

## Simultaneous Determination of Mesotrione and Nicosulfuron in OD Formulations

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### Abstract

In this study an isocratic high-performance liquid chromatographic method with diode array detection was developed for simultaneous determination of mesotrione and nicosulfuron active ingredients content in pesticide products formulated as oil dispersion (OD). For the analysis, LC system an Agilent Technologies 1100 was used. Good separation was achieved on a Zorbax SB-C18 column using a mobile phase consisting of 0.1% CH<sub>3</sub>COOH/acetonitrile (75:25), at a flow rate of 0.9 ml/minute and UV detection at 245 nm. Column temperature was 40 °C, injected volume was 1 µl. Retention times for nicosulfuron and mesotrione were 3.009 min and 4.363 min, respectively. Validation of the method was performed according to IAEA guidelines. The obtained results showed that the linear coefficients were 0.9999 for both analyzed herbicides in the mixture. The repeatability of the method expressed as relative standard deviations (%RSDr) was 0.33% for mesotrione and 0.18% for nicosulfuron. The accuracy of the proposed method was determined from recovery experiments. The obtained results for mesotrione and nicosulfuron were 100.5-102.9% and 99.3-103.9%, respectively, proved to be acceptable. Precision of the method expressed as %RSD was 0.38% for mesotrione and 0.99% for nicosulfuron and are lower than the values calculated by the Horwitz equation.

### Introduction

For the successful pest control, use of pesticide products of known quality is essential. Recommended methods for active ingredients content in pesticide products are collaboratively tested and published by Association of Official Analytical Chemists (AOAC) and Collaborative International Pesticides Analytical Council (CIPAC). Shortage of the available standard methods for certain active ingredients in pesticide products of different formulations conditions the development of the appropriate methods in a laboratory. This refers especially to more frequent use of one or more active ingredients combinations, matters in the formulated product, and to the lack of the methods for simultaneous determination of their content.

Positive experiences on nicosulfuron (the herbicide from sulfonylurea group) and mesotrione (the herbicide belonging to the group of triketones) compatibility and efficiency led to formulation of the products in combination of these two active ingredients. Determination of nicosulfuron by CIPAC MT 709 method refers to the determination of technical matter purity and to the pesticide products formulated as water dispersible granules (WG). It is based on the use of the reverse-phase high-performance liquid chromatography with the application of detectors with a variety of photosensitive diodes [1].

For the analysis of mesotrione, as well as for the simultaneous determination of these two active ingredients in oil dispersion pesticide formulations (OD), corresponding methods are not available.

The objective of this work has been the development of a simple and fast HPLC-DAD procedure which could be applied to the routine quality control analysis of pesticide formulated product (OD) containing mesotrione and nicosulfuron as active ingredients.

### Experimental

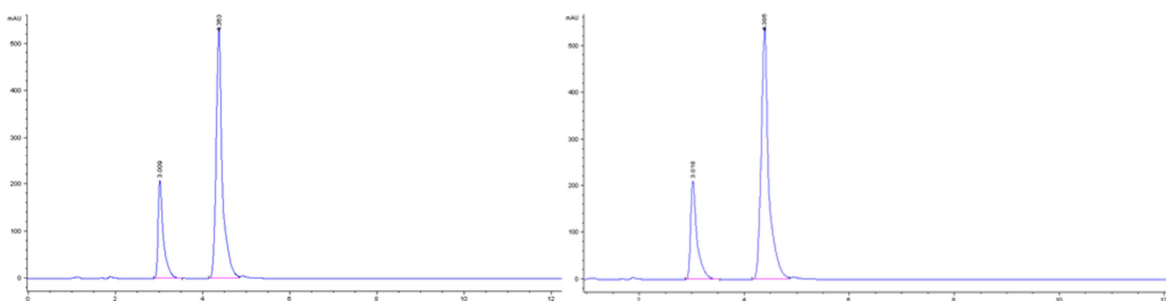
Stock solutions of each active ingredient were prepared by diluting appropriate amount of analytical standards mesotrione (99.9%, Dr Ehrenstorfer, Germany) and nicosulfuron (97%, Dr Ehrenstorfer, Germany) in acetonitrile. Final concentration was ~ 1.0 mg/ml. Working standard solutions were prepared by diluting mixture of stock solution in acetonitrile, in concentration ranged between 0.04-0.27 mg/ml for mesotrione and 0.03-0.25 mg/ml for nicosulfuron.

