

**Triflumuron**  
**rel. Impurities**  
**1,3-bis(4-trifluoromethoxyphenyl)urea**  
**and**  
**4-(trifluoromethoxy)aniline**

**HPLC Method 5091/m**

**CIPAC Peer Validation**

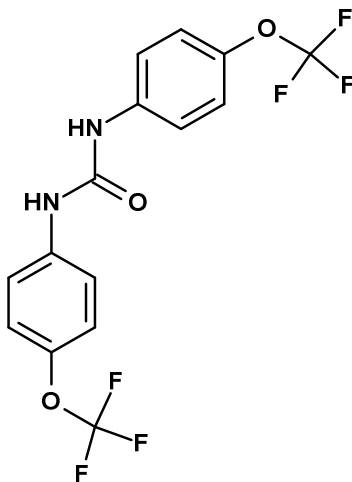
by

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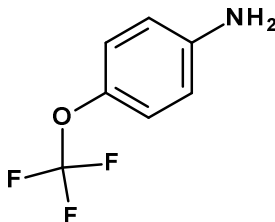
**TRIFLUMURON, rel. Impurities****1,3-bis(4-trifluoromethoxyphenyl)urea and 4-(trifluoromethoxy)aniline**

Report Name 1,3-bis(4-trifluoromethoxyphenyl)urea  
Synonyms N,N'-bis-[4-(trifluoromethoxy)phenyl]urea,  
AE B143886, BCS-AD26894  
Three letter code -  
Structural formula



Empirical Formula  $C_{15} H_{10} F_6 N_2 O_3$   
Molecular Weight 380.2 g/mol  
CAS no 78015-49-3

Report Name 4-(trifluoromethoxy)aniline  
Synonyms AE F069069, BCS-AC49934  
Three letter code -  
Structural formula



Empirical Formula  $C_7 H_6 F_3 N O$   
Molecular Weight 177.1 g/mol  
CAS no 461-82-5

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**TRIFLUMURON TECHNICAL  
5091/TC/M/-**

**1 Sampling.** Take at least 100 g\*. Grind the sample thoroughly in a mortar.

\*(for this trial less amount is provided; please grind the entire sample)

**2 Identity tests.**

**2.1 HPLC.** Use the HPLC method described below. The relative retention time of 1,3-bis(4-trifluoromethoxyphenyl)urea and 4-(trifluoromethoxy)aniline in the sample solution should not deviate by more than 2% from that of the calibration solution.

**2.2 UV spectrometry.** Record the UV spectrum during the HPLC determination. The UV spectrum obtained from the sample should not differ significantly from that of the standard. (Fig. 1 and Fig. 2)

**3 1,3-bis(4-trifluoromethoxyphenyl)urea and 4-(trifluoromethoxy)aniline.**

OUTLINE OF THE METHOD.

1,3-bis(4-trifluoromethoxyphenyl)urea and 4-(trifluoromethoxy)aniline contents are determined (g/kg) by reversed phase high performance liquid chromatography using UV detection at 226 nm and 258 nm and external standard calibration.

**3.1 Determination of 1,3-bis(4-trifluoromethoxyphenyl)urea and 4-(trifluoromethoxy)aniline by reversed phase HPLC**

REAGENTS

1,3-bis(4-trifluoromethoxyphenyl)urea and 4-(trifluoromethoxy)aniline reference standards of known content

Acetonitrile (HPLC grade)

Purified water (HPLC grade)

Eluent A: purified water 40% (v/v)

Eluent B: acetonitrile 60% (v/v)

APPARATUS

High performance liquid chromatograph equipped with an injection system capable to inject 5 µL and an UV spectrophotometric detector operated at 226 nm and 258 nm.

Liquid chromatography column, stainless steel, 125 x 4 (i.d.) mm, packed with Nucleosil 120-3 C 18; 3 µm or equivalent with the same selectivity.

