

# Broflanilide

994

994/TC/M/-

## Method Extension for Broflanilide UL

Studies for Method Extension of existing CIPAC method for Broflanilide UL.

By  
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## 1. Introduction

The CIPAC 994/TC/M/- broflanilide content method was extended to the UL formulation type, that contains broflanilide, with one modification. This report was prepared to demonstrate the validity of the extension of the CIPAC 994/TC/M/- for total broflanilide in UL formulations. The analysis was performed by two separate laboratories.

A selective identity test using the HPLC chromatography acquired in the assay was performed to confirm the identity of broflanilide in the UL formulation.

## 2. Method Description

**Outline of CIPAC Method:** Broflanilide is determined by reversed phase high performance liquid chromatography using UV detection at 254nm and external standardization.

### Reagents

*Broflanilide* reference standard with known content

*Water* HPLC Grade

*Acetonitrile* HPLC Grade

*Dilution Solution* Mobile Phase

*Mobile Phase* Acetonitrile – Water (65 + 35) (v/v)

### Calibration Solution

Weigh in duplicate (to the nearest 0.1mg) 50 mg (s mg) of the broflanilide standard into separate 100 mL volumetric flasks. Fill it up to the mark with dilution solution (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 20 µL.

Column 250 x 4.6 mm (i.d.), Waters XSelect CSH C18 5 $\mu$ m, or equivalent material

Electronic Integrator or data system

Ultrasonic bath

Filtration unit equipped with a PTFE membrane, 0.45 $\mu$ m

## Procedure

### a. Chromatographic Conditions (typical)

Parameter	Specification
Column Temperature	40 °C
Flow Rate	1.0 mL/min
Measuring Wavelength	254 nm
Injection Volume	20 $\mu$ L
Run Time	Approx. 15min
Retention Time	Approx. 11.5min

### b. System Equilibration

Inject 20  $\mu$ L portions of the calibration solution C<sub>1</sub> and repeat the injections until peak areas deviate by less than  $\pm 1.0\%$  of two consecutive injections. Then inject consecutively two 20  $\mu$ L portions of the second calibration solution (C<sub>2</sub>). The mean response factor for this solution should not deviate by more than 1.0% from that of the first calibration solution (C<sub>1</sub>) otherwise prepare new calibration solutions

### c. Sample preparation

Prepare sample solutions in duplicate. Weigh (to the nearest 0.01mg) enough sample to

contain 50 mg Broflanilide into separate 100 mL volumetric flasks. Fill it up to the mark with dilution solution (Sample solutions UL<sub>1-A</sub>, UL<sub>1-B</sub>, UL<sub>1-A</sub> and UL<sub>1-B</sub>).

*d. Determination*

Inject in duplicate 20 µL portions of each sample solution bracketing them by injections of the calibration solutions as follows;

C<sub>1</sub>, UL<sub>1-A</sub>, UL<sub>1-A</sub>, C<sub>2</sub>, UL<sub>1-B</sub>, UL<sub>1-B</sub>, C<sub>1</sub>, UL<sub>2-A</sub>, UL<sub>2-A</sub>, C<sub>2</sub>...

Determine the relevant peak areas.

*e. Calculation*

$$f_i = s * P / H_s$$

Where:

$f_i$  = individual response factor

$H_s$  = peak area of broflanilide in the calibration solution

$s$  = mass of broflanilide in the calibration solution (mg)

$P$  = purity of broflanilide standard (g/kg)

$$\text{Broflanilide content (g/kg)} = H_w * f / w$$

Where:

$f$  = mean response factor

$H_w$  = peak area of broflanilide in the sample solution

$w$  = mass of sample taken (mg)

### 3. Method Assessment

According to the CIPAC method extension guideline, the method extension of the CIPAC 994/TC/M/- for total broflanilide in UL formulations was investigated.

One UL formulation, CMP132-022, was subjected to this assessment. This assessment was performed by two separate laboratories:

**Laboratory 1:**

Clarke Mosquito Control Company  
Research Development & Engineering Laboratory  
675 Sidwell Court  
St. Charles, Illinois 60174  
United States of America

**Laboratory 2:**

Department of Agriculture, Ireland  
Pesticide Formulations Laboratory  
Department of Agriculture, Food and the Marine  
Backweston, Celbridge, Co. Kildare W23X3PH  
Ireland

The nominal content of total broflanilide in the UL formulation tested is 10.0 g/kg

a. **Check the availability of a CIPAC method for the formulation concerned (Step 1)**

The formulation of interest is a single active ingredient formulation. There is no existing CIPAC method available for the UL formulation type containing broflanilide. The formulation of interest, CMP132-022, contains broflanilide. The method extension of CIPAC 994/TC/M/- was investigated.

b. **Check whether the concentration of the analyte is inside or outside the acceptability range covered by the samples of the original trial (Step 2)**

CIPAC 994/TC/M/- was originally evaluated for concentrations of 997/kg (0.5 mg/ml, in final sample solutions) The broflanilide content in the formulation of interest is 1.0 g/kg. This is within the acceptability range of the existing CIPAC method.

