LOT-2:** Bedaquiline Appearance: Off-white solid	
19.	Submit for purity analysis (50 mg) (Related Substance) and keep aside control sample (300 mg) of solid (**HDPE-4**) rest amount (500 mg; **CROP-II**) mixed together with **CROP-I** (Step-7) and forwarded to the next step.	350.0 mg	350.0 mg	Purity by HPLC & SFC for Information only	

HDPE-2 (Table No. 5, Step-8) [CROP-I]

Sl. No.	Batch ID	Input	Output	Remarks	Sign
1	CR592-20218-18	130.0 g HPLC A %: **D-I** = 77.74, **D-II** = 5.7 Assay: **D-I** = 76.4 %, **D-II** = 6.1 % SFC: **BDQ** = 74.2; BDQ enan = 20.65; Other two = 2.55 & 2.57	91.1 g HPLC A %: **D-I** = 91.55, **D-II** = 7.06 Assay: **D-I** = 92.7 %, **D-II** = 6.1 % SFC: **BDQ** = 71.45; BDQ enan = 23.15; Other two = 2.76 & 2.63	Assay corrected yield of **BDQ** = 47.4 % (w/w)	

HDPE-4 (Table No. 5, Step No. 9 to 19) [CROP-II]

Sl. No.	Batch ID	Input Seed BDQ	Output			Sign
			Gross Wt.	Net Wt.	Used for mixing with CROP-I	
2	CR592-20218-20	0.75 g	0.85 g HPLC A %: **D-I** = 99.76, **D-II** = 0.06 Assay: NA SFC: **BDQ** = 96.45; BDQ enan = 1.93; Other two = ND	(0.85 - 0.75 g) = 0.1 g	0.5 g	

Total Output = CROP-I + CROP-II (Net wt.) = 91.1 g + 0.1 g = 91.2 g

Material used for chiral resolution: step 90.5 g from CROP-I and 0.5 g from CROP- II = 91 g.

Note: These results show that recovery of BDQ from ethanol mother liquor can be avoided as long as precipitation of the desired diastereomer (**D-I**) takes place efficiently.

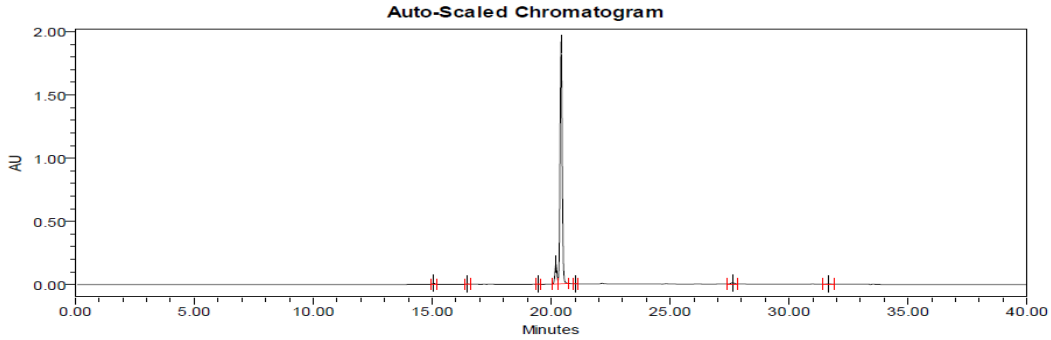
Results

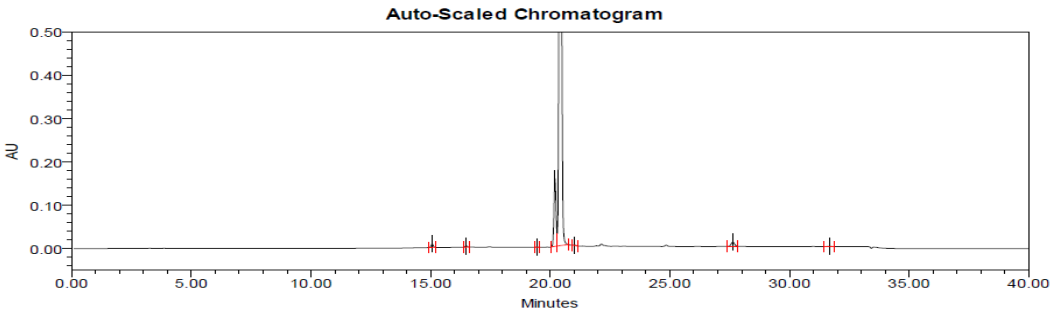
HPLC, Assay and SFC of D-I (Table No. 5, Step-8 [CROP-I])

HPLC of CROP-I

SAMPLE INFORMATION

Sample Name:	CR592-20218-18-D1	Acquired By:	PG0112811
Sample Type:	Unknown	Sample Set Name:	BDQ_CP_210323_02
Vial:	2	Acq. Method Set:	BDQ_CP_LC43_01
Injection #:	1	Processing Method	BDQ_CP_220323_01
Injection Volume:	10.00 ul	Channel Name:	W2489 ChA
Run Time:	40.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 225nm
		Column Name:	SHIMPACK C18(250X4.6)mm,5um
Date Acquired:	21-03-2023 23:59:22 IST		
Date Processed:	22-03-2023 06:40:58 IST		





Reported by User: Sibnath Das (SD0113647)
Report Method: RS _ REPT
Report Method ID: 1015
Page: 1 of 2

Project Name: 2023\Mar\BDQ
Date Printed:
22-03-2023
09:51:45 Asia/Kolkata

Peak Results

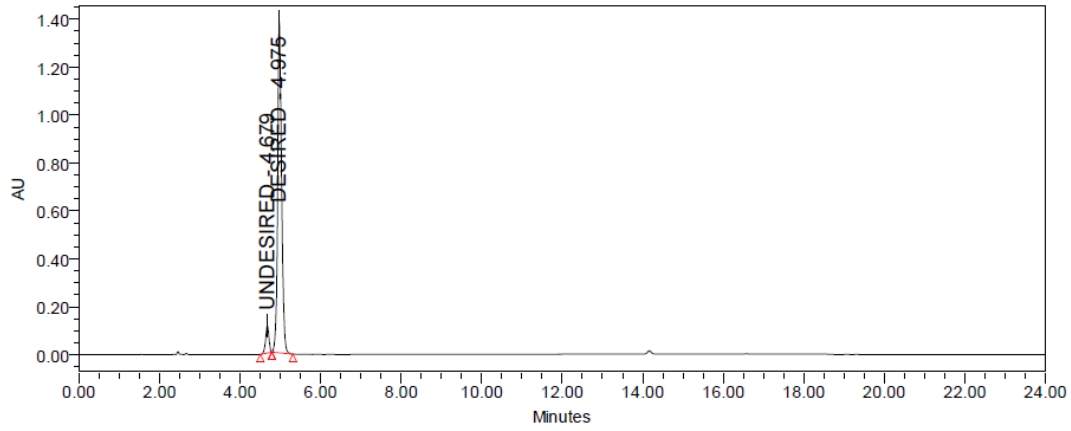
	Name	RT	Area	% Area	RT Ratio
1	KSM-II	15.066	48969	0.37	0.738
2	Peak2	16.487	18111	0.14	0.807
3	Peak3	19.442	2860	0.02	0.952
4	UNDESIRED	20.201	924140	7.06	0.989
5	DESIRED	20.419	11977118	91.55	1.000
6	Peak6	21.019	6262	0.05	1.029
7	KSM-I	27.631	97709	0.75	1.353
8	Peak8	31.670	7400	0.06	1.551

Assay of CROP-I

SAMPLE INFORMATION

Sample Name:	CR592-20218-18-D1-assay	Acquired By:	PG0112811
Sample Type:	Unknown	Sample Set Name:	BDQ_CP_210323_02
Vial:	5	Acq. Method Set:	BDQ_AS_LC43_02
Injection #:	1	Processing Method	BDQ_AS_220323_01
Injection Volume:	10.00 ul	Channel Name:	W2489 ChA
Run Time:	24.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 225nm
Date Acquired:	22-03-2023 03:10:51 IST	Column Name:	SHIMPACK C18(250X4.6)mm,5um
Date Processed:	22-03-2023 07:00:07 IST		