Electronic integrator or data system

Ultrasonic bath

**PROCEDURE***(a) Operating conditions (typical):*

Flow rate: 1 mL/min  
Column temperature: 40°C  
Injection volume: 5 µL  
Detector wavelength: 0.0 – 3.0 min: 226 nm (for 4-(trifluoromethoxy)aniline)  
3.0 – 3.7 min: 258 nm  
3.7 – 5.0 min: 300 nm (for triflumuron)  
5.0 – 9.0 min: 258 nm (for 1,3-bis(4-trifluoromethoxyphenyl)urea)

Retention time: approx. 2.2 min for 4-(trifluoromethoxy)aniline  
approx. 5.2 min for 1,3-bis(4-trifluoromethoxyphenyl)urea

Total run time: approx. 8 min

*(b) Equilibration of the system.* Pump sufficient mobile phase through the column to equilibrate the system. Inject 5 µL portions of the calibration solution C1 and repeat the injections until retention times and peak areas deviate by less than  $\pm 1$  % from the mean for three successive injections.

*(c) Calibration solution.* Weigh in duplicate (to the nearest 0.1 mg) approximately 20 mg (s mg) of the reference standard of 1,3-bis(4-trifluoromethoxyphenyl)urea and 4-(trifluoromethoxy)aniline into separate volumetric flasks (50 mL). Add 20 mL acetonitrile and place the flasks in an ultrasonic bath for 15 min. Make up the flasks with acetonitrile to just below the calibration mark and allow to cool to ambient temperature. Fill to the mark with acetonitrile and mix thoroughly.

Transfer 5 mL of this solution into a separate volumetric flask (50 mL) and make up the flasks with acetonitrile to just below the calibration mark. Allow to cool to ambient temperature, fill to the mark with acetonitrile and mix thoroughly.

Depending on the final concentrations of the analytes in the sample solution, transfer 3 mL of this solution into a separate volumetric flask (50 mL) and make up the flasks with acetonitrile to just below the calibration mark. Allow to cool to ambient temperature, fill to the mark with acetonitrile and mix thoroughly (Calibration solutions C1, C2) (Fig. 3).

*(d) Sample preparation.* Prepare sample solutions in duplicate for each sample. Weigh (to the nearest 0.1 mg) sufficient sample (*w* mg) to contain about approximately 0.12 mg (*w* mg) of 1,3-bis(4-trifluoromethoxyphenyl)urea and 4-(trifluoromethoxy)aniline into separate volumetric flasks (50 mL). Add 20 mL acetonitrile and place the flasks in an ultrasonic bath for 15 min. Make up the flasks with acetonitrile to just below the calibration mark and allow to cool to ambient temperature. Fill to the mark with acetonitrile and mix thoroughly (Sample solutions S1, S2) (Fig. 4).

*(e) Determination.* Inject in duplicate each sample solution and bracket a series of sample solution injections by injections of the calibration solutions as follows: calibration solution 1, calibration solution 2, calibration solution 1, sample solution 1, sample solution 1, sample solution 2, sample solution 2, calibration solution 1, ... (C1, C2, C1, S1, S1, S2, S2, C1, ...).

Determine the peak areas of 1,3-bis(4-trifluoromethoxyphenyl)urea and 4-(trifluoromethoxy)aniline.

*(f) Calculation*

Calculate the response factors from the calibration solutions bracketing the injections of the sample solutions. Average the response factors of the calibration solutions preceding and following the sample solution injections. These must agree within  $\pm 1\%$  of the average otherwise repeat the determination. Calculate the content of the sample solutions.

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

1,3-bis(4-trifluoromethoxyphenyl)urea and 4-(trifluoromethoxy)aniline

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

Where:

- $f_i$  = single response factor
- $f$  = average response factor
- $H_s$  = peak area of 1,3-bis(4-trifluoromethoxyphenyl)urea and 4-(trifluoromethoxy)aniline standard in the calibration solution
- $H_w$  = peak area of 1,3-bis(4-trifluoromethoxyphenyl)urea and 4-(trifluoromethoxy)aniline in the sample solution
- $s$  = weight of the 1,3-bis(4-trifluoromethoxyphenyl)urea and 4-(trifluoromethoxy)aniline standard in the calibration solution (mg)
- $w$  = weight of the sample (mg)
- $P$  = purity of the 1,3-bis(4-trifluoromethoxyphenyl)urea and 4-(trifluoromethoxy)aniline standard (g/kg)

### TRIFLUMURON SUSPENSION CONCENTRATE 5091/SC/M/-

**1 Sampling.** Take at least 500 mL\*. Shake the sample well prior to weighing.

\*(for this trial less amount is provided)

