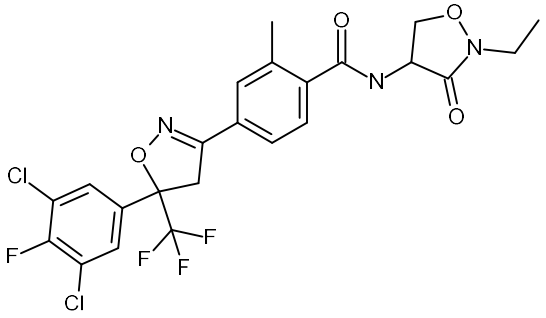
ISOCYCLOSERAM

XXX



ISO Common Name	Isocycloseram
Chemical Name	4-(5-(3,5-dichloro-4-fluorophenyl)-5-(trifluoromethyl)-4,5-dihydro-1,2-oxazol-3-yl)-N-(2-ethyl-3-oxo-1,2-oxazolidin-4-yl)-2-methylbenzamide
CAS Number	2061933-85-3
Empirical formula	$C_{23}H_{19}Cl_2F_4N_3O_4$
Molecular mass	548.3
m.p.	138.9°C (412.0 K) AT 100.1 to 103.0 kPa
b.p.	*178 °C (451 K) AT 100.2 to 102.2 kPa*
v.p	*< 6.2 * 10^{-6} Pa*
Solubility	*acetone 270 g/l* *methanol 75 g/l* *dichloromethane 400 g/l* *octanol 17 g/l* *ethyl acetate 190 g/l* *toluene 33 g/l* *hexane 39 mg/I* *water 21 mg/l*

Stability	*stable at room temperature*
Description	*white to beige solid*
Formulation	*WP*

Note: Isocycloseram is a mixture of the isomers (5*S*,4*R*), (5*S*,4*S*), (5*R*,4*R*) and (5*R*,4*S*).

ISOCYCLOSERAM TECHNICAL

[*]**XXX**/TC/(M)/-

1 Sampling. Take at least 5 g.

2 Identity tests

2.1 Infrared. Prepare potassium bromide pellets of the sample and of pure isocycloseram and scan from 4000 to 600 cm^{-1}. The spectrum obtained from the sample should not differ significantly from that of the reference grade material. (Fig 1)

2.2 HPLC. Use the reversed phase HPLC method below. The relative retention time of the respective peak in the sample solution should not deviate by more than 2% from that of the calibration solution. The UV spectrum measured from this peak should match that obtained from the calibration substance (Fig 2).

REAGENTS

Isocycloseram: reference standard containing the four isomers.

Acetonitrile: HPLC grade

Methanol: HPLC grade

Deionized water

Standard solution. Weigh (to the nearest 0.1 mg) 45 – 55 mg (*s* mg) of isocycloseram reference standard into a 100 ml volumetric flask. Dissolve with 50 ml acetonitrile and treat for approx. 5 minutes in an ultrasonic bath or until completely dissolved. Cool down to ambient temperature and make up to volume with acetonitrile. If necessary, filter a portion of the solution using a PTFE 0.45 µm filter.

[*] Provisional CIPAC method 2024. Based on a method supplied by Syngenta Crop Protection AG, Switzerland

APPARATUS

High performance liquid chromatograph equipped with an ultraviolet spectrophotometric detector and an injecting system capable of injecting 5 µl.

Column stainless steel, 150 x 4.6 mm (i.d.), packed with Chiralpak IG-3 (Daicel), 4.6 mm, or equivalent material with the same selectivity.

Ultrasonic bath

Dispensable PTFE filters, solvent compatible, 25 mm, porosity 0.45 µm.

Electronic integrator or *data system*

PROCEDURE

(a) Liquid chromatographic conditions (typical):

Mobile phase	Water / Acetonitrile / Methanol = 25/50/25
Column temperature	40 °C
Flow rate	1.0 ml/min
Injection volume	5 µl
Detector wavelength	265 nm
Run time	15 min
Retention time	approximately 4.7 min for (5*S*,4*S*) isomer approximately 6.9 min for (5*R*,4*S*) isomer approximately 8.9 min for (5*S*,4*R*) isomer approximately 12.5 min for (5*R*,4*R*) isomer

(c) System equilibration. Inject 5 µl portions of the standard solution and repeat the injections until the peak areas obtained for two consecutive injections differ by less than 1 % for isomer (5*S*,4*R*), and less than 5 % for the other three isomers.