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	% Area
1	UNDESIRED	4.679	649796	6.48
2	DESIRED	4.975	9379184	93.52

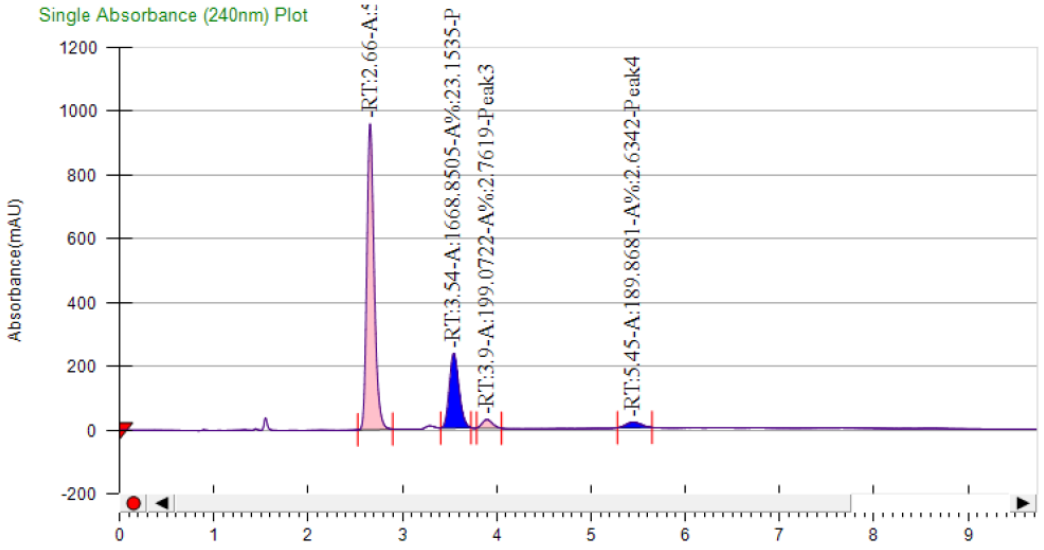
ASSAY:-

DESIRED:92.7% W/W

UNDESIRED:6.1%W/W

SFC of CROP-I

Waters
C:\Program Files (x86)\ChromScope IE\Investigator\Projects\MARCH_2023\DataFiles\3_22_2023\CR592-20218-18-D1_22-2023 9-15-25 AM.tta



General Information

Log Author	Log Date	Report By	Report Date	Notes
Administrator	3/22/2023 9:15:25 AM	Administrator	3/22/2023	

Run Information

Instrument Method	Inj. Vol.	Solvent	Column	Sample	Well Location	Temp.	Flow	% Modifier	Pressure
M_4-35	10	0.3 % IPamine MeOH	CHIRALPAK IC	CR592-20218-18-D1	27F	35	4	35	100

Peak Information

Peak No	% Area	Area	Ret. Time	Height	Cap. Factor
1	71.4504	5149.9888	2.66 min	958.852	0
2	23.1535	1668.8505	3.54 min	234.2593	0
3	2.7619	199.0722	3.9 min	26.936	0
4	2.6342	189.8681	5.45 min	17.2912	0

S. No.	Batch ID	Table No. 5		
		Step-8		
		HPLC	**Assay**	**SFC**
1.	CR592-20218-18 (BDQ-desired diastereomer, **D-I**)	Step-8_HPLC	Step-8_Assay	Step-8_SFC

HPLC SFC of D-I (Table No. 5, Step-19) [CROP-II]

HPLC of CROP-II

SAMPLE INFORMATION

Sample Name:	CR592-20218-20-Seed	Acquired By:	SD0113647
Sample Type:	Unknown	Sample Set Name:	BDQ_CP_240323_01
Vial:	56	Acq. Method Set:	BDQ_CP_LC43_01
Injection #:	1	Processing Method:	BDQ_CP_240323_01
Injection Volume:	10.00 ul	Channel Name:	W2489 ChA
Run Time:	40.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 225nm
		Column Name:	SHIMPACK C18(250X4.6)mm,5um
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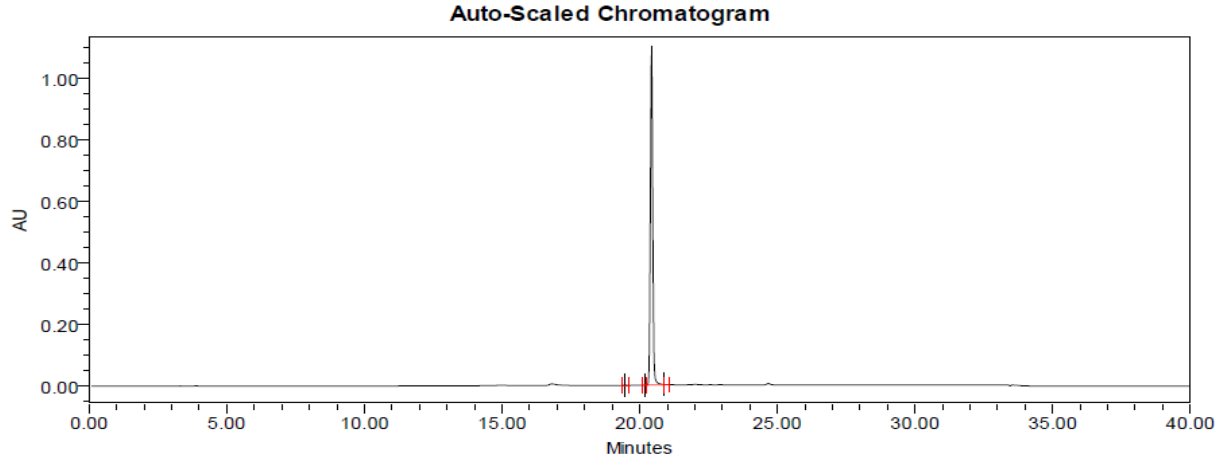
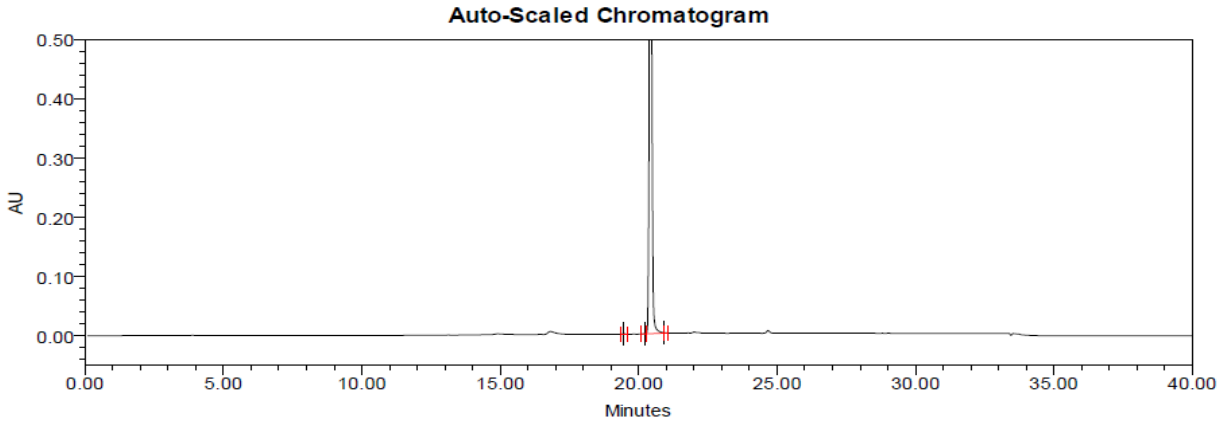
Date Processed:	24-03-2023 13:43:36 IST		





Peak Results

	Name	RT	Area	% Area	RT Ratio
1	Peak1	19.461	7127	0.11	0.953
2	UNDESIRED	20.215	3973	0.06	0.989
3	DESIRED	20.430	6540310	99.76	1.000
4	Peak4	20.900	4475	0.07	1.023

SFC of CROP-II

Waters
THE SCIENCE OF WHAT'S POSSIBLE.