**2 Identity tests.**

**2.1 HPLC.** As for 1,3-bis(4-trifluoromethoxyphenyl)urea and 4-(trifluoromethoxy)aniline in Triflumuron 5091/TC/M/-

**2.2 UV spectrometry.** As for 1,3-bis(4-trifluoromethoxyphenyl)urea and 4-(trifluoromethoxy)aniline in Triflumuron 5091/TC/M/-

**3 1,3-bis(4-trifluoromethoxyphenyl)urea and 4-(trifluoromethoxy)aniline.**

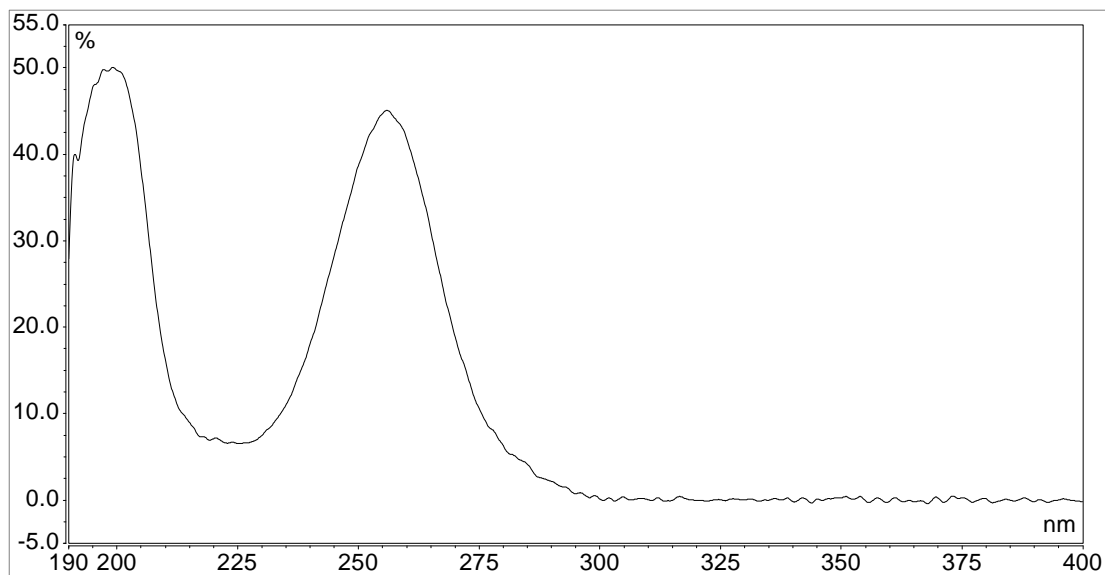
Same approach as for 1,3-bis(4-trifluoromethoxyphenyl)urea and 4-(trifluoromethoxy)aniline in Triflumuron 5091/TC/M/-

**3.1 Determination of 1,3-bis(4-trifluoromethoxyphenyl)urea and 4-(trifluoromethoxy)aniline by reversed phase HPLC**

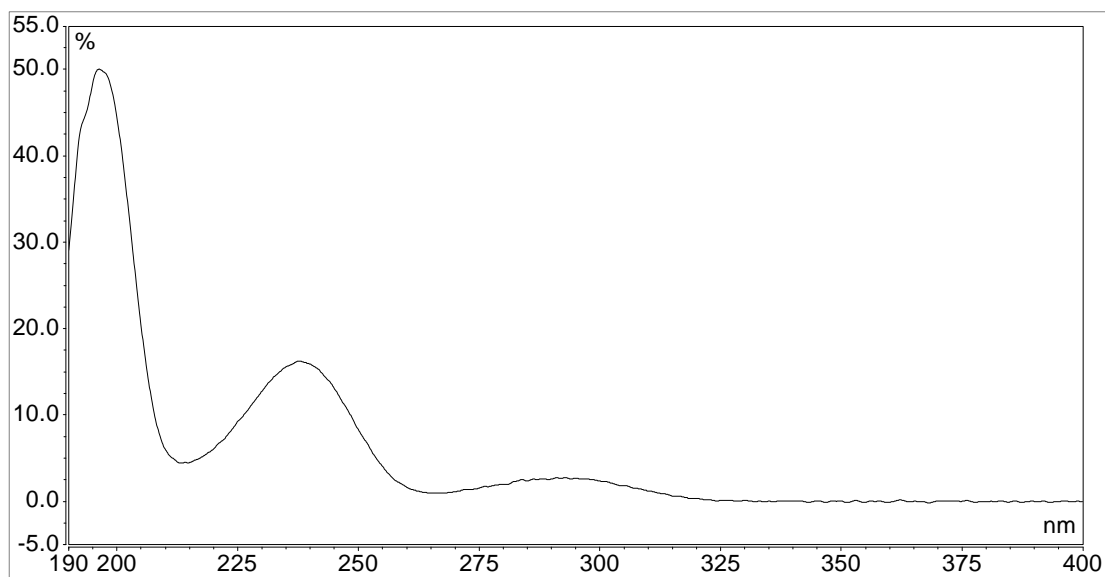
As for 1,3-bis(4-trifluoromethoxyphenyl)urea and 4-(trifluoromethoxy)aniline in Triflumuron 5091/TC/M/- except