In the preparation of the sample, the broflanilide concentration in the sample solution was set to 0.5 mg/ml as described in the sample preparation section of the existing CIPAC method. This is the identical concentration of broflanilide that is present in the calibration solutions, thus the analysis of the sample solution per the CIPAC method falls within the acceptable linearity range.

c. **Modification of method has to be changed in order to be specific (Step 4)**

In order to apply the CIPAC 994/TC/M/- methodology to the formulation of interest, CMP132-022, the following modification was applied:

- i. The dilution solution was changed from Mobile Phase to neat Acetonitrile

This change is considered to be a minor modification.

The Clarke UL formulation of interest, CMP132-022 was observed, through multiple analyses, to exhibit the formation a bilayer system when diluted using the Mobile Phase. This bi-layer system demonstrated a higher concentration of Broflanilide content in the top layer leading to an artificially high recovery of total Broflanilide content in the test samples.

In order to achieve acceptable and accurate recoveries of broflanilide in the formulation, it was necessary to eliminate the formation of the bilayer system through the utilization of neat acetonitrile as the diluent.

Standard addition is a common and widely used analytical technique to account for matrix effects observed through sample analysis by spiking sample preparations of the UL formulation with varying volumes of a stock standard to generate a known range of standard concentrations in each of the sample solutions, which also contain the unknown amount from the formulation.

The change in diluent has no impact on the quantification or analysis of Broflanilide in the formulation.

The following text is proposed for the inclusion of UL formulations in the existing method:

*Sample preparation*

Prepare sample solutions in duplicate. Weigh (to the nearest 0.01mg) enough sample to contain 50 mg Broflanilide into separate 100 mL volumetric flasks. Fill it up to the mark with acetonitrile and invert several times to mix (Sample solutions UL<sub>1-A</sub>, UL<sub>1-B</sub>, UL<sub>1-A</sub> and UL<sub>1-B</sub>).

d. **Validation Study (Step 5)**

Specificity and precision tests were conducted.

- i. **Specificity**

The sample solutions and a blank solution were prepared identically. A comparative (refer to chromatogram figures) evaluation of the sample solution, blank solution, and the standard solution show that there is no interference with the analysis of broflanilide.

## ii. Precision (repeatability, r)

The UL sample was prepared in 5 replicates (5 separate sub samples) and analyzed according to the specified chromatographic conditions with the exception of the modifications listed above in **3(C)**. Per the Horwitz equation, the acceptable %RSD for a sample with a nominal 1.0% concentration is as follows:

$$\%RSD = 2^{(1-0.5*\log(C))}$$

C = concentration of analyte expressed as a decimal

For a 1.0% concentration, this equates to  $(2^{(1-0.5*\log(0.01))})$  which is 2.83%. As shown in the table below, the repeatability of this method was satisfactory with a %RSD of 0.89% for Lab 1 and 1.46% for Lab 2.

Lab 1 - Replicate	Broflanilide Content (g/	% Recovery
1	9.90	99.0
2	9.65	96.5
3	10.05	100.5
4	9.90	99.0
5	9.85	98.5
<b>Average</b>	9.87	98.7

<b>SD</b>	0.144	
<b>%RSD</b>	1.46	

<b>Lab 2 - Replicate</b>	<b>Broflanilide Content (g/</b>	<b>% Recovery</b>
1	9.93	99.3
2	9.90	99.0
3	10.08	100.8
4	10.10	101.0
5	10.03	100.3
<b>Average</b>	10.01	100.1
<b>SD</b>	0.089	
<b>%RSD</b>	0.89	

When combining all results, the overall %RSD between all analyses was 1.36%.

When applying the Horwitz Ratio (%RSD of the results / Predicted %RSD from the Horwitz equation) we get a ratio of 0.5 which is considered fully acceptable.

Overall %RSD	1.36
%RSD per Horwitz Equation	2.83
HorRat	0.5

#### 4. Conclusion

In order to apply the CIPAC 994/TC/M/- to UL formulations containing broflanilide, the method required a change in extraction diluent. This is considered a minor modification.

The data shown demonstrates that the method is specific and has an acceptable precision (repeatability, r). Therefore, the modified method is considered appropriate for the determination of Broflanilide in a UL formulation and the extension of CIPAC 994/TC/M/- to UL formulations is proposed by Clarke.