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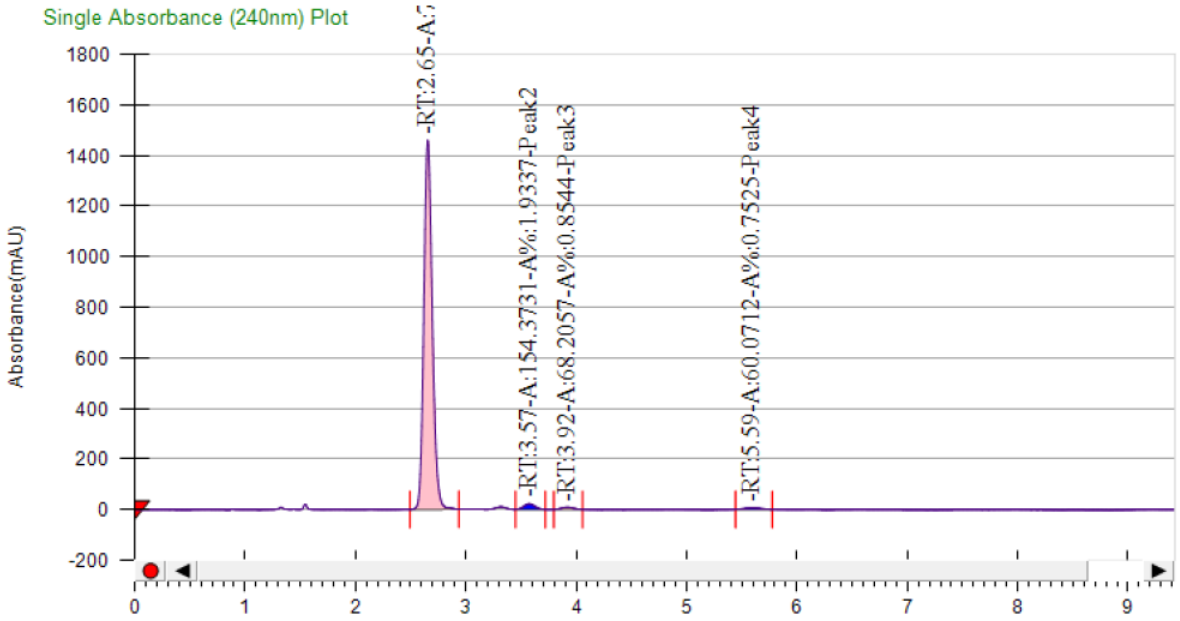


General Information

Log Author	Log Date	Report By	Report Date	Notes
Administrator	3/27/2023 10:47:01 AM	Administrator	3/27/2023	

Run Information

Instrument Method	Inj. Vol.	Solvent	Column	Sample	Well Location	Temp.	Flow	% Modifier	Pressure
M_4-35	10	0.3 % IPamine MeOH	CHIRALPAK IC	CR592-20218-20-Seeding	21F	35	4	35	100

Peak Information

Peak No	% Area	Area	Ret. Time	Height	Cap. Factor
1	96.4595	7700.5846	2.65 min	1459.4746	0
2	1.9337	154.3731	3.57 min	22.2796	0
3	0.8544	68.2057	3.92 min	9.048	0
4	0.7525	60.0712	5.59 min	5.512	0

Stepwise purification details (Output and yield of BDQ)

130 g of Crude BDQ has been used for purification out of 136 g of crude BDQ obtained from the 1,2-addition step.

Table No. 6A (purification has been carried out using 130 g of Crude BDQ)

Sl. No.	Batch ID	Input	Assay corrected yield of BDQ		
			Net Wt.	Total Output	
1	CR592-20218-18	130.0 g	91.1 g (**CROP-**I) + 0.1 g (**CROP-II**)	91.2 g	Assay corrected yield of BDQ = 47.4 % (w/w)

S. No.	Batch ID	Table No. 5		
		Step-19		
		HPLC	**Assay**	**SFC**
1.	CR592-20218-20 (BDQ-desired diastereomer, **D-I**)	Step-19_HPLC	NA	Step-19_SFC

ii) Procedure-B (BDQ Salt formation with (*R*)-(−)-1,1′-Binaphthyl-2,2′-diyl hydrogen phosphate) - RBF-12 to RBF-17

Actual batch size and quantity:

S. No.	Reagent		Unit	Qty	Mol Wt.	mol	Mol Ratio / wt. times vol	Source
1	HDPE-2 (CROP-I)		g	90.5	555.52	0.163	1.0 eq	CR592-20218-18 **(HDPE-2)**
2	HDPE-4 (CROP-II)		g	0.50	555.52		1.0 eq	CR592-20218-20 **(HDPE-4)**
	(*R*)-(−)-1,1′-Binaphthyl-2,2′-diyl hydrogen phosphate		g	68.4	348.2	0.196	1.2 eq	BLD Pharma
3	DMSO		mL	135.0	-	-	1.5 V	Rankem
3	Acetone	Lot-1	mL	775.0	-	-	8.5 V	Rankem
		Lot-2		275.0			3.0 V	
4	Water	Lot-1	mL	910 mL	-	-	10.0 V	Raw water
		Lot-2		910 mL			10.0 V	
5	DCM	Lot-1	mL	910 mL	-	-	10.0 V	Rankem
		Lot-2		200 mL			2.0 V	
		Lot-3		450 mL			5.0 V	
		Lot-4		90.0 mL			1.0 V	
6	Celite		g	10.0	-		-	Spectro chem
7	K_2CO_3		g	91.0	-	-	-	Rankem
8	Na_2SO_4		g	15.0	-		-	Avra

Process Information [Chiral Resolution Step]

Table No. 7:

S. No.	Procedure	Required Qty (units)	Actual Qty (units)	Remarks	Sign
1.	Check the cleanliness of the reaction **RBF-12** (5 L 4-neck flask) fitted with an internal temperature probe, overhead stirrer, and condenser.				
2.	Charge through funnel solid from **HDPE-2** and **HDPE-4** into **RBF-12**.	91.0 g	91.0 g	Mixed together (**HDPE-2** & **HDPE-4**)	
3.	Charge **Acetone-Lot-1** into the **RBF-12** at 25-30 °C.	775 mL	775 mL		
4.	Stir (100-120 RPM) the mixture at 25-30 °C for ~10-15 min. **Note:** Heterogeneous mixture				
5.	Raise the oil bath temperature of **RBF-12** to 40-45 °C. **Note:** Heterogeneous mixture.			Internal temperature (40-45 °C) Cloudy reaction mass is still observed	
6.	Charge (*R*)-(−)-1,1′-Binaphthyl-2,2′-diyl hydrogen phosphate (*R*-Chiral phosphoric acid) through solid funnel into clean and dry **RBF-13** (3-neck RBF) at 25-30 °C.	68.4 g	68.4 g		
7.	Charge DMSO through graduated measuring cylinder into **RBF-13** at 25-30 °C.	135.0 mL	135.0 mL		
8.	Stir (100-120 RPM) the **RBF-13** mixture at 25-30 °C for 10-15 min. **Note:** Solid (*R*-Chiral phosphoric acid) was slowly dissolved in DMSO.			Clear solution observed	
9.	Charge DMSO solution (*R*-Chiral phosphoric acid in DMSO) from **RBF-13** through **addition funnel-1** into **RBF-12** at 40-45 °C for 10-15 min.				
10.	Raise the oil bath temperature of **RBF-12** to 50-55 °C. **Note:** Reaction mixture becomes homogeneous (after ~ 10-15 min.			Internal temperature = 50 °C	
11.	Stir (100-120 RPM) the mixture at 50-55 °C for 25-30 min.				

