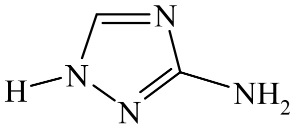
AMITROLE
90



ISO common name	Amitrole
Chemical name	1-*H*-1,2,4-triazol-3-ylamine (IUPAC); 1-*H*-1,2,4-triazol-3-amine (CA; *000061-82-5*)
Empirical formula	$C_2H_4N_4$
RMM	84.1
m.p.	155 °C (technical below 145 °C)
Dissociation const.	pK_1: 4.0; pK_2: 11.0 at 20 °C
Solubility	In water: 264 g/l at pH 7, 261 g/l at pH 10; amitrole hydrochloride: 1384 g/l at pH 4; methanol: 133-160 g/l; acetone: 2.9-3.3 g/l; ethyl acetate: 1 g/l; 1,2-dichloroethane: less than 0.1 g/l; heptane and xylene: much less than 0.1 g/l
Description	White crystalline powder
Stability	Stable under normal conditions
Formulations	Wettable powders, water soluble concentrates and suspension concentrates (usually mixed formulations)

AMITROLE TECHNICAL

[*]**90**/TC/M/-

1 Sampling. Take at least 100 g. If the material consists of flakes, grind a subsample (20 g) into a powder.

2 Identity tests

2.1 HPLC. Use the method described below. The retention time of amitrole for the sample solution should not deviate by more than 2% from that of the calibration solution.

2.2 Infrared. Prepare potassium bromide discs of amitrole hydrochloride from the sample and amitrole standard substance using the following conditions:

Reference solution	Dissolve amitrole standard substance (5 mg) in water (2 ml).
Sample solution	Weigh sufficient sample to contain 5 mg of amitrole and dissolve in water (2 ml).
First clean up	Wash two cartridges OnGuard-RP DIONEX, art No P/N 039595 (or equivalent RP material) with water (8 ml). Then percolate the one with the reference solution and the other with the sample solution at a flow rate of 2 ml/min.
Second cleanup ion exchange	Wash two cartridges OnGuard-H DIONEX, art No P/N 039596 (or equivalent SCX material) with water (2 ml) and percolate the one with the percolate of the first cleanup step of the reference solution and the other with that of the sample solution at a flow rate of 1 ml/min. Rinse the cartridges with water (6 ml).
Elution	Elute the SCX cartridges with hydrochloric acid and collect the eluate from each column in an evaporating dish.
Drying	Dry the eluates in a ventilated oven at 105 °C.
Infrared spectra	Weigh about 1 mg of the residue and add potassium bromide (about 100 mg). Grind and dry the mixed powder in an oven at 80 °C for 5 min. Press the powder to a disc and scan the discs from 600 to 4000 cm^{-1}. The spectrum obtained from the sample should not differ significantly from that of the reference material apart from possible bands of ammonium chloride at 1400 cm^{-1} and between 3000 and 3500 cm^{-1}.

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[*] CIPAC method 1999. Prepared by PAC-F. Chairman: B Declercq. Based on a method developed by Bayer, Elf-Atochem and CFPI-Agro.

3 Amitrole

OUTLINE OF METHOD Amitrole is determined by normal phase high performance liquid chromatography (HPLC) on a silica diol phase with quantification by external standard.

REAGENTS

Acetonitrile HPLC grade
Water HPLC grade
Amitrole standard of known purity
Ammonium acetate
Ammonium acetate solution 1 % in HPLC grade water
Mobile phase Weigh ammonium acetate (400 ± 10 mg) into a beaker (125 ml), add HPLC grade water (40 ml) and shake until dissolved completely. Transfer the contents of the beaker to a volumetric flask (1000 ml) and rinse the beaker several times with acetonitrile. Fill to the mark with acetonitrile and mix thoroughly. Filter the solution through 0.45 µm filter before use.
Calibration solution. Weigh (to the nearest 0.1 mg) into two volumetric flasks (100 ml) amitrole reference material (85 to 100 mg, *s* mg). Dissolve in ammonium acetate solution (4 ml). Add to each flask acetonitrile (80 ml) and mix thoroughly. Allow to warm to room temperature (mixing water and acetonitrile is an endothermic process) and make up to volume with acetonitrile. Invert the flasks at least ten times to homogenise (solutions C_1 and C_2). Filter the solution through a 0.45 µm filter before HPLC analysis.