(b) Sample preparation. Prepare solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) 45 – 55 mg of the sample into a 100 ml volumetric flask. Dissolve with 50 ml acetonitrile and place in an ultrasonic bath for approx. 5 minutes or until completely dissolved. Cool down to ambient temperature and make up to volume with acetonitrile. If necessary, filter a portion of the solution using a PTFE 0.45 µm filter.

(d) Determination. Inject 5 µl of the sample solution and determine the respective peak areas.

(e) Calculation. Calculate the content of each isomer using the area ratio of the four isomers and the content of isocycloseram determined in **XXX**/TC/(M)/3:

$$Content\ (5S,4S)\ \text{isomer} = \frac{H_{(5S,4S)}}{H_{(5S,4R)} + H_{(5S,4S)} + H_{(5R,4R)} + H_{(5R,4S)}}\ \text{x}\ C \quad \text{g/kg}$$

$$Content\ (5R,4S)\ \text{isomer} = \frac{H_{(5S,4R)}}{H_{(5S,4R)} + H_{(5S,4S)} + H_{(5R,4R)} + H_{(5R,4S)}}\ \text{x}\ C \quad \text{g/kg}$$

$$Content\ (5S,4R)\ \text{isomer} = \frac{H_{(5S,4R)}}{H_{(5S,4R)} + H_{(5S,4S)} + H_{(5R,4R)} + H_{(5R,4S)}}\ \text{x}\ C \quad \text{g/kg}$$

$$Content\ (5R,4R)\ \text{isomer} = \frac{H_{(5S,4R)}}{H_{(5S,4R)} + H_{(5S,4S)} + H_{(5R,4R)} + H_{(5R,4S)}}\ \text{x}\ C \quad \text{g/kg}$$

Where:

$H_{5S,4S}$ = peak area of the (5*S*,4*S*) isomer
$H_{5R,4S}$ = peak area of the (5*R*,4*S*) isomer
$H_{5S,4R}$ = peak area of the (5*S*,4*R*) isomer
$H_{5R,4R}$ = peak area of the (5*R*,4*R*) isomer
C = content of isocycloseram determined in **XXX**/TC/(M)/3 (g/kg)

3 Isocycloseram

OUTLINE OF METHOD

Isocycloseram is determined by reversed phase high performance liquid chromatography using UV detection at 265 nm and external standardization (Fig 3).

REAGENTS

Isocycloseram: reference standard with known content

Acetonitrile: HPLC grade

Deionized water

Formic acid: analytical grade

Calibration solution. Weigh in duplicate (to the nearest 0.1 mg) 45 – 55 mg (*s* mg) of isocycloseram reference standard into separate 100 ml volumetric flasks. Dissolve with 60 ml acetonitrile and treat for approx. 5 minutes in an ultrasonic bath or until completely dissolved. Cool down to ambient temperature and make up to volume with acetonitrile. Dilute 10.0 ml of this solution into a 50 ml volumetric flask and make up to volume with acetonitrile at ambient temperature. If necessary, filter a portion of the solution using a PTFE 0.45 µm filter (solutions C_A and C_B).

APPARATUS

High performance liquid chromatograph equipped with an ultraviolet spectrophotometric detector and an injecting system capable of injecting 5 µl.

Column stainless steel, 100 x 4.6 mm (i.d.), packed with Kinetex C18, 2.6 µm, or equivalent material with the same selectivity.

Ultrasonic bath

Dispensable PTFE filters, solvent compatible, 25 mm, porosity 0.45 µm.

Electronic integrator or *data system*

PROCEDURE

(a) Liquid chromatographic conditions (typical):

Column temperature	40 °C
Flow rate	1.0 ml/min
Detector wavelength	265 nm
Injection volume	5 µl
Run time	10 min
Retention time	approximately 4.8 min

Gradient

time (min)	% acetonitrile	0.1% v/v formic acid in water
0	60	40
4	60	40
4.1	95	5
6.9	95	5
7	60	40
10	60	40

(b) System equilibration. Prepare two calibration solutions. Inject 5 µl portions of solution C_A until the response factors obtained for two consecutive injections differ by less than 1 %. Then inject a 5 µl portion of the solution C_B. The response factor for this solution should not deviate by more than 5 % from that of solution C_A, otherwise prepare new calibration solutions.