S. No.	Procedure	Required Qty (units)	Actual Qty (units)	Remarks	Sign
	Note: Over time (after ~10-15 min) reaction mass becomes heterogeneous and a white solid precipitate has been observed.				
12.	Cool the **RBF-12** to 25-30 °C.				
13.	Stir (100-120 RPM) the mixture at 25-30 °C for 8 h. **Note:** More white solid precipitate has been observed.				
14.	Filter the reaction mass through Buchner funnel (**FLT-3**).			Keep MLR in **HDPE-5** and discard.	
15.	Wash residue with **Acetone-Lot-2** and suck dry the solid for 1-2 h.	275.0 mL	275.0 mL		
16.	Unload solid through solid funnel and transfer into **RBF-14** and dry under vacuum at 45-50 °C for 3-4 h.			2L 1-neck RBF	
17.	Unload solid through solid funnel store in dedicated **HDPE-6** (tared) and record the weight.	95.08 g	95.08 g	BDQ-Chiral phosphoric salt has been kept in **HDPE-6**. Appearance: white solid	
18.	Submit sample for purity analysis (Related Substance) of bedaquiline salt.	80.0 mg	80.0 mg	Purity by HPLC & SFC for Information only	
19.	Check the cleanliness of the reaction **RBF-15** (3 L 4-neck flask) fitted with an internal temperature probe, overhead stirrer, and condenser with addition funnel.				
20.	Charge solid from **HDPE-6** (Bedaquiline salt) through a solid funnel into **RBF-15** at 25-30 °C.				
21.	Charge **Water-Lot-1** through graduated measuring cylinder into **RBF-15** at 25-30 °C.	910 mL	910 mL		
22.	Stir (100-120 RPM) the mixture at 25-30 °C for ~10-15 min. **Note:** Heterogeneous solution.			BDQ salt was not soluble in water	
23.	Check the cleanliness of the reaction **RBF-16** (3 L 4-neck flask) fitted with an internal temperature probe, and overhead stirrer.				

S. No.	Procedure	Required Qty (units)	Actual Qty (units)	Remarks	Sign
24.	Charge **Water-Lot-2** through graduated measuring cylinder into the **RBF-16** at 25-30 °C	910 mL	910 mL		
25.	Charge **K_2CO_3** through solid funnel into **RBF-16** at 25-30 °C.	91.0 g	91.0 g		
26.	Stir (100-120 RPM) the mixture at 25-30 °C for 10-15 min. **Note**: Solution becomes cloudy.				
27.	Charge the 10 % K_2CO_3 solution from **RBF-16** to **RBF-15** via **Addition funnel-2** at 25-30 °C for 25-30 min.	910 mL	910 mL		
28.	Stir (120-140 RPM) the mixture at 25-30 °C for 10-15 min. **Note:** Solution becomes cloudy.				
29.	Charge **DCM-Lot-1** through graduated measuring cylinder into **RBF-15** at 25-30 °C.	910 mL	910 mL		
30.	Stir (100-120 RPM) the mixture at 25-30 °C for 10-15 min. **Note:** Solution is still cloudy.				
31.	Filter the reaction mass through celite pad.	10.0 g	10.0 g	Keep the Filtrate in **HDPE-7**	
32.	Wash celite pad with **DCM-Lot-2** and suck dry the solid. **Note:** Filtrate MLR is transferred into **HDPE-7.**	200.0 mL	200.0 mL		
33.	Charge Filtrate-MLR (**HDPE-7**) into separating funnel **(Sep funnel-1)**.				
34.	Settle the solution for layer separation (5-10 min).				
35.	Separate the DCM layer by separatory funnel and store it in dedicated **HDPE-8** container. Aqueous layer goes to the next step-36.			Organic layer contains desired product	
36.	Charge aqueous layer through funnel into separatory funnel.				
37.	Charge **DCM-Lot-3** into the aqueous layer for a second extraction.	450 mL	450 mL		
38.	Settle the solution for layer separation (10-15 min).				
39.	Separate the DCM layer by separatory funnel and store it in dedicated **HDPE-8**. Aqueous layer transfer into **HDPE-9**.			Organic layer contains desired product, and aqueous layer kept aside for analysis	
40.	Charged Na_2SO_4 through solid funnel into dedicated **HDPE-8** container	15 g	15 g		

S. No.	Procedure	Required Qty (units)	Actual Qty (units)	Remarks	Sign
41.	Filter the organic layer through Buchner funnel (**FLT-4**), followed by washing successively with **DCM-Lot-4** and suck dry the solid Na_2SO_4.	100 mL	100 mL		
42.	Remove DCM under reduced pressure (Rotavapor) at 25-30 °C.				
43.	Unload solid through solid funnel into **RBF-17** and dry under vacuum at 45-50 °C for 3-4 h. **Note:** White color solid.			1-neck RBF Vacuum: 740 - 750 mmHg	
44.	Unload solid through solid funnel and store in dedicated **HDPE-10** (tared) and record the weight.	57.09 g	57.09 g	**HDPE-10** (Bedaquiline-free base) Appearance: Off-white solid	
45.	Submit sample for purity analysis (Related Substance) of solid (**HDPE-10**).	90.0 mg	90.0 mg	Purity by HPLC & SFC for Information only	

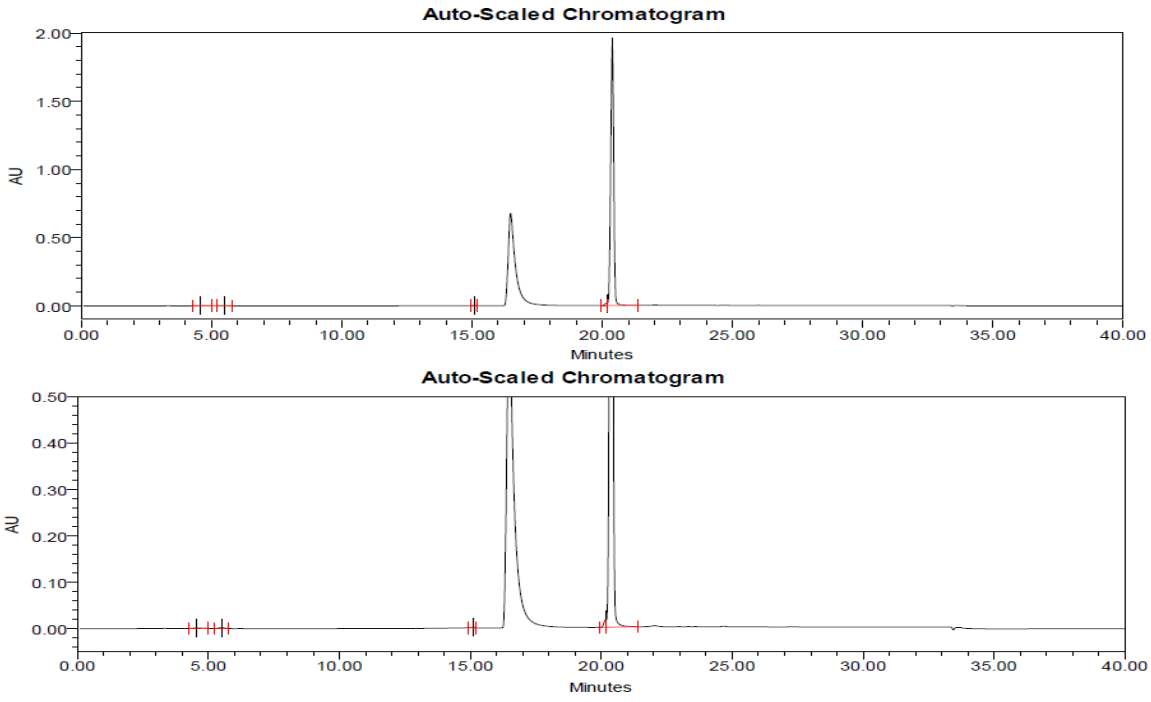
Results

HPLC, Assay and SFC of bedaquiline BNP salt

HPLC bedaquiline BNP salt (Table No. 7, Step-18)

SAMPLE INFORMATION

Sample Name:	CR592-20218-19-Phosphatesalt-R	Acquired By:	SS0113466
Sample Type:	Unknown	Sample Set Name:	BDQ_CP_280323_01
Vial:	26	Acq. Method Set:	BDQ_CP_LC43_01
Injection #:	1	Processing Method	BDQ_CP_280323_01
Injection Volume:	10.00 ul	Channel Name:	W2489 ChA
Run Time:	40.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 225nm
		Column Name:	SHIMPACK C18(250X4.6)mm,5um
Date Acquired:	28-03-2023 18:41:19 IST		
Date Processed:	28-03-2023 19:25:24 IST		