APPARATUS

Liquid chromatograph equipped with an automatic loop injector (5 µl) and a UV detector capable of measuring the absorbance at 215 nm and fitted with a 14 µl cell (path length: 8 mm).
Liquid chromatographic column LiChroCART 250-4 cartridge, 250 × 4 mm (i.d.) packed with LiChrospher 100 DIOL, 5 µm (Merck art. No. 50836), or equivalent. Condition a new column as prescribed by the manufacturer.
Pre-column LiChroCART 4-4 LiChrospher 100 DIOL, 5 µm (Merck art. No. 1.50960.0001), or equivalent
Electronic integrator or *data system*
Filtration system Millipore HVLP 04700-0.45 µm or equivalent
Disposable filters Millipore SSLCR 013 NL-0.45 µm, or equivalent

PROCEDURE

(a) Operating conditions (typical):

Flow rate	1.2 ml/min
Column temperature	40 °C
Injection volume	5 µl. The peak height should not exceed 1000 milli absorbance units
Detector wavelength	215 nm
Chromatographic duration	25 min
Retention times	amitrole: 7.2 min

(b) Preparation of sample. If necessary grind the sample (about 20 g). Weigh (to the nearest 0.1 mg) into a volumetric flask (100 ml) sufficient sample to contain 85 to 90 mg amitrole (*w* mg). Dissolve in ammonium acetate solution (4 ml). Add acetonitrile (80 ml) and mix thoroughly. Allow to warm to room temperature (mixing water and acetonitrile is an endothermic process) and make up to volume with acetonitrile. Invert the flask at least ten times to homogenise (solution S_1). Filter the solution through a 0.45 µm filter before HPLC analysis.

(c) Column conditioning. Before injecting condition the column with mobile phase for at least two hours under the given operating conditions.

(d) Determination. Inject 5 µl portions of the two calibration solutions C_1 and C_2 until the response factor varies by less than 1% of the mean. Then inject 5 µl aliquots of the calibration and sample solutions in the following order:

$$C_1, S_1, S_1, C_2, S_2, S_2, C_2, \ldots\text{etc.}$$

Determine the peak areas. Calculate the response factors (f_1 and f_2) for the pair of calibration solutions that bracket the sample solution injections and calculate the mean (f).

(d) Calculation

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

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

where:

f_i = response factor
f = average response factor
H_s = area of amitrole peak in the calibration solution
H_w = area of amitrole peak in the sample solution
s = mass of amitrole in the calibration solution (mg)
w = mass of sample taken (mg)
P = purity of amitrole reference substance (g/kg)

Repeatability r	=	18 g/kg at 921 g/kg active ingredient content
	=	27 g/kg at 913 g/kg active ingredient content
Reproducibility R	=	19 g/kg at 921 g/kg active ingredient content
	=	37 g/kg at 913 g/kg active ingredient content

AMITROLE WETTABLE POWDERS

[*] **90**/WP/M/-

SCOPE The method is also applicable to amitrole in mixtures with diuron.

1 Sampling. Take at least 500 g.

2 Identity tests
2.1 HPLC. As for amitrole technical **90**/TC/M/2.1.
2.2 Infrared. As for amitrole technical **90**/TC/M/2.2.

3 Amitrole. As for amitrole technical **90**/TC/M/3 together with:
(a) Operating conditions (typical):

Retention times	diuron: 2.5 min

Note: Diuron is soluble in the mobile phase, but some formulants are not.

Repeatability r	=	9 g/kg at 235 g/kg active ingredient content
Reproducibility R	=	16 g/kg at 235 g/kg active ingredient content

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AMITROLE SOLUBLE CONCENTRATES
*90/SL/M/-

SCOPE The method is also applicable to amitrole in mixtures with ammonium thiocyanate.

1 Sampling. Take at least 500 ml.

2 Identity tests
2.1 HPLC. As for amitrole technical **90**/TC/M/2.1.
2.2 Infrared. As for amitrole technical **90**/TC/M/2.2.