(c) Sample preparation. Prepare solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) 45 – 55 mg (w mg) of the sample into a 100 ml volumetric flask. Dissolve with 60 ml acetonitrile and place in an ultrasonic bath for approx. 5 minutes or until completely dissolved. Cool down to ambient temperature and make up to volume with acetonitrile. Dilute 10.0 ml of this solution into a 50 ml volumetric flask and make up to volume with acetonitrile at ambient temperature. If necessary, filter a portion of the solution using a PTFE 0.45 µm filter (solutions S_A and S_B).

(d) Determination. Inject in duplicate 5 µl portions of each sample solution, bracketing them with duplicate injections of the calibration solution as follows: calibration solution C_A, calibration solution C_B, calibration solution C_A, sample solution $S1_A$, sample solution $S1_B$, calibration solution C_A, sample solution $S2_A$, sample solution $S2_B$, calibration solution C_A, and so on for further samples. Measure the relevant peak areas.

(e) Calculation. Calculate the mean value of each pair of calibration response factors, bracketing the two injections of a sample, and use this value for calculating the isocycloseram contents of the bracketed sample injections.

$$f = \frac{s \times P}{H_s}$$

$$\text{Content of isocycloseram} = \frac{f \times H_w}{w} \qquad \text{g/kg}$$

where:

f = mean response factor
H_s = peak area of isocycloseram in the calibration solution
H_w = peak area of isocycloseram in the sample solution
s = mass of isocycloseram reference standard in the calibration solution (mg)
w = mass of sample taken (mg)
P = purity of isocycloseram reference standard (g/kg)

Repeatability r = 16.5 to 20.1 g/kg at an active ingredient content of 986 to 989 g/kg

Reproducibility R = 16.8 to 29.1 g/kg at an active ingredient content of 986 to 989 g/kg

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ISOCYCLOSERAM WETTABLE POWDERS
[*]**XXX**/WP/(M)/-

1 Sampling. Take at least 20 g.

2 Identity tests

2.1 Infrared. As for technical **XXX**/TC/(M)/2.1.

2.2 HPLC. As for technical **XXX**/TC/(M)/2.2 and Fig 4., except:

(b) Sample preparation. Prepare solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) 320 - 340 mg of the sample into a 100 ml volumetric flask. Dissolve with 50 ml acetonitrile and place in an ultrasonic bath for approx. 5 minutes or until completely dissolved. Cool down to ambient temperature and make up to volume with acetonitrile. If necessary, filter a portion of the solution using a PTFE 0.45 µm filter.

3 Isocycloseram. As for isocycloseram technical **XXX**/TC/(M)/3 and Fig 5., except:

PROCEDURE

(c) Sample preparation. Prepare solutions in duplicate for each sample. Homogenize the test sample thoroughly. Weigh (to the nearest 0.1 mg) sufficient sample to contain 45 – 55 mg (w mg) of isocycloseram (equal to 150 – 180 mg of isocycloseram formulation WP (15)) into a 50 ml volumetric flask. Dissolve with 35 ml acetonitrile and treat for approx. 5 minutes in an ultrasonic bath or until completely dissolved. Cool down to ambient temperature and make up to volume with acetonitrile. Dilute 10.0 ml of this solution into a 50 ml volumetric flask and make up to volume with acetonitrile at ambient temperature. Filter a portion of the solution using a PTFE 0.45 µm filter, discarding the first 1 ml (sample solutions S_A and S_B).

(e) Calculation. Calculate the mean value of each pair of calibration response factors, bracketing the two injections of a sample, and use this value for calculating the isocycloseram contents of the bracketed sample injections.

[*] Provisional CIPAC method 2023. Based on a method supplied by Syngenta Crop Protection AG, Switzerland

$$f = \frac{s \times P}{H_s}$$

$$\text{Content of isocycloseram} = \frac{f \times H_w}{w * 2} \qquad \text{g/kg}$$

where

f = mean response factor
H_s = peak area of isocycloseram in the calibration solution
H_w = peak area of isocycloseram in the sample solution
s = mass of isocycloseram reference standard in the calibration solution (mg)
w = mass of sample taken (mg)
P = purity of isocycloseram reference standard (g/kg)

Repeatability r = 3.6 to 5.2 g/kg at an active ingredient content of 147 to 152 g/kg
Reproducibility R = 4.6 to 6.7 g/kg at an active ingredient content of 147 to 152 g/kg

4 Suspensibility

REAGENTS AND APPARATUS As for **XXX**/TC/(M)/ and MT 184.

