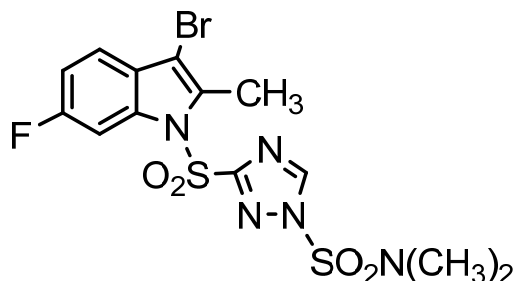


**AMISULBROM****789**

<i>ISO common name</i>	Amisulbrom
<i>Chemical name</i>	3-(3-bromo-6-fluoro-2-methylindol-1-ylsulfonyl)- <i>N,N</i> -dimethyl-1,2,4-triazole-1-sulfonamide (IUPAC) 3-[(3-bromo-6-fluoro-2-methyl-1 <i>H</i> -indol-1-yl)sulfonyl]- <i>N,N</i> -dimethyl-1 <i>H</i> -1,2,4-triazole-1-sulfonamide (CA)
<i>CAS No.</i>	348635-87-0
<i>Empirical formula</i>	C <sub>13</sub> H <sub>13</sub> BrFN <sub>5</sub> O <sub>4</sub> S <sub>2</sub>
<i>RMM</i>	466.3
<i>m.p.</i>	128.6 – 130.0 °C
<i>v.p.</i>	1.8 × 10 <sup>-8</sup> Pa (25 °C)
<i>Solubility</i>	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 20 °C
<i>Description</i>	White to pale yellow solid
<i>Stability</i>	DT50 in water: 88 d (pH 4), 76 d (pH 7), 7.1 d (pH 9) at 25 °C
<i>Formulations</i>	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  $\text{cm}^{-1}$ . 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<sub>1</sub> and C<sub>2</sub>).

**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  $\mu$ l.

*Column* stainless steel, 250  $\times$  4.6 mm (i.d.) packed with YMC Pack Pro C18 5  $\mu$ m, or equivalent.

*Electronic integrator or data system*

*Filtration unit* equipped with a PFTE membrane, 0.45  $\mu$ m.

*Ultrasonic bath*

## PROCEDURE

(a) *Liquid chromatographic conditions* (typical):

<i>Column</i>	stainless steel, 250 $\times$ 4.6 mm (i.d.), packed with YMC Pack Pro C18 5 $\mu$ m, or equivalent.
<i>Mobile phase</i>	acetonitrile – 0.01% v/v aqueous phosphoric acid, 75+25 (v/v)
<i>Temperature</i>	40 $^{\circ}$ C
<i>Flow rate</i>	1.0 ml/min
<i>Detection wavelength</i>	254 nm
<i>Injection volume</i>	5 $\mu$ l
<i>Retention time</i>	amisulbrom: about 9 min.

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

(c) *System equilibration.* Inject 5  $\mu$ l portions of the calibration solution  $C_1$  and repeat the injections until peak areas deviate by less than  $\pm 1.0\%$  of the mean for two consecutive injections. Then inject consecutively two 5  $\mu$ L 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  $\mu$ l portions of each sample solution bracketing them by injections of the calibration solutions as follows;

$C_1, S_{TC1-1}, S_{TC1-1}, C_2, S_{TC1-2}, S_{TC1-2}, C_1, S_{TC2-1}, S_{TC2-1}, C_2 \dots$

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** = 17 to 20 g/kg at 1002 g/kg active ingredient content

**Reproducibility R** = 19 to 27 g/kg at 1002 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<sub>WG-1</sub> and S<sub>WG-2</sub>).

**Repeatability r** = 5 g/kg at 507 g/kg active ingredient content

**Reproducibility R** = 5 g/kg at 507 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<sub>SC1-1</sub>, S<sub>SC1-2</sub>, S<sub>SC2-1</sub> and S<sub>SC2-2</sub>).