3 Amitrole. As for amitrole technical **90**/TC/M/3 together with:

(a) Operating conditions (typical):
Retention times ammonium thiocyanate: 1.8 min
Note: Ammonium thiocyanate is soluble in the mobile phase.

Repeatability r = 11 g/kg at 207 g/kg active ingredient content
Reproducibility R = 13 g/kg at 207 g/kg active ingredient content

AMITROLE SUSPENSION CONCENTRATES
*90/SC/M/-

SCOPE The method is also applicable to amitrole in mixtures with terbutylazine.

1 Sampling. Take at least 500 ml.

2 Identity tests
2.1 HPLC. As for amitrole technical **90**/TC/M/2.1.
2.2 Infrared. As for amitrole technical **90**/TC/M/2.2.

3 Amitrole. As for amitrole technical **90**/TC/M/3 together with:
(a) Operating conditions (typical):
Retention times terbutylazine: 2.1 min
Note: Terbutylazine is soluble in the mobile phase. Nevertheless some small lumps are visible in the solution consisting of a minor formulant which is insoluble in the mobile phase.

Repeatability r = 8 g/kg at 167 g/kg active ingredient content
Reproducibility R = 10 g/kg at 167 g/kg active ingredient content

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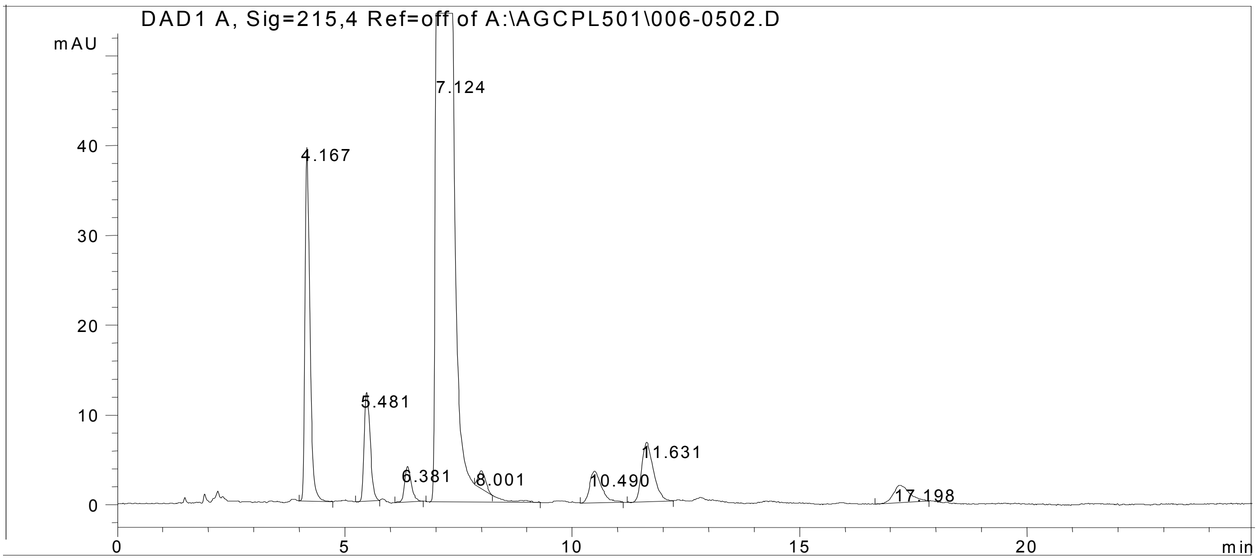