## 5. **Broflanilide Identity**

To verify and confirm the identity of Broflanilide, HPLC was used. The instrument parameters of the original CIPAC method were utilized, using the proposed acetonitrile diluent as described above.

Using these parameters, the retention time of the broflanilide peak in the calibration solution C<sub>1</sub> was compared to the retention time of broflanilide in the sample solution. The retention times did not deviate by more than 2%.

The following ID test is proposed as part of the scope extension of the existing CIPAC method to UL formulations:

**HPLC.** Use the HPLC method described. The Retention time of Broflanilide for the sample solution should not deviate by more than 2% from that of the calibration solution.

Figure 1. Injection of Diluent – HPLC analysis

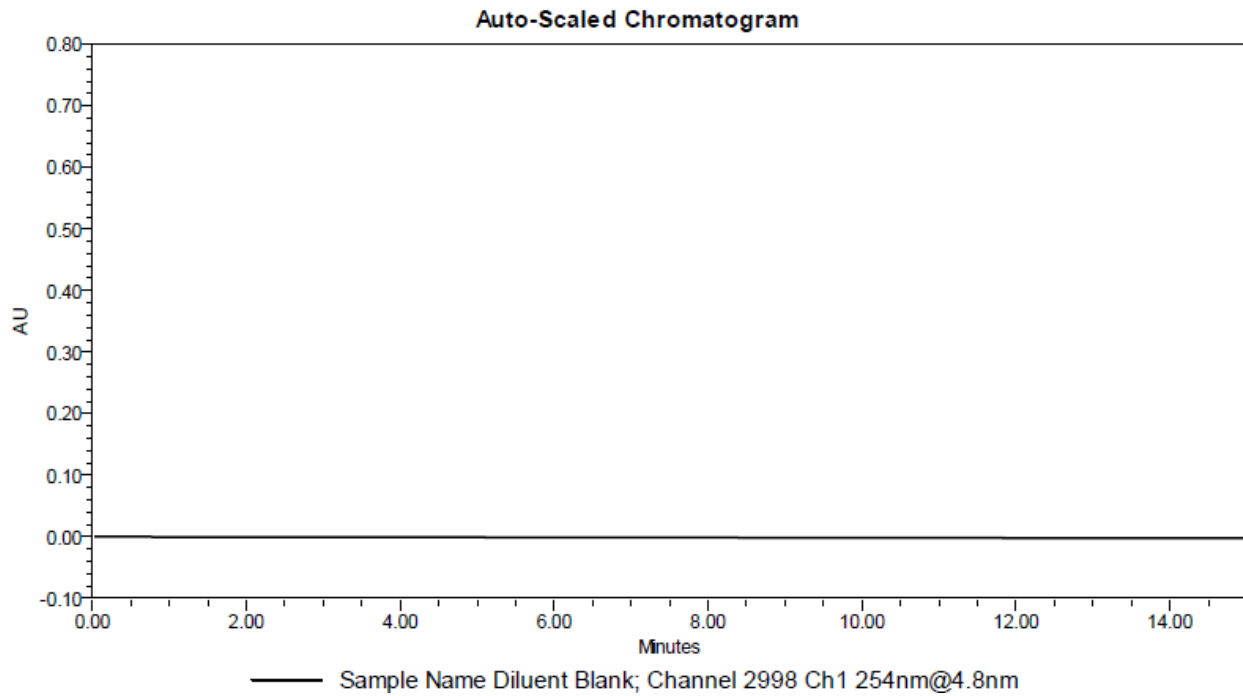


Figure 2. Injection of Blank preparation – HPLC analysis

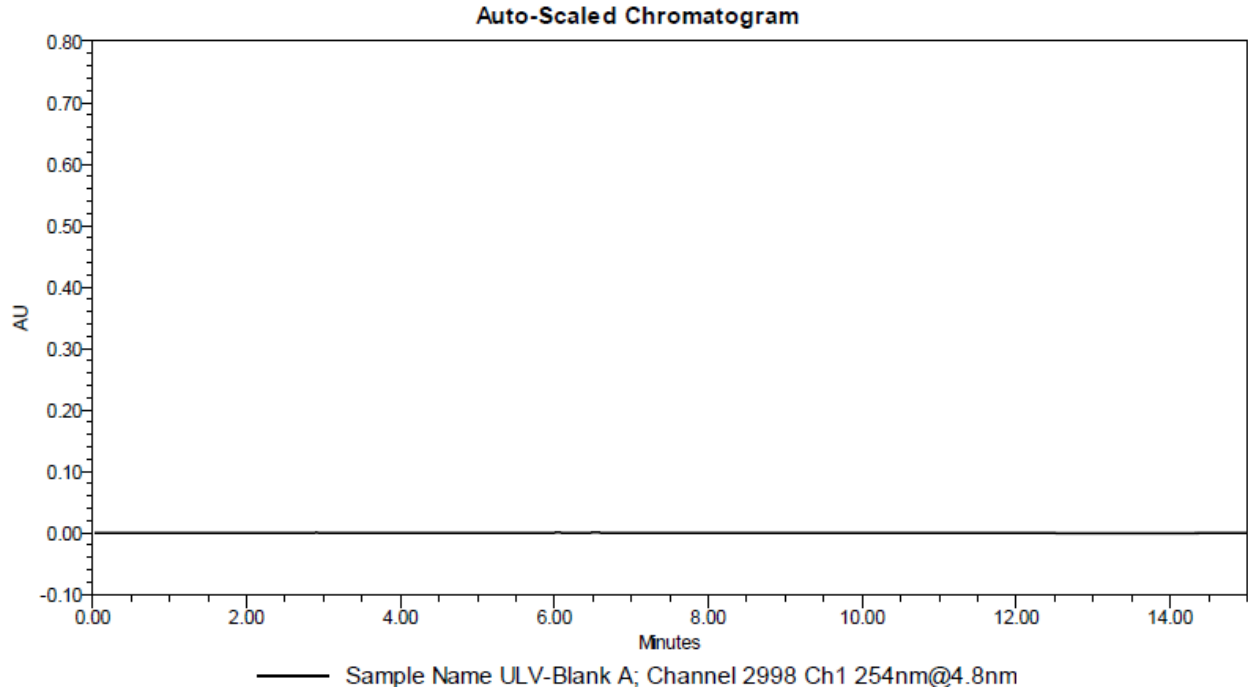


Figure 3. Injection of Calibration Solution – HPLC analysis

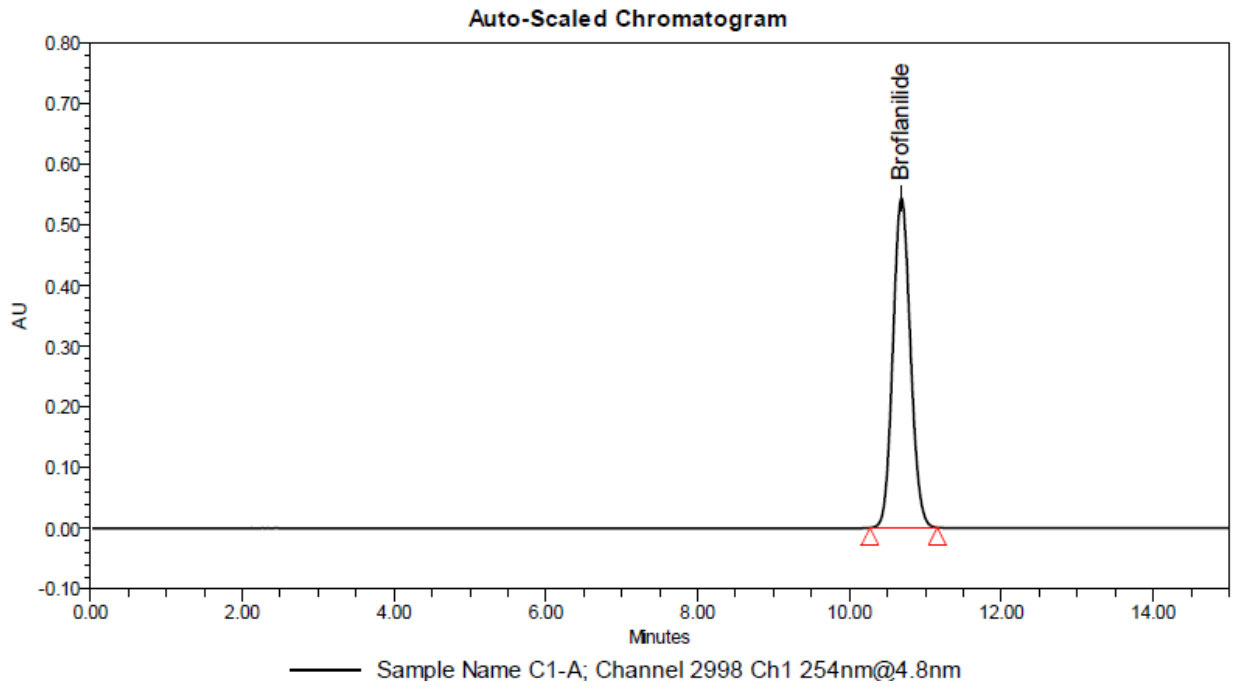


Figure 4. Injection of Sample preparation – HPLC Analysis