Reported by User: Sandip Shyam (SS0113466)
Report Method: RS _ REPT
Report Method ID: 1015
Page: 1 of 2

Project Name: 2023\Mar\BDQ
Date Printed:
28-03-2023
19:26:22 Asia/Kolkata

Peak Results

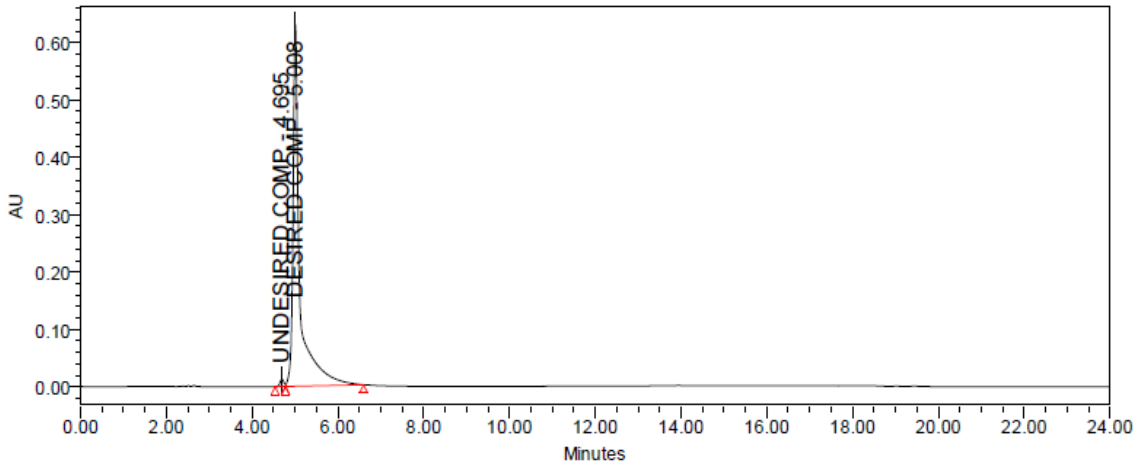
	Name	RT	Area	% Area	RT Ratio
1	Peak1	4.557	20986	0.13	0.224
2	Peak2	5.511	28296	0.18	0.270
3	KSM-II	15.095	12873	0.08	0.740
4	UNDESIRED COMP	20.173	108841	0.69	0.990
5	DESIRED COMP	20.387	15690494	98.92	1.000

Assay bedaquiline BNP salt (Table No. 7, Step-18)

SAMPLE INFORMATION

Sample Name:	CR592-20218-19-Phosphatesalt-Ass	Acquired By:	PG0112811
Sample Type:	Unknown	Sample Set Name:	BDQ_AS_240323_02
Vial:	53	Acq. Method Set:	BDQ_AS_LC43_02
Injection #:	1	Processing Method	BDQ_ASSAY_250323_01
Injection Volume:	10.00 ul	Channel Name:	W2489 ChA
Run Time:	24.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 225nm
Date Acquired:	24-03-2023 20:50:51 IST	Column Name:	SHIMPACK C18(250X4.6)mm,5um
Date Processed:	25-03-2023 09:38:31 IST		

Auto-Scaled Chromatogram



Peak Results

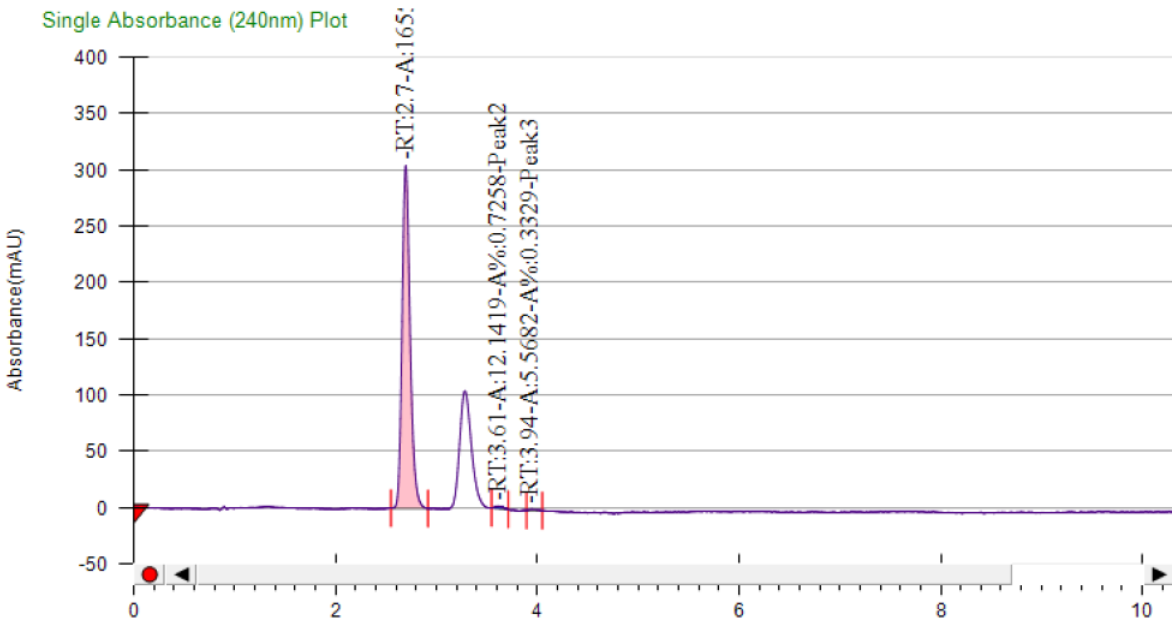
	Name	RT	Area	% Area
1	UNDESIRED COMP	4.695	78385	1.10
2	DESIRED COMP	5.008	7060303	98.90

DESSIRED COMP=64.9%W/W
UNDESIRED COMP=0.7%W/W

SFC bedaquiline BNP salt (Table No. 7, Step-18)

Waters

C:\Program Files (x86)\ChromScope IE\Investigator\Projects\MARCH_2023\DataFiles\3_29_2023\CR592-20218-19-Phosphatesalt-R_3-29-2023 10-49-30 AM.tta



General Information

Log Author	Log Date	Report By	Report Date	Notes
Administrator	3/29/2023 10:49:30 AM	Administrator	3/29/2023	

Run Information

Instrument Method	Inj. Vol.	Solvent	Column	Sample	Well Location	Temp.	Flow	% Modifier	Pressure
M_4-35	10	0.3 % IPamine MeOH	CHIRALPAK IC	CR592-20218-19-Phosphatesalt-R	16C	35	4	35	100

Peak Information

Peak No	% Area	Area	Ret. Time	Height	Cap. Factor
1	98.9413	1655.1118	2.7 min	304.2911	0
2	0.7258	12.1419	3.61 min	2.0936	0
3	0.3329	5.5682	3.94 min	1.0841	0

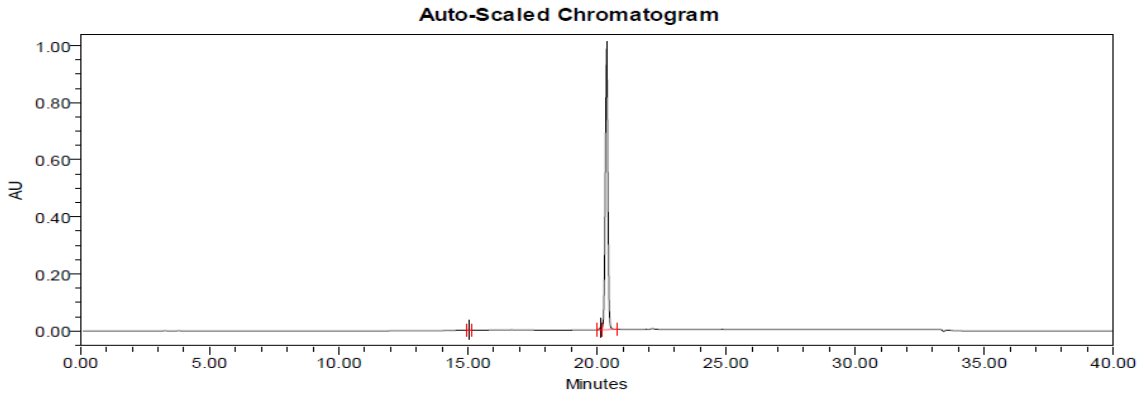
S. No.	Batch ID	Table No. 7		
		Step-18		
		HPLC	**Assay**	**SFC**
1.	CR592-20218-19 (BDQ-BNP salt)	Step-18_HPLC	Step-18_Assay	Step-18_SFC