As the certified referent material for the accuracy determination was not available, samples with a known content of the studied active ingredients were enriched by the known quantity of mesotrione and nicosulfuron analytical standards. The appropriate quantity of a sample was weighted into volumetric flask (25 ml), dissolved in acetonitrile and fortified with standards mixture of mesotrione (0.07, 0.14 and 0.27 mg/ml) and nicosulfuron (0.06, 0.12 and 0.25 mg/ml). All solutions were filtered through membrane filter of 0.45 µm and analyzed by HPLC-DAD.

### Results and discussion

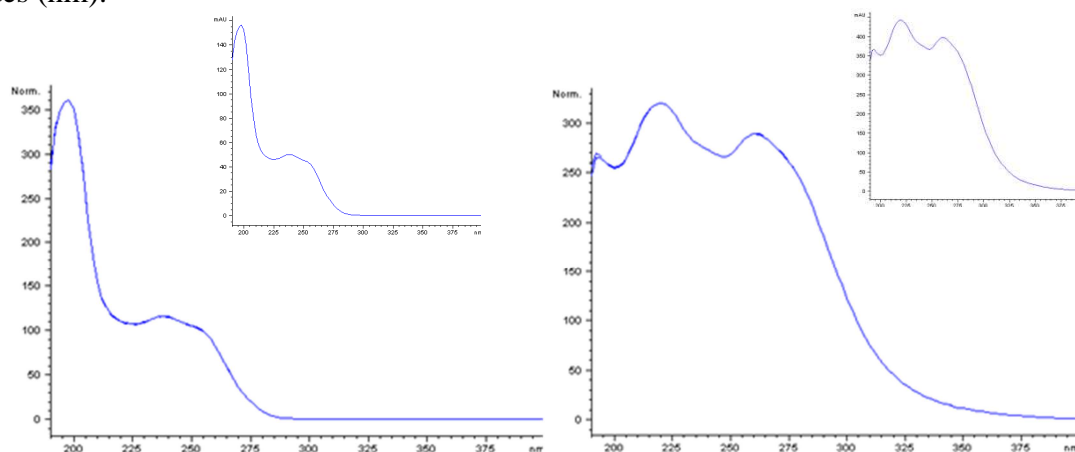
#### HPLC analysis

To achieve adequate separation factor value and sensitivity of the method it is necessary to select the appropriate column, the detector wavelength of the detector, the mobile phase, volume of injection and column temperature. For the analysis of mesotrione and nicosulfuron, HPLC system an Agilent Technologies 1100 was used. The best determination was achieved on a Zorbax SB-C18 column using a mobile phase consisting of 0.1% CH<sub>3</sub>COOH/acetonitrile (75:25), at a flow rate of 0.9 ml/minute and UV detection at 245 nm. Column temperature was 40 °C, injected volume was 1 µl. Qualitative HPLC analysis of a certain ingredient presence was carried out on the basis of retention times, while the quantitative ones were based on the size of the analytical signal. Chromatogram of mesotrione and nicosulfuron standard mixture and sample are presented in Figure 1. Retention times of mesotrione and nicosulfuron were established at 4.330 min and 3.000 min, respectively.



**Figure 1.** The chromatogram of nicosulfuron and mesotrione in standards solution and In OD formulation sample

Component identification was achieved on the basis of  $R_t$  and also by comparison of UV-VIS spectrum of unknown peak with the spectrum of the referent standard [2]. Figure 2 presents spectra of mesotrione and nicosulfuron from analytical standards and sample solution. On y-axis are given values of the current signal detector (mAU), while on the x-axis are wavelength values (nm).



**Figure 2.** UV spectra of nicosulfuron and mesotrione peak from the standards and the sample

#### Validation of the method

According to IAEA guidelines, validation was performed checking the following analytical performance parameters - linearity of detector response, repeatability of injections, precision of the method and accuracy, as well as by determination of Horwitz's limit.

The linearity was established by the scope of the analytical method and it is presented by the regression equation. The linearity of detector response was determined at five levels of concentrations in triplicate, by injecting 1  $\mu$ L of the standard mixture. Calibration curve was defined as dependence of the peak area from concentration and expressed by regression equation with correlation coefficient ( $R^2$ ) (Table 1).

**Table 1.** Linearity parameters

	Active ingredient	
	Mesotrione	Nicosulfuron
concentration range (mg/ml)	0.04-0.27	0.03-0.25
regression equation	$y=55.72x-71.91$	$y=45.86x-43.57$
correlation coefficient ( $R^2$ )	0.9996	0.9999

The obtained values suggest high susceptibility of mesotrione and nicosulfuron determination by this method.

Repeatability of mesotrione and nicosulