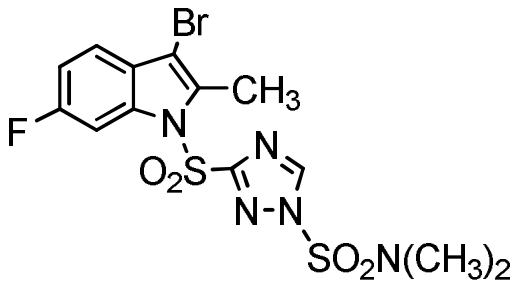
AMISULBROM
789



ISO common name	Amisulbrom
Chemical name	3-(3-bromo-6-fluoro-2-methylindol-1-ylsulfonyl)-*N*,*N*-dimethyl-1,2,4-triazole-1-sulfonamide (IUPAC) 3-[(3-bromo-6-fluoro-2-methyl-1*H*-indol-1-yl)sulfonyl]-*N*,*N*-dimethyl-1*H*-1,2,4-triazole-1-sulfonamide (CA)
CAS No.	348635-87-0
Empirical formula	$C_{13}H_{13}BrFN_5O_4S_2$
RMM	466.3
m.p.	128.6 – 130.0ºC
v.p.	1.8×10^{-8} Pa (25ºC)
Solubility	In water: 0.11 mg/l, *n*-hexane: 0.26 g/l, *n*-octanol: 2.60 g/l, methanol: 10.1 g/l, toluene: 88.6 g/l, dichloromethane, acetone acetonitrile and ethyl acetate: >250 g/l at 20ºC
Description	White to pale yellow solid
Stability	DT50 in water: 88 d (pH 4), 76 d (pH 7), 7.1 d (pH 9) at 25ºC
Formulations	Water dispersible granules and suspension concentrates

AMISULBROM
789/TC/m/-

1. Sampling. Take at least 100 g.

2. Identity tests.

2.1 HPLC. Use the HPLC method described below. The retention time of amisulbrom for the sample solution should not deviate by more than 0.2 min from that of the calibration solution.

2.2 Infrared. Take 1 mg of sample and 100 mg of dry KBr powder to form a KBr disc and scan the disks from 4000 to 400 cm^{-1}. The spectrum produced from the sample should not differ significantly from that of the standard (Figure 1).

3. Amisulbrom

OUTLINE OF METHOD Amisulbrom is determined by reversed phase high performance liquid chromatography using UV detection at 254 nm and external standardisation.

REAGENTS

Acetonitrile HPLC grade

Water HPLC grade

Phosphoric acid

Amisulbrom standard of known purity

Mobile phase Acetonitrile – 0.01% v/v aqueous phosphoric acid (75+25) (v/v)

Calibration solution. Weigh in duplicate (to the nearest 0.1 mg) 100 mg (*s* mg) of amisulbrom standard into separate volumetric flasks (100 ml). Add mobile phase (about 90 ml) and place the flask in an ultrasonic bath for 1 min. Fill to the mark with mobile phase. Mix thoroughly (calibration solutions C_1 and C_2).

APPARATUS

High performance liquid chromatograph equipped with a detector suitable for operation at 254 nm, constant-temperature column

compartment and an injector capable of delivering 5 µl.

Column stainless steel, 250 × 4.6 mm (i.d.) packed with YMC Pack Pro C18 5 µm, or equivalent.

Electronic integrator or data system

Filtration unit equipped with a PTFE membrane, 0.45 µm.

Ultrasonic bath

PROCEDURE

(a) Liquid chromatographic conditions (typical):

Column	stainless steel, 250 × 4.6 mm (i.d.), packed with YMC Pack Pro C18 5 µm, or equivalent.
Mobile phase	acetonitrile – 0.01% v/v aqueous phosphoric acid, 75+25 (v/v)
Temperature	40°C
Flow rate	1.0 ml/min
Detection wavelength	254 nm
Injection volume	5 µl
Retention time	amisulbrom: about 9 min.

(*b) Linearity check.* Check the linearity of the detector response by injecting 5 µl of amisulbrom reference standard solutions at concentrations of 0.5, 1 and 2 times that of the calibration solution.

(c) System equilibration. Inject 5 µl portions of the calibration solution C_1 and repeat the injections until peak areas deviate by less than ±1.0% of the mean for two consecutive injections. Then inject consecutively two 5 µL portions of the second calibration solution (C_2). The mean response factor for this solution should not deviate by more than 1.0% from that of the first calibration solution (C_1), otherwise prepare new calibration solutions.

(d) Sample preparation. Prepare sample solutions in duplicate. Weigh (to the nearest 0.1 mg) 100 mg of amisulbrom technical into separate 100 ml volumetric flasks. Add mobile phase (about 90 ml) and

place the flask in an ultrasonic bath for 1 min. Fill to the mark with mobile phase. Mix thoroughly (sample solutions S_{TC1}-1, S_{TC1}-2, S_{TC2}-1 and S_{TC2}-2).

(e) Determination. Inject in duplicate 5 µl portions of each sample solution bracketing them by injections of the calibration solutions as follows;

C_1, S_{TC1}-1, S_{TC1}-1, C_2, S_{TC1}-2, S_{TC1}-2, C_1, S_{TC2}-1, S_{TC2}-1, C_2 ...

Determine the relevant peak areas.

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

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

$$\text{Amisulbrom content} = \frac{f \times H_w}{w} \text{ (g/kg)}$$

Where,

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

Repeatability r = 16 to 18 g/kg at 993 to 995 g/kg active ingredient content

Reproducibility R = 25 to 33 g/kg at 993 to 995 g/kg active ingredient content

AMISULBROM WATER DISPERSIBLE GRANULES
789/WG/m/-

1. Sampling. Take at least 100 g.

2. Identity tests.

2.1 HPLC. As for amisulbrom technical **789**/TC/m/2.1.

2.2 Infrared. Take 0.5 g of sample and shake vigorously for 2 minutes with acetone (2 ml). Filter to remove any residue. To 1 ml of the filtrate add water (9 ml) and mix in a centrifuging tube. Centrifuge the precipitate of amisulbrom. Remove the supernatant and add 10 ml of water to the residue. Shake the mixture and centrifuge. Remove the supernatant and dry the residue at 60°C under vacuum for three hours. Proceed as for amisulbrom technical **789**/TC/m/2.2.

3. Amisulbrom. As for 789/TC/m/3 except:
change 'PROCEDURE *(d) Sample preparation.*' as follows:
Prepare sample solutions in duplicate. Weigh (to the nearest 0.1 mg) 200 mg of WG formulation into separate 100 ml volumetric flasks. Add mobile phase (about 90 ml) and place the flask in an ultrasonic bath for 10 min. Allow to cool to ambient temperature and fill to the mark with mobile phase. Mix thoroughly. Clear each solution from the formulated product by filtration through a 0.45 μm PTFE (polytetrafluoroethylene) filter (sample solutions S_{WG}-1 and S_{WG}-2).

Repeatability r = 11 g/kg at 501 g/kg active ingredient content
Reproducibility R = 18 g/kg at 501 g/kg active ingredient content

AMISULBROM SUSPENSION CONCENTRATES
789/SC/m/-

1. Sampling. Take at least 500 ml.

2. Identity tests.

2.1 HPLC. As for amisulbrom technical **789**/TC/m/2.1.

2.2 Infrared. Take 1 ml of sample and shake vigorously for 2 minutes with acetone (2 ml). Filter to remove any residue. To 1 ml of the filtrate add water (9 ml) and mix in a centrifuging tube. Centrifuge the precipitate of amisulbrom. Remove the supernatant and add 10 ml of water to the residue. Shake the mixture and centrifuge. Remove the supernatant and add 1 ml of methanol and 9 ml of water to the residue. Place it in ultrasonic bath for 2 minutes and centrifuge. Remove the supernatant and dry the residue at 60°C under vacuum for three hours. Proceed as for amisulbrom technical **789**/TC/m/2.2.