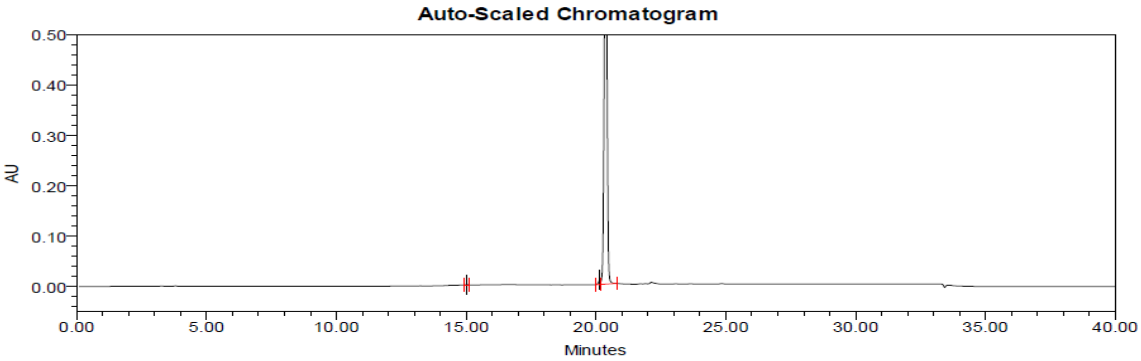
HPLC, Assay and SFC of Bedaquiline free base (Table No. 7, Step-45)

HPLC of BDQ free base

SAMPLE INFORMATION

Sample Name:	CR592-20218-23-BDQ	Acquired By:	SS0113466
Sample Type:	Unknown	Sample Set Name:	BDQ_CP_290323_02
Vial:	26	Acq. Method Set:	BDQ_CP_LC43_01
Injection #:	1	Processing Method:	BDQ_CP_280323_01
Injection Volume:	10.00 ul	Channel Name:	W2489 ChA
Run Time:	40.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 225nm
		Column Name:	SHIMPACK C18(250X4.6)mm,5um
Date Acquired:	29-03-2023 19:41:23 IST		
Date Processed:	29-03-2023 20:26:52 IST		





Reported by User: Sandip Shyam (SS0113466)
Report Method: RS _ REPT
Report Method ID: 1015
Page: 1 of 2

Project Name: 2023\Mar\BDQ
Date Printed:
29-03-2023
20:27:44 Asia/Kolkata

Peak Results

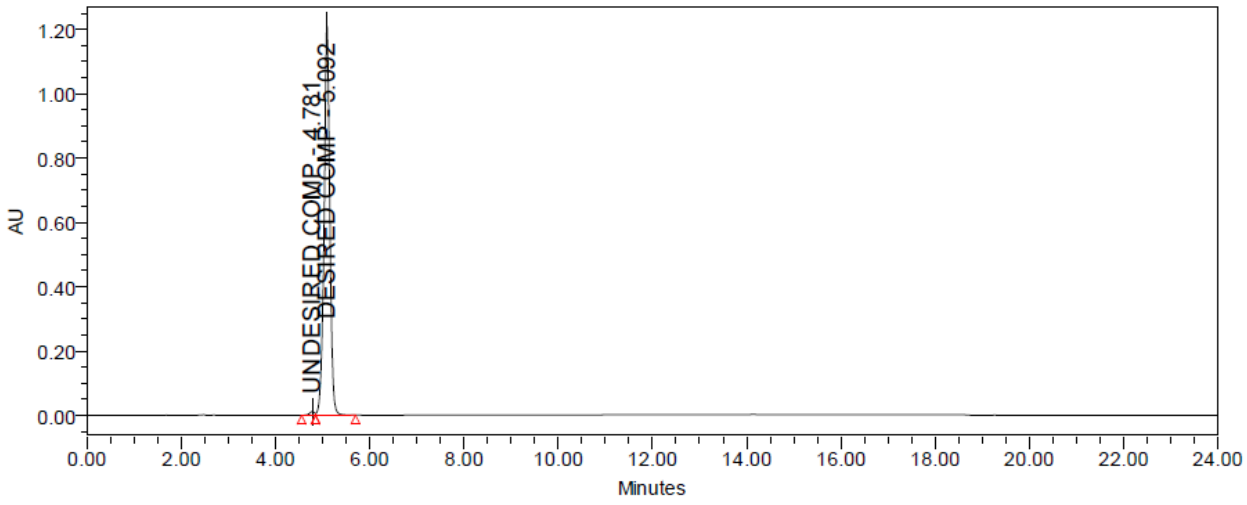
	Name	RT	Area	% Area	RT Ratio
1	KSM-II	15.028	4719	0.07	0.738
2	UNDESIRED COMP	20.149	53802	0.77	0.989
3	DESIRED COMP	20.372	6904761	99.16	1.000

Assay of BDQ free base

SAMPLE INFORMATION

Sample Name:	CR592-20218-23-BDQ-Assay	Acquired By:	SS0113466
Sample Type:	Unknown	Sample Set Name:	BDQ_AS_290323_02
Vial:	27	Acq. Method Set:	BDQ_AS_LC43_02
Injection #:	1	Processing Method	BDQ_AS_300822_01
Injection Volume:	10.00 ul	Channel Name:	W2489 ChA
Run Time:	24.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 225nm
Date Acquired:	30-03-2023 07:11:43 IST	Column Name: SHIMPACK C18(250X4.6)mm,5um	
Date Processed:	30-03-2023 08:35:07 IST		

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	% Area
1	UNDESIRED COMP	4.781	86326	0.84
2	DESIRED COMP	5.092	10224952	99.16

UNDESIRED= 0.8%(W/W)
DESIRED COMP= 98.4%(W/W)

SFC of BDQ free base

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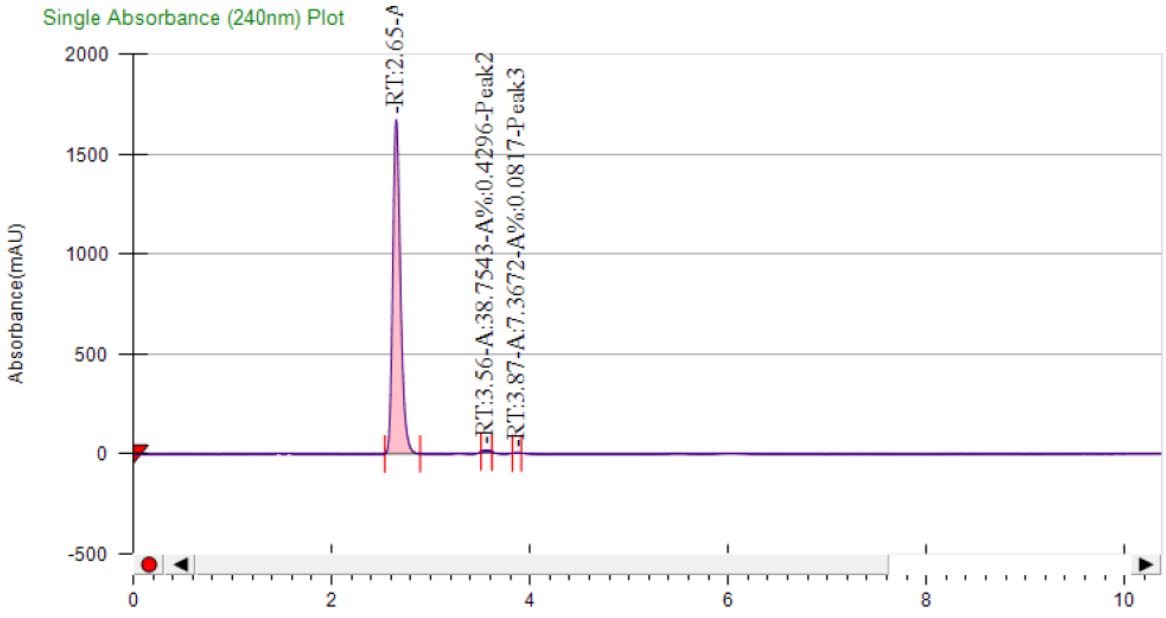