Retention time: 7.12 min, amitrole. Other retention times, process impurities

Fig. 1 Chromatogram of amitrole technical

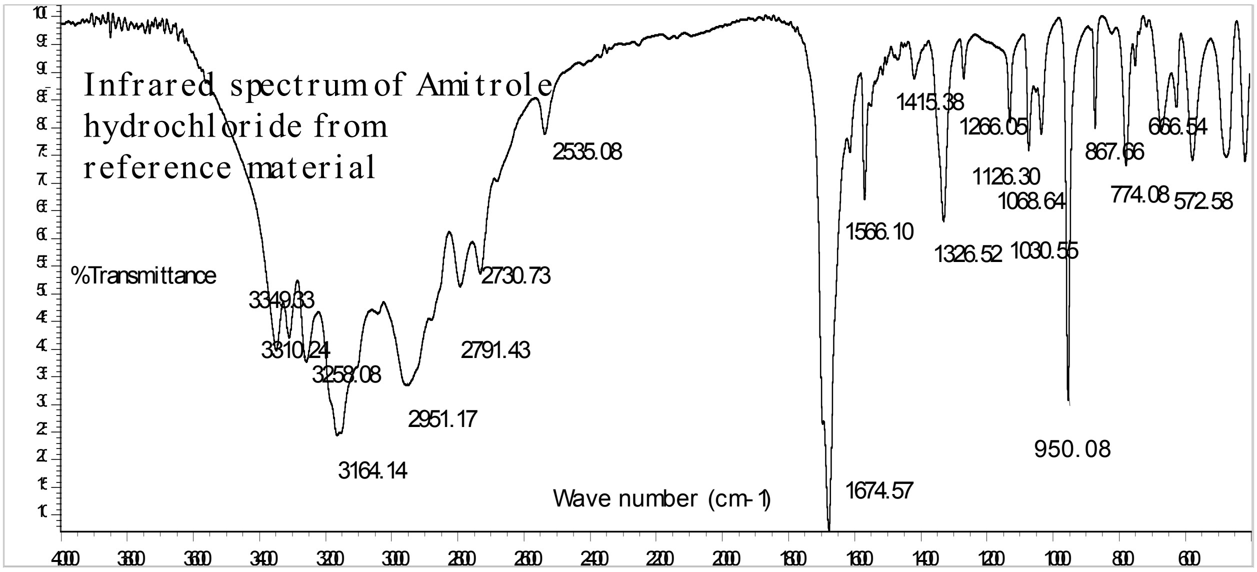
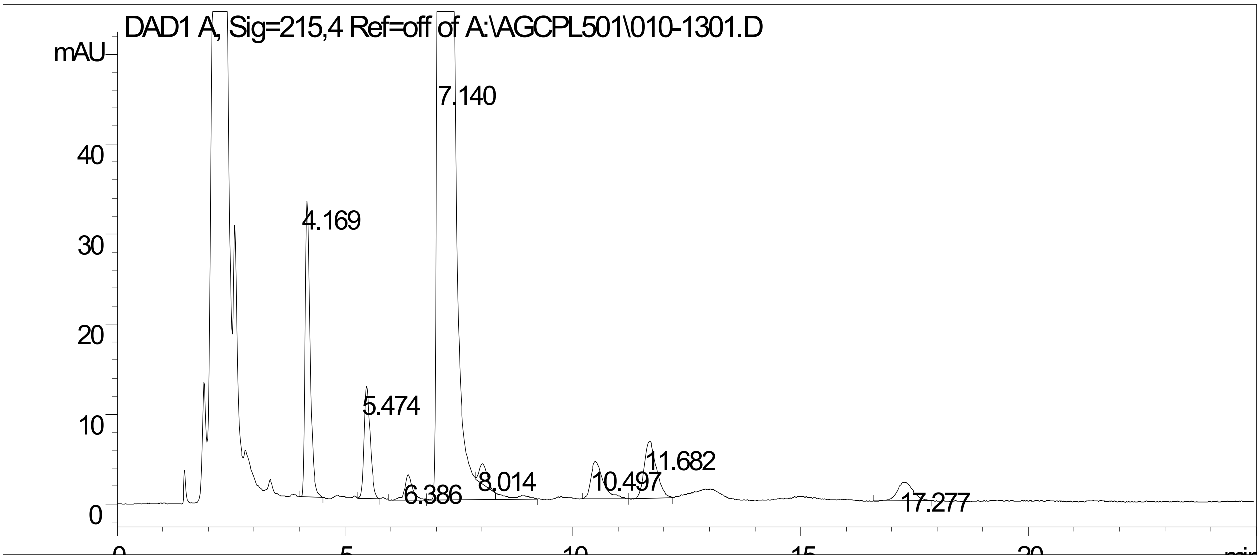