3. Amisulbrom. As for **789**/TC/m/3 except:

Change 'PROCEDURE *(d) Sample preparation.*' as follows:

Homogenise each sample by vigorous shaking for 10 min. Prepare sample solutions in duplicate. Weigh (to the nearest 0.1 mg) 500 mg of SC formulation into separate 100 ml volumetric flasks. Add mobile phase (about 90 ml) and place the flask in an ultrasonic bath for 10 min. Allow to cool to ambient temperature and fill to the mark with mobile phase. Mix thoroughly. Clear each solution from the formulated products by filtration through a 0.45 µm PTFE (polytetrafluoroethylene) filter (sample solutions S_{SC1}-1, S_{SC1}-2, S_{SC2}-1 and S_{SC2}-2).

Repeatability r	= 3 g/kg at 177 to 179 g/kg active ingredient content
Reproducibility R	= 5 g/kg at 177 to 179 g/kg active ingredient content

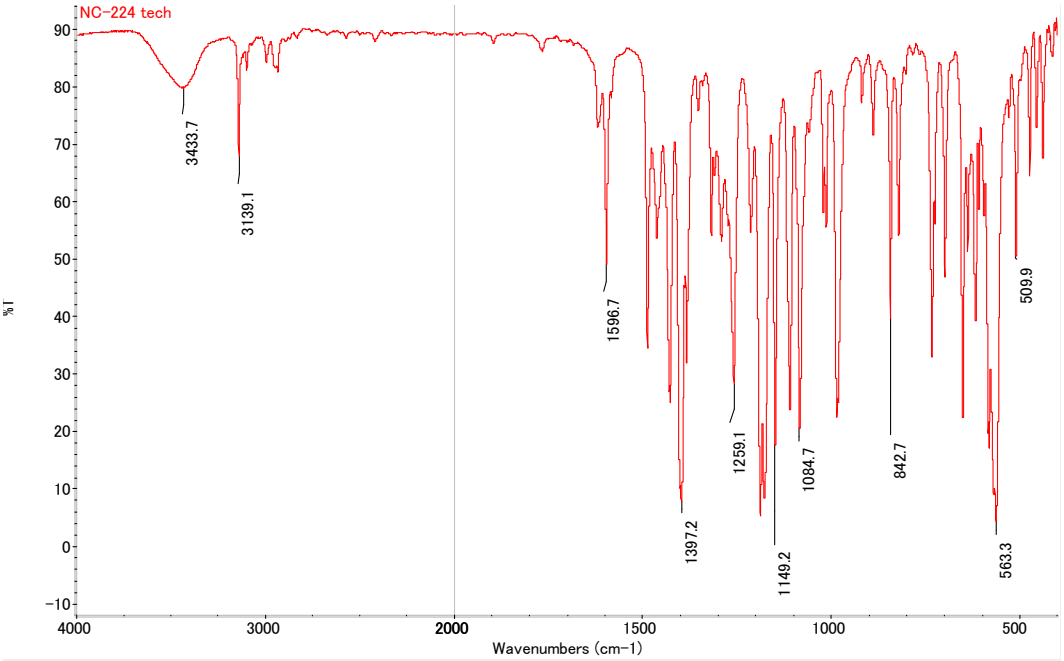


Figure 1 Infrared Spectrum of amisulbrom

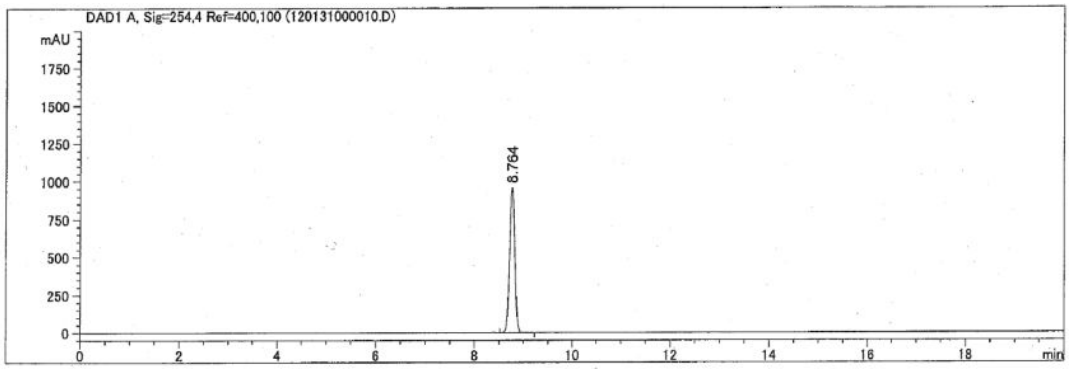


Figure 2 Chromatogram of amisulbrom TC

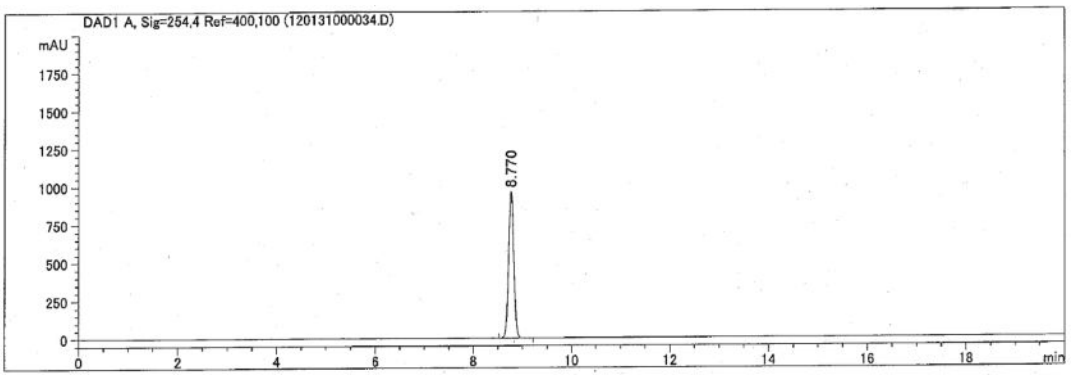


Figure 3 Chromatogram of amisulbrom WG

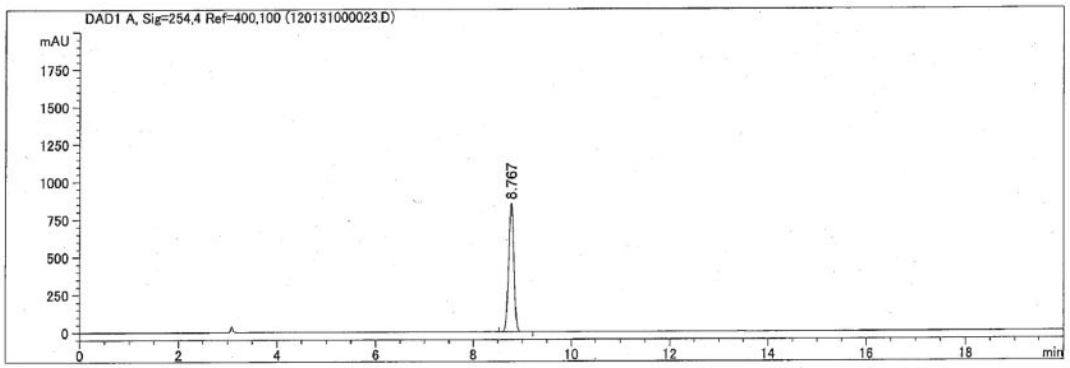


Figure 4 Chromatogram of amisulbrom SC