General Information

Log Author	Log Date	Report By	Report Date	Notes
Administrator	3/30/2023 1:25:29 PM	Administrator	3/30/2023	

Run Information

Instrument Method	Inj. Vol.	Solvent	Column	Sample	Well Location	Temp.	Flow	% Modifier	Pressure
M_4-35	5	0.3 % IPamine MeOH	CHIRALPAK IC	CR592-20218-23-BDQ	15E	35	4	35	100

Peak Information

Peak No	% Area	Area	Ret. Time	Height	Cap. Factor
1	99.4887	8975.2029	2.65 min	1670.7369	0
2	0.4296	38.7543	3.56 min	9.4403	0
3	0.0817	7.3672	3.87 min	2.1692	0

S. No.	Batch ID	Table No. 7		
1.	CR592-20218-23 (BDQ-free base)	Step-45		
		HPLC  Step-45_HPLC.pdf	**Assay**  Step-45_Assay.pdf	**SFC**  Step-45_SFC.pdf

Results

Table No. 8A:
Bedaquiline chiral phosphoric salt (BDQ-Salt) [Table No. 7, Step No. 18]

Sl. No.	Batch ID	Input	Output (Including sample for analysis)	Assay corrected yield of BDQ	Sign
1	CR592-20218-19	91.0 g HPLC A %: **D-I** = 91.55, **D-II** = 7.06 Assay: **D-I** = 92.7 %, **D-II** = 6.1 % SFC: BDQ = 71.45 BDQ enan = 23.15; Other two = 2.76 & 2.63	95.08 g HPLC A %: **D-I** = 98.92, **D-II** = 0.69 Assay: **D-I** = 64.9 % SFC: BDQ = 98.94 BDQ enan = 0.72; Other two = 0.33 & ND	48.06 % (w/w)	

Table No. 8B:
Bedaquiline free base (BDQ-free base) [Table No. 7, Step-45]:

Sl. No.	Batch ID	Input (BNP Salt)	Output (Free Base) (Including sample for analysis)	Assay corrected yield of BDQ	Sign
1	CR592-20218-23	95.0 g HPLC A %: **D-I** = 98.92, **D-II** = 0.69 Assay: **D-I** = 64.9 SFC: BDQ = 98.94 BDQ enan = 0.72; Other two = 0.33 & ND	57.09 g HPLC A %: **D-I** = 99.16, **D-II** = 0.77 Assay: **D-I** = 98.4, **D-II** = 0.8 SFC: BDQ = 99.48 BDQ enan = 0.42; Other two = 0.08 & ND	43.96 % (w/w)	

iii) Procedure-C (Bedaquiline fumarate) [RBF-18]

Actual batch size and quantity:

S. No.	Reagent		Unit	Qty	Mol Wt.	mol	Mol Ratio / wt. times vol	Source
1	Bedaquiline		g	57.0	555.5	0.099	1.0 eq	**CR592-20218-23 HDPE-10**
2	Fumaric acid		g	13.7	116.0	0.118	1.2 eq	Alfa-Aesar
3	IPA	Lot-1	mL	825.0	-	-	15 V	Rankem
		Lot-2		275.0			5 V	

Process Information: [Fumarate Salt Formation]

Table No. 9:

S. No.	Procedure	Required Qty (units)	Actual Qty (units)	Remarks	Sign
1.	Check the cleanliness of the reaction **RBF-18** (3 L 4-neck flask) fitted with an internal temperature probe, overhead stirrer, and condenser.				
2.	Charge through graduated cylinder **IPA-Lot-1** at 25-30 °C.	825.0 mL	825.0 mL		
3.	Charge through solid funnel **bedaquiline-free base** from **HDPE-10** into **RBF-18** at 25-30 °C.	57.0 g	57.0 g	CR592-20218-23	
4.	Stir (100-120 RPM) the mixture at 25-30 °C for ~10-15 min. **Note:** Heterogeneous mixture.				
5.	Raise the oil bath temperature of **RBF-18** to 50-55 °C. **Note:** Solution becomes cloudy.				
6.	Charge through solid funnel fumaric acid into **RBF-18** at 50-55 °C.	13.7 g	13.7 g		
7.	Raise the oil bath temperature of **RBF-18** at 75-80 °C.			Internal temperature = 70-75 °C	

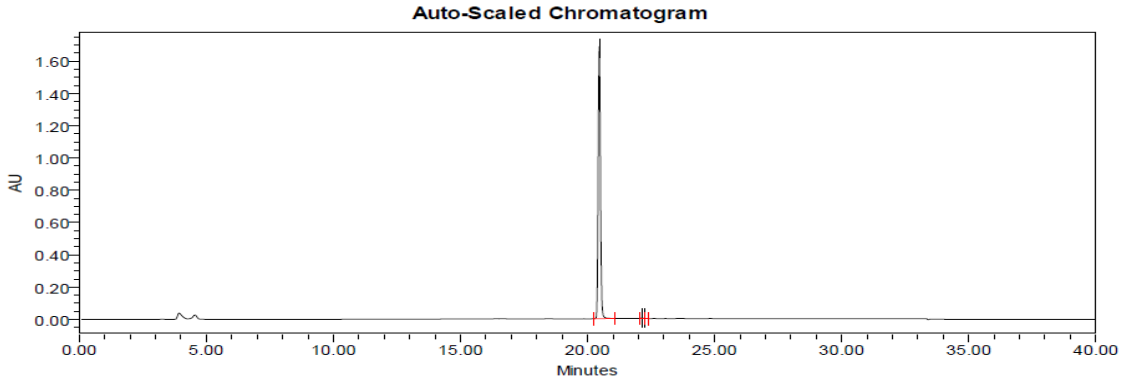
	Note: Reaction mixture becomes homogeneous, and after ~5-10 min solid precipitation is observed.				
8.	Stir (100-120 RPM) the mixture at 80-85 °C for 25-30 min.			Internal temperature = 75-80 °C	
9.	Cool the **RBF-18** up to 25-30 °C				
10.	Stir (100-120 RPM) the mixture at 25-30 °C for 8-10 h. **Note:** Further precipitation of white solid observed.				
11.	Filter the reaction mass through Buchner funnel **(FLT-5)**.				
12.	Wash residue with **IPA-Lot-2** and suck dry the solid for 1-2 h.	275.0 mL	275.0 mL	MLR was kept into **HDPE-11** container and a sample submitted for analysis (recovery of BDQ)	
13.	Unload solid through solid funnel and transfer into **RBF-19** and dry under vacuum at 45-50 °C for 3-4 h.			1-neck RBF Vacuum: 740 - 750 mmHg	
14.	Unload solid through solid funnel and store in dedicated **HDPE-12** (tared) and record the weight.	59.12 g	59.12 g	Bedaquiline fumarate salt Appearance: Off-white solid	
15.	Submit for purity analysis (Related Substance) of solid (**HDPE-12**).	120.0 mg	120.0 mg	IPC: Purity by HPLC & SFC for Information only	

HPLC, Assay and SFC of bedaquiline fumarate (Table No. 9, Step-15)

HPLC bedaquiline fumarate

SAMPLE INFORMATION

Sample Name:	CR592-20218-24-BDQ-Fumarate	Acquired By:	SS0113466
Sample Type:	Unknown	Sample Set Name:	BDQ_CP_290323_02
Vial:	27	Acq. Method Set:	BDQ_CP_LC43_01
Injection #:	1	Processing Method	BDQ_CP_300323_01
Injection Volume:	10.00 ul	Channel Name:	W2489 ChA
Run Time:	40.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 225nm
		Column Name:	SHIMPACK C18(250X4.6)mm,5um
Date Acquired:	30-03-2023 18:30:10 IST		
Date Processed:	30-03-2023 19:30:32 IST		