Disposable PTFE syringe filter compatible with organic solvents and a 0.45 µm pore diameter or centrifuge.

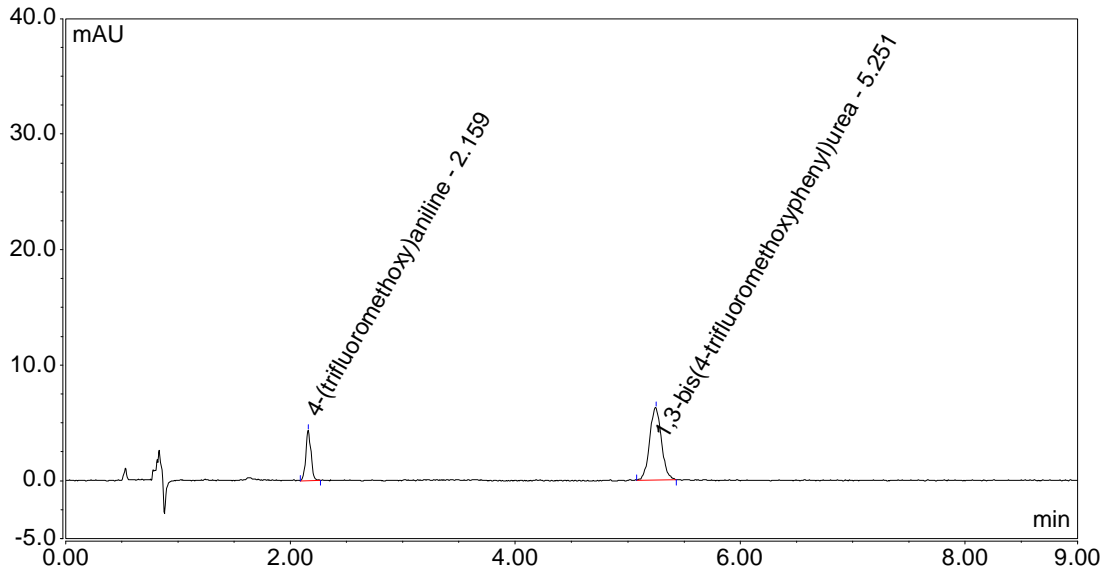
(d) *Sample preparation.* As for 1,3-bis(4-trifluoromethoxyphenyl)urea and 4-(trifluoromethoxy)aniline in Triflumuron 5091/TC/M/- except filter the sample solution through a disposable filter or centrifuge the sample solution (Sample solutions S3, S4) (Fig. 5).