PROCEDURE

(a) Preparation of suspension and determination of sedimentation. MT 184

(b) Determination of isocycloseram in the bottom 25 ml of suspension. After removal of the top 225 ml suspension transfer the 25 ml remaining quantitatively with acetonitrile to a volumetric flask (100 ml). Mix thoroughly and sonicate solution. Allow to cool to room temperature and dilute to volume with acetonitrile. Filter until the solution is transparent. Take an aliquot of the solution and determine the mass of isocycloseram (Q g) by **XXX**/TC/(M)/3 in the calculation of isocycloseram content.

(c) Calculation.

$$Suspensibility = \frac{111(c - Q)}{c} \%$$

where:

c = mass of isocycloseram in the sample taken for the preparation of the suspension (g)

Q = mass of isocycloseram in the bottom 25 ml of suspension (g)

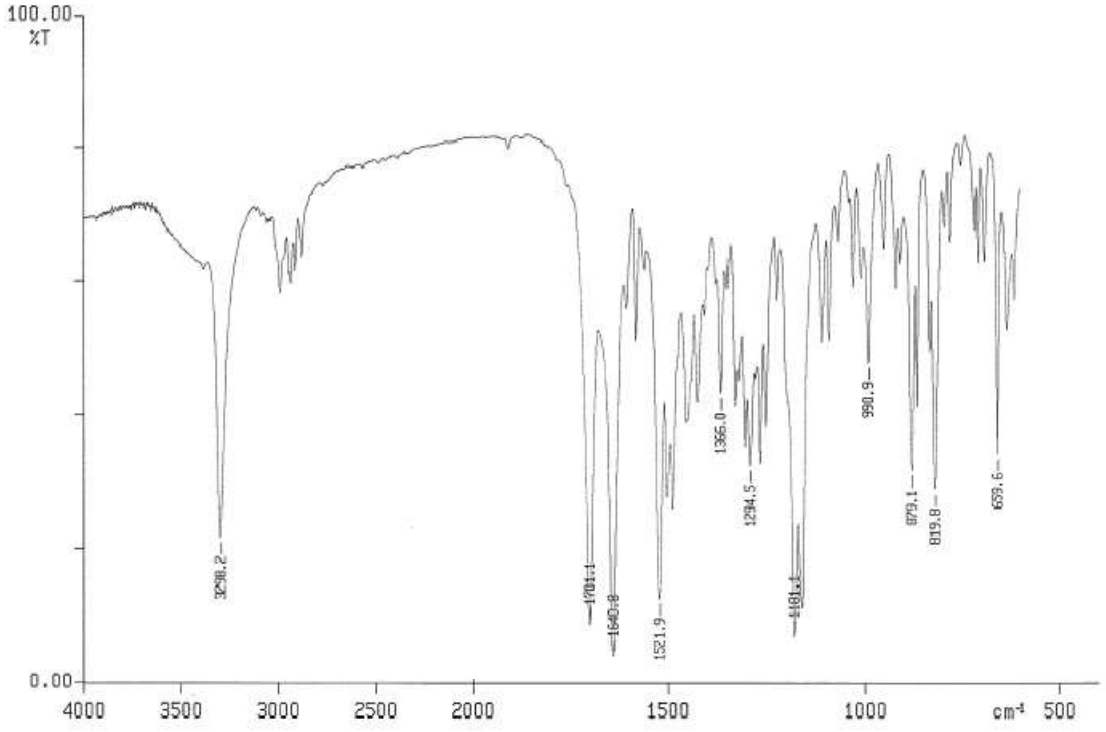

Fig 1 Typical IR spectrum according to **XXX**/TC/(M)/2.1