Fig. 2 IR spectrum of amitrole hydrochloride from reference material



Retention time: 7.14 min, amitrole; < 4 min, wettable powder formulants (mainly diuron, retention time: 2.30 min). Other retention times, process impurities and wettable powder formulants

Fig. 3 Chromatogram of wettable powder

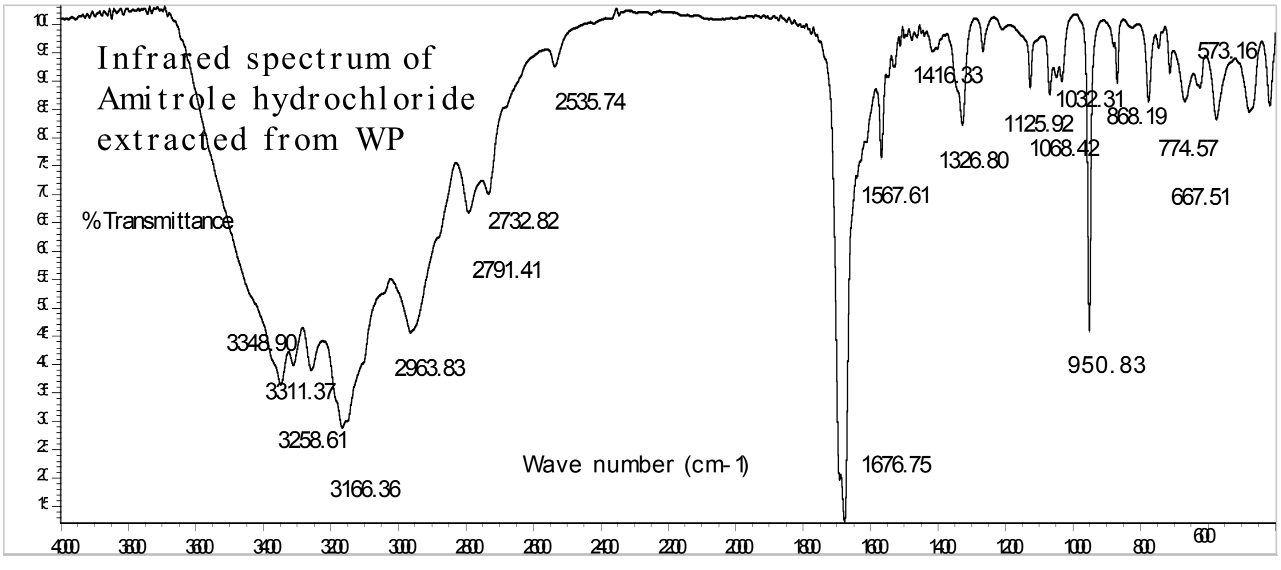
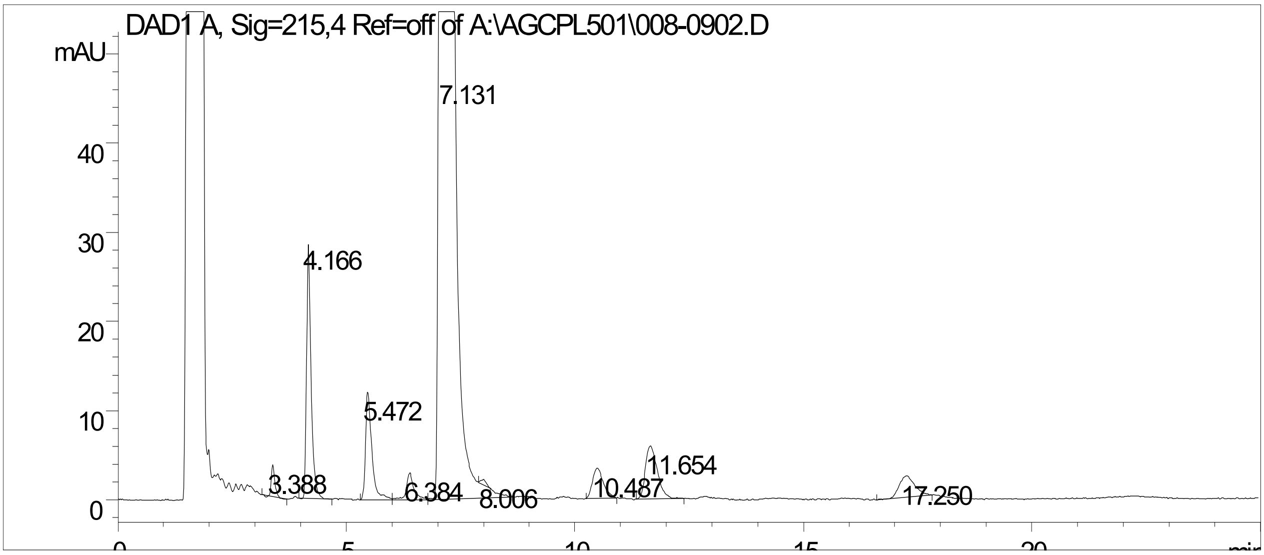