Reported by User: Sandip Shyam (SS0113466)
Report Method: RS _ REPT
Report Method ID: 1015
Page: 1 of 2

Project Name: 2023\Mar\BDQ
Date Printed:
30-03-2023
21:21:17 Asia/Kolkata

Peak Results

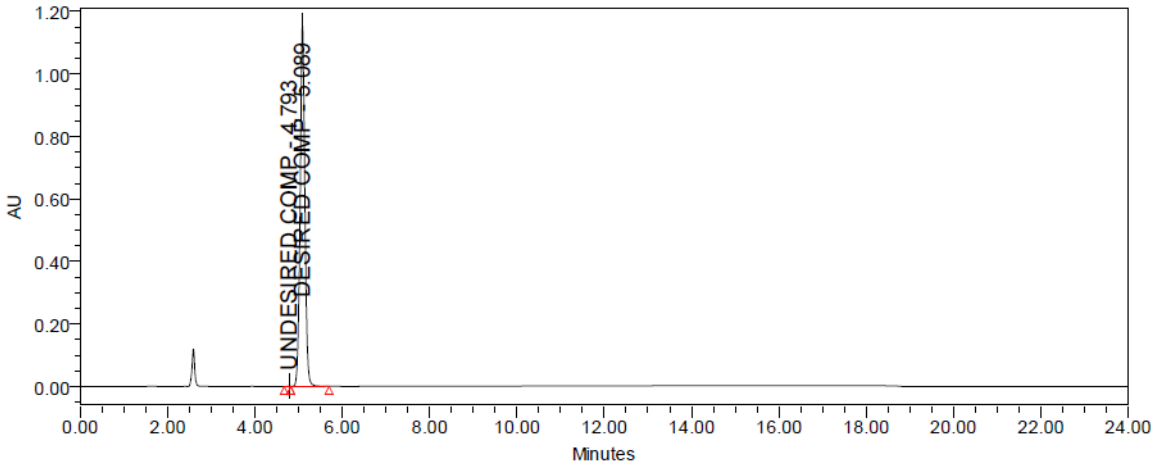
	Name	RT	Area	% Area	RT Ratio
1	DESIRED COMP	20.462	10561130	99.74	1.000
2	Peak2	22.138	20229	0.19	1.082
3	Peak3	22.233	7378	0.07	1.087

Assay bedaquiline fumarate

SAMPLE INFORMATION

Sample Name:	CR592-20218-24-BDQ-Fumarate-Assa	Acquired By:	SD0113647
Sample Type:	Unknown	Sample Set Name:	BDQ_AS_310323_01
Vial:	51	Acq. Method Set:	BDQ_AS_LC43_02
Injection #:	1	Processing Method	BDQ_AS_310822_03
Injection Volume:	10.00 ul	Channel Name:	W2489 ChA
Run Time:	24.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 225nm
Date Acquired:	31-03-2023 12:21:43 IST	Column Name: SHIMPACK C18(250X4.6)mm,5um	
Date Processed:	31-03-2023 12:49:39 IST		

Auto-Scaled Chromatogram



Peak Results

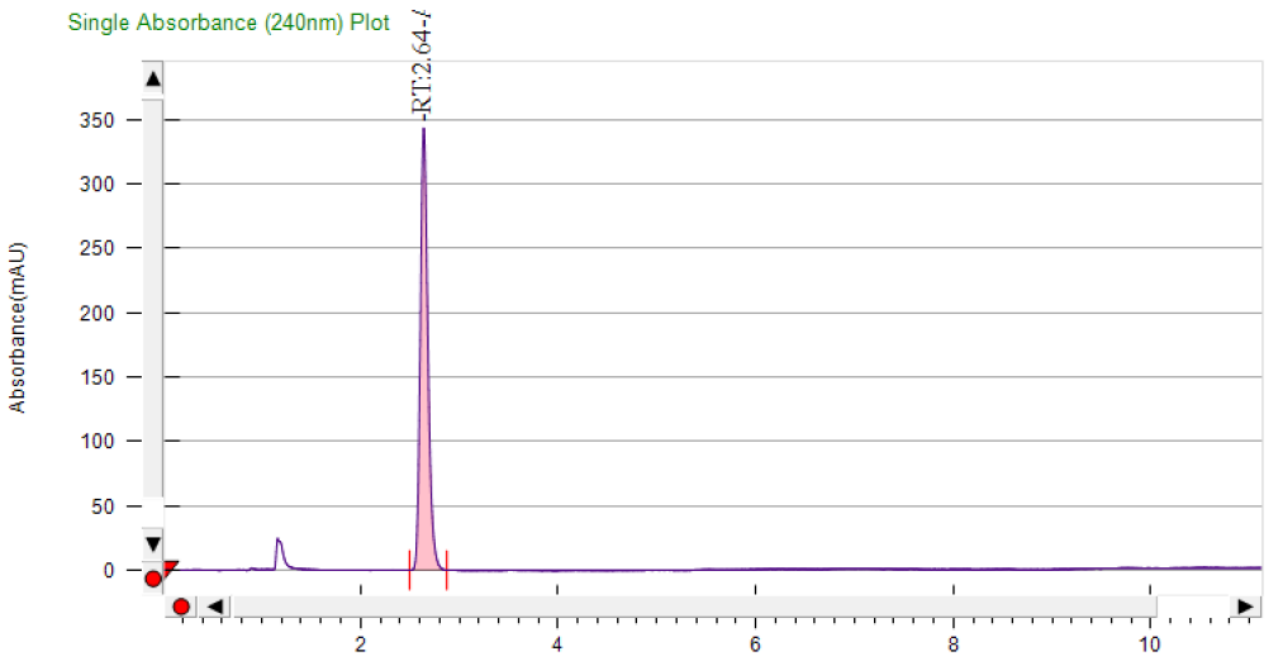
	Name	RT	Area	% Area
1	UNDESIRED COMP	4.793	1488	0.02
2	DESIRED COMP	5.089	8716356	99.98

UNDESIRED = NOT DETECTED

DEASIRED= 84.8%(W/W)

SFC bedaquiline fumarate

C:\Program Files (x86)\ChromScope IE\Investigator\Projects\MARCH_2023\DataFiles\3_30_2023\CR592-20218-24-BDQ-Fumarate_3-30-2023 5-14-01 PM.tta



General Information

Log Author	Log Date	Report By	Report Date	Notes
Administrator	3/30/2023 5:14:01 PM	Administrator	3/30/2023	

Run Information

Instrument Method	Inj. Vol.	Solvent	Column	Sample	Well Location	Temp.	Flow	% Modifier	Pressure
M_4-35	15	0.3 % IPamine MeOH	CHIRALPAK IC	CR592-20218-24-BDQ-Fumarate	21A	35	4	35	100

Peak Information

Peak No	% Area	Area	Ret. Time	Height	Cap. Factor
1	100	1828.16	2.64 min	343.6663	2639

S. No.	Batch ID	Table No. 9		
		Step-15		
1.	CR592-20218-24 (BDQ fumarate)	**HPLC** Step-15_HPLC.pdf	**Assay** Step-15_Assay.pdf	**SFC** Step-15_SFC.pdf

Results

[Fumarate salt formation]: (Table No. 9, Step No. 15)

Table 10A:

Sl. No.	Batch ID	Input	Output (Including sample for analysis)	Assay corrected yield of BDQ	Sign
1	CR592-20218-24	57.0 g HPLC A %: **D-I** = 99.16, **D-II** = 0.77 Assay: **D-I** = 98.4, **D-II** = 0.8 SFC: BDQ = 99.48 BDQ enan = 0.42; Other two = 0.08 & ND	59.12 g HPLC A %: **BDQ** = 99.74**, D-II** = ND Assay: BDQ= 84.8, **D-II** = ND SFC: BDQ = 100.0 BDQ enan = ND; Other two = ND & ND	39.46 % (w/w)	