**Repeatability r** = 2 to 3 g/kg at 179 to 180 g/kg active ingredient content

**Reproducibility R** = 2 to 3 g/kg at 179 to 180 g/kg active ingredient content

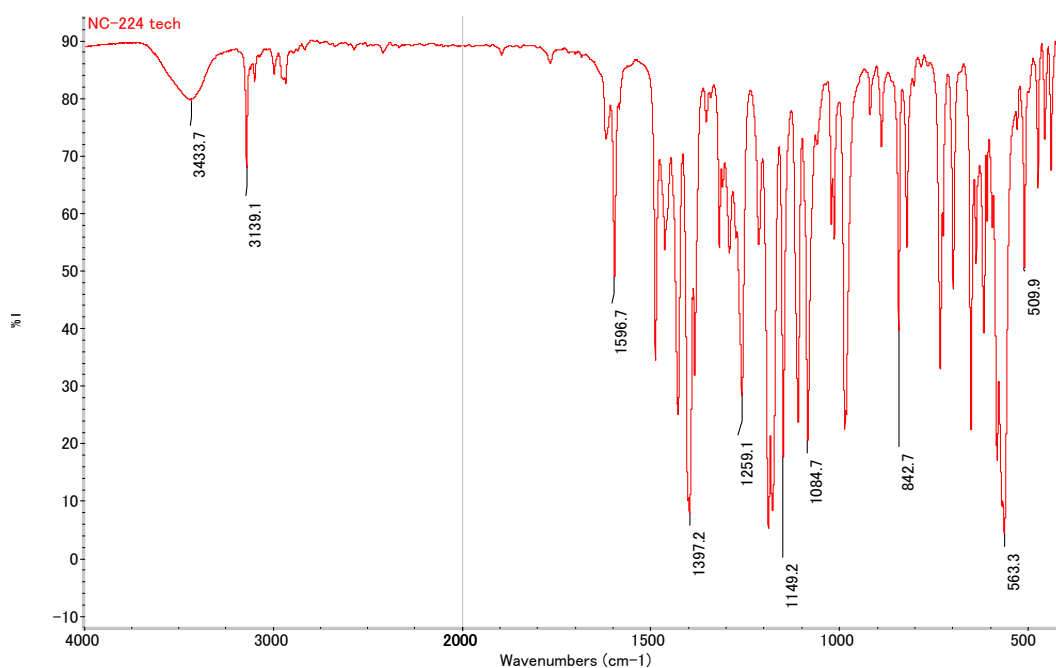


Figure 1 Infrared Spectrum of amisulbrom

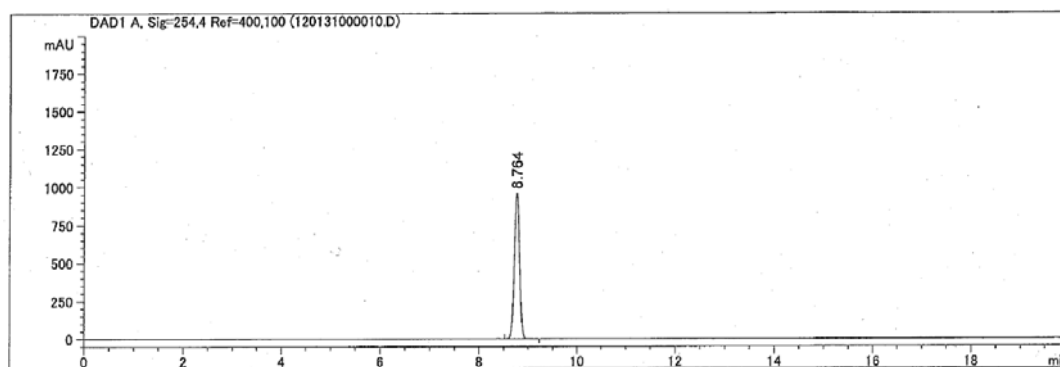


Figure 2 Chromatogram of amisulbrom TC

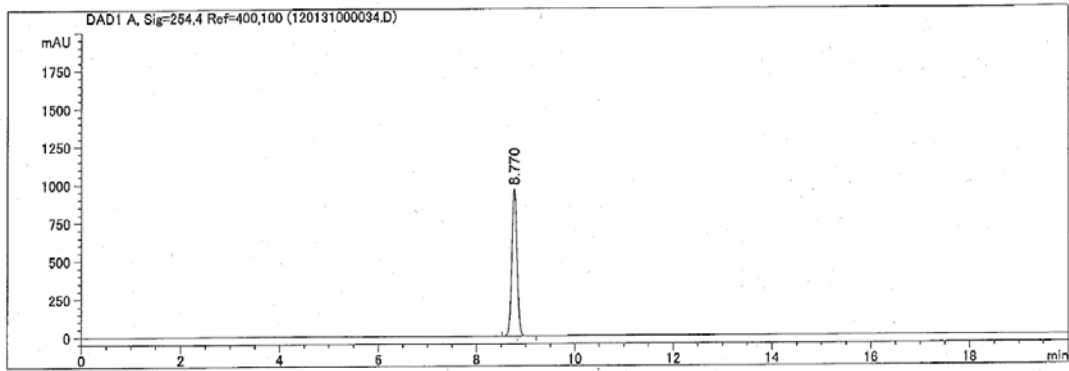


Figure 3 Chromatogram of amisulbrom WG

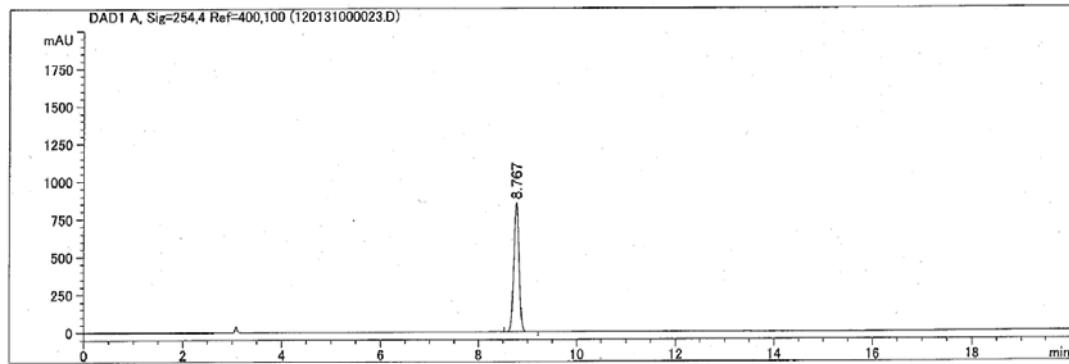


Figure 4 Chromatogram of amisulbrom SC