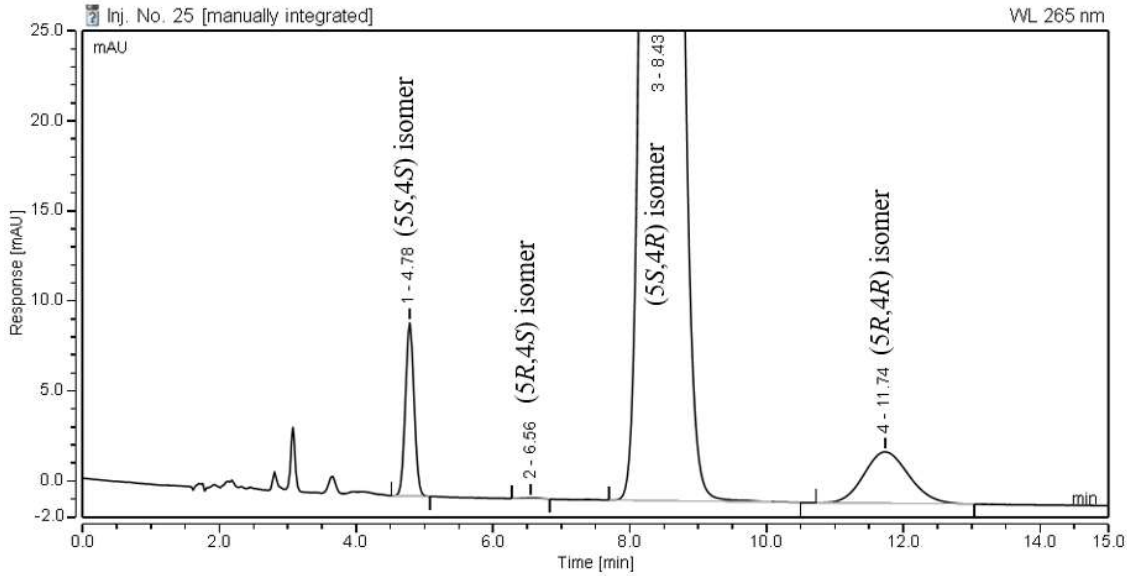

Fig 2 Typical chromatogram of Isocycloseram TC according to **XXX**/TC/(M)/2.2

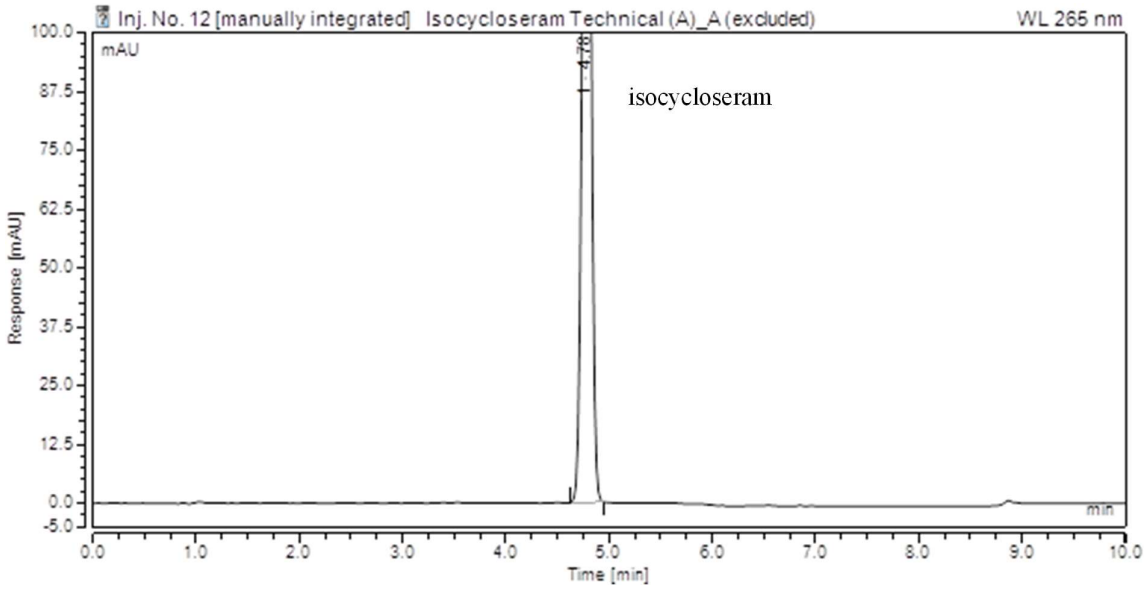

Fig 3 Typical chromatogram of Isocycloseram TC according to **XXX**/TC/(M)/3

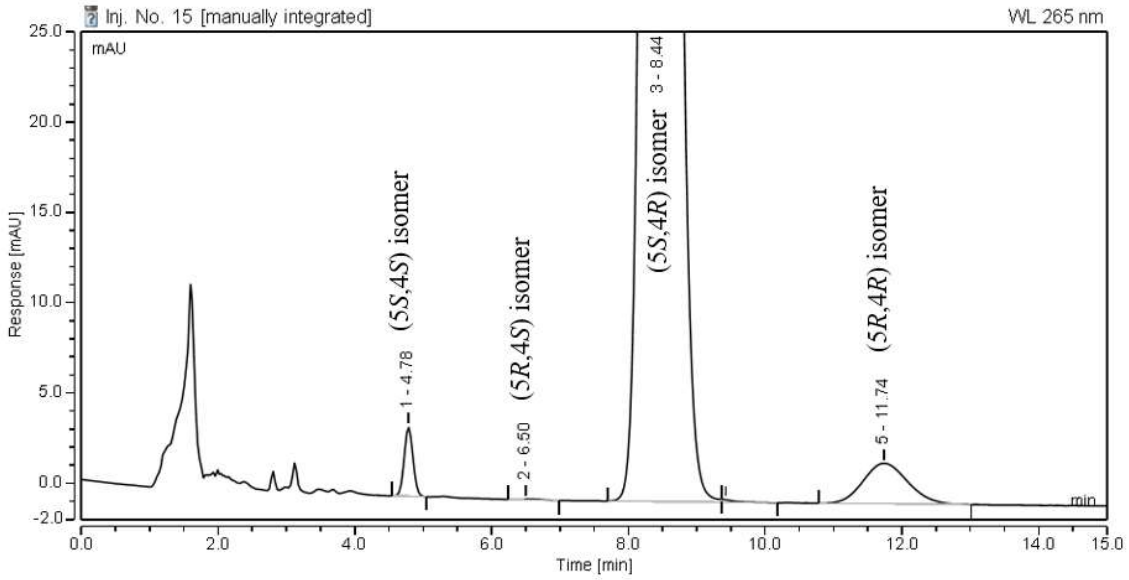

Fig 4 Typical chromatogram of Isocycloseram WP according to **XXX**/WP/(M)/2.2

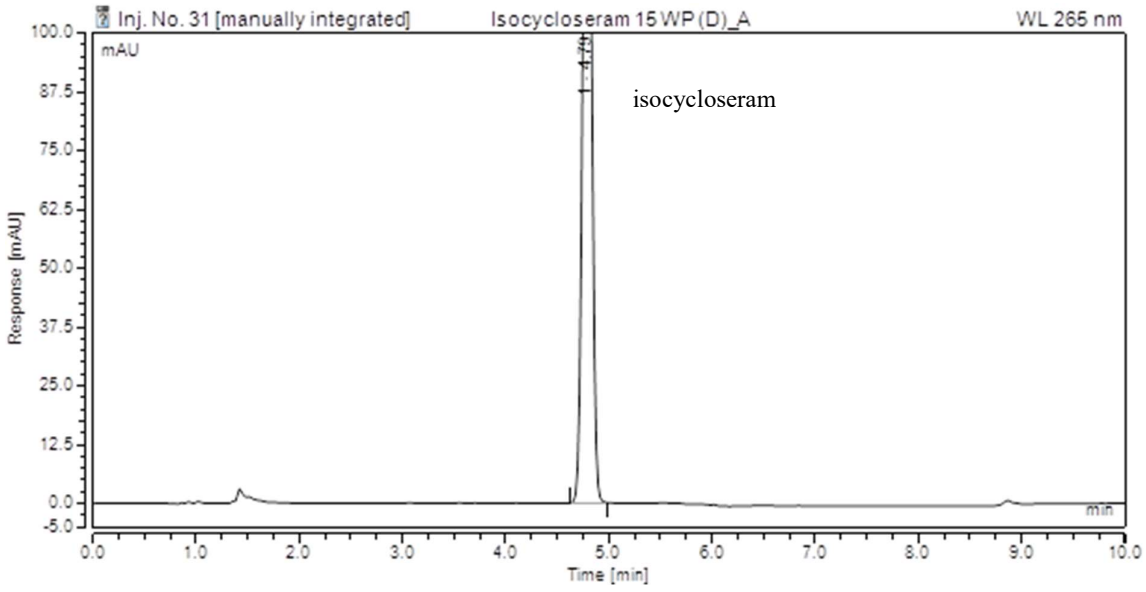

Fig 5 Typical chromatogram of Isocycloseram WP according to xxx/WP/M/3