Fig. 4 IR spectrum of amitrole hydrochloride extracted from wettable powder



Retention time: 7.13 min, amitrole; < 4 min, solution concentrate formulants (mainly ammonium thiocyanate, retention time: 1.8 min). Other retention times, process impurities

Fig. 5 Chromatogram of soluble concentrate

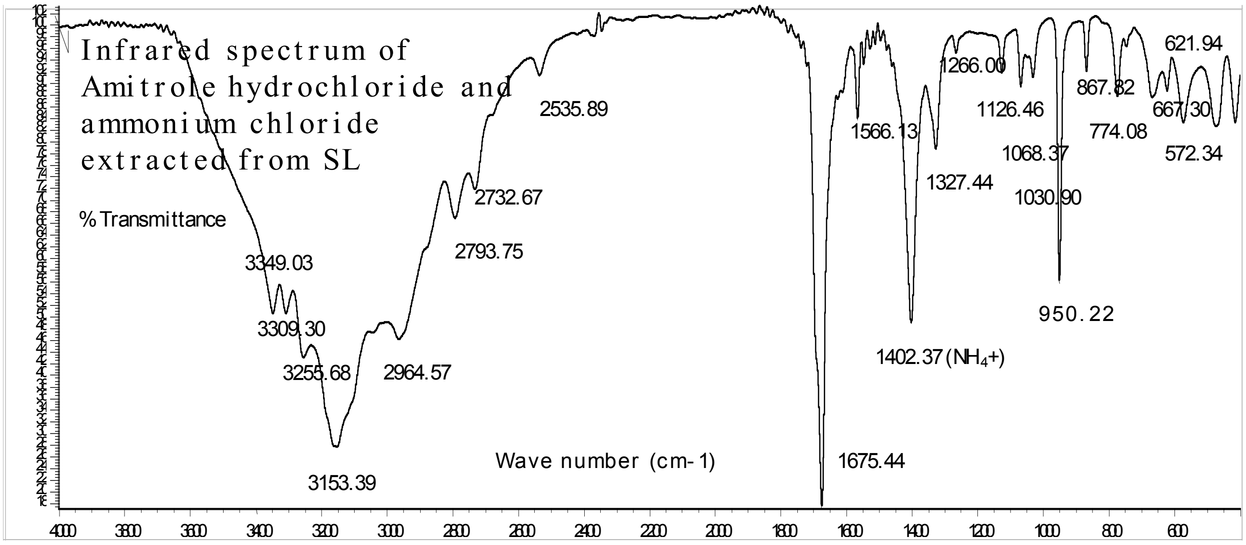
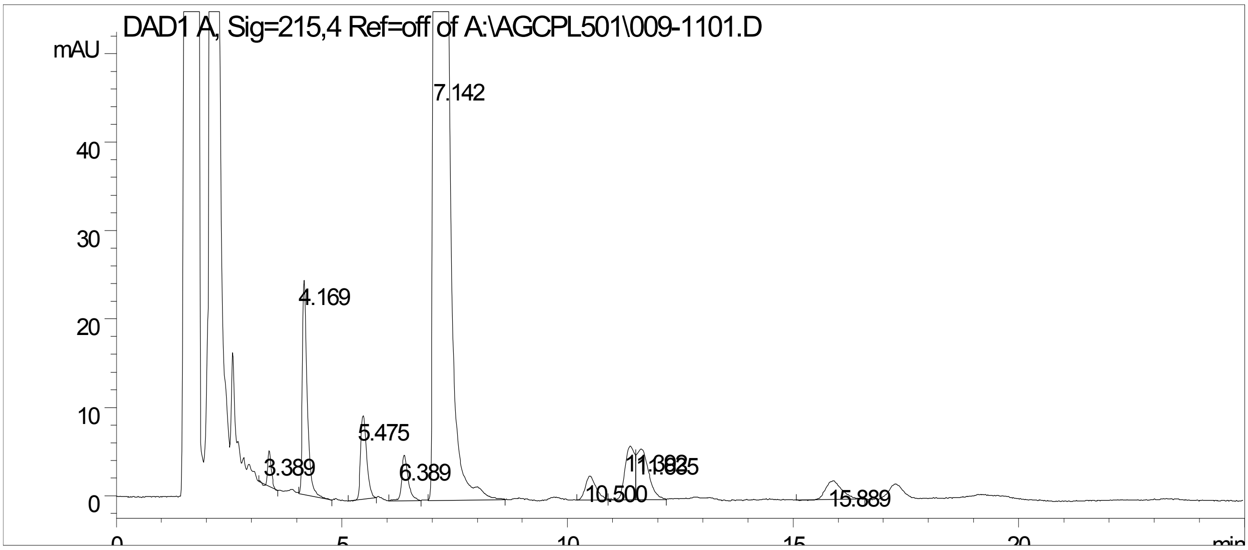


Fig. 6 IR spectrum of amitrole hydrochloride and ammonium hydrochloride extracted from soluble concentrate



Retention time: 7.14 min, amitrole; < 4 min, suspension concentrate formulants (mainly ammonium thiocyanate, retention time: 1.8 min and terbuthylazine, retention time: 2.1 min). Other retention times, process impurities and suspension concentrate formulants

Fig. 7 Chromatogram of suspension concentrate

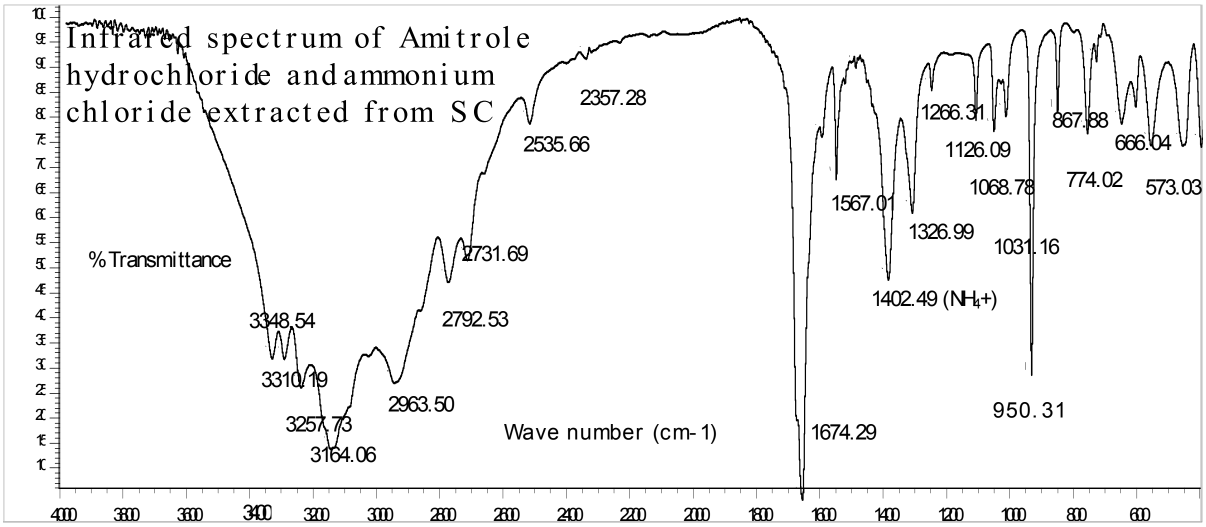


Fig. 8 IR spectrum of amitrole hydrochloride and ammonium chloride extracted from suspension concentrate