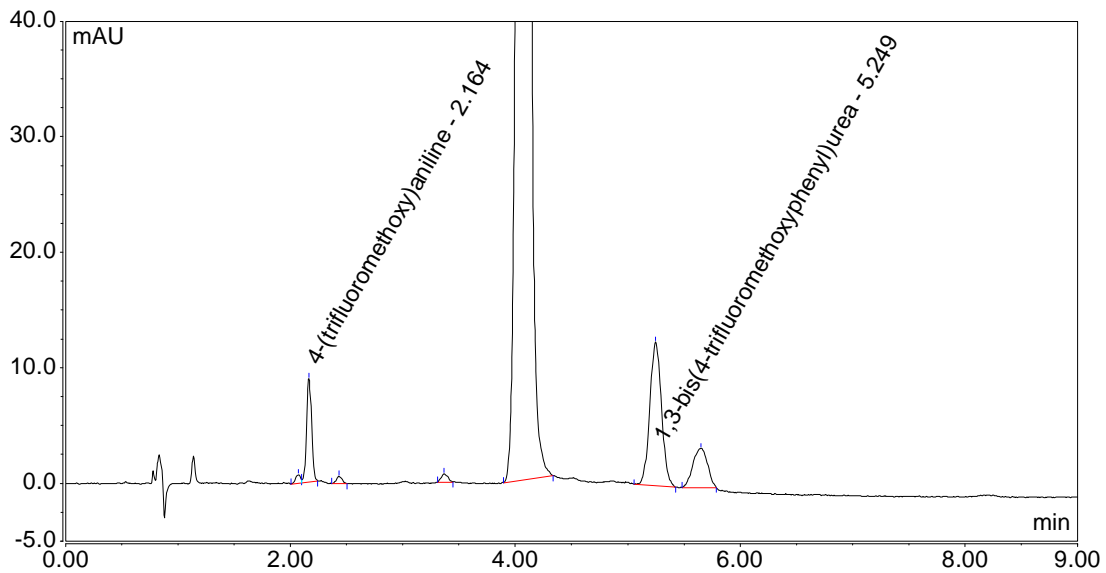
**Fig. 1 UV-Spectrum of 1,3-bis(4-trifluoromethoxyphenyl)urea**



**Fig. 2 UV-Spectrum of 4-(trifluoromethoxy)aniline**

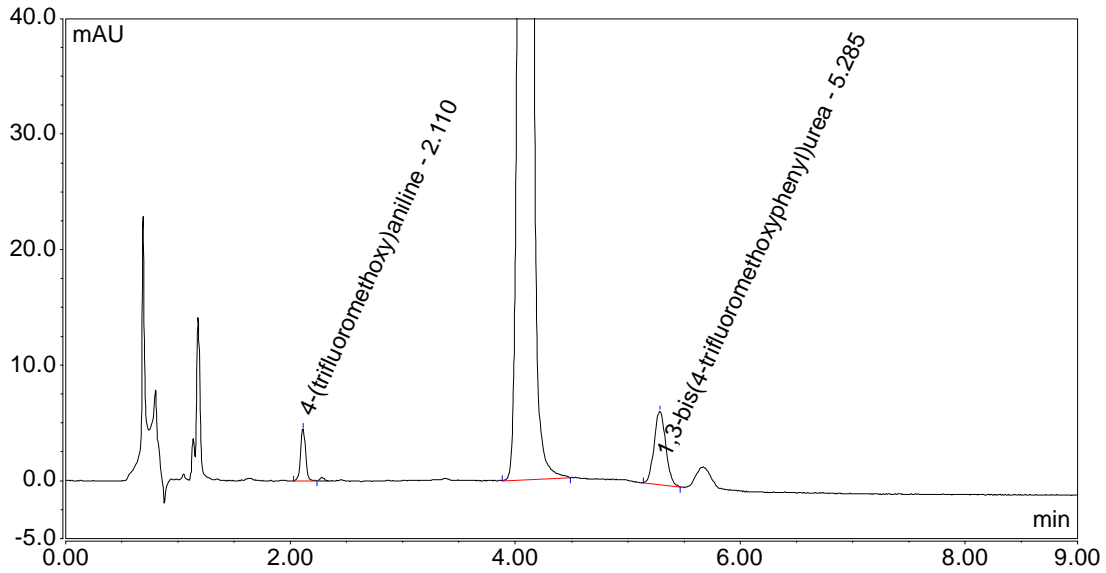


**Fig. 3 Chromatogram of 1,3-bis(4-trifluoromethoxyphenyl)urea and 4-(trifluoromethoxy)aniline Analytical Standards**



**Fig. 4 Chromatogram of Triflumuron TC spiked with 1,3-bis(4-trifluoromethoxyphenyl)urea and 4-(trifluoromethoxy)aniline**





**Fig. 5 Chromatogram of Triflumuron SC spiked with 1,3-bis(4-trifluoromethoxyphenyl)urea and 4-(trifluoromethoxy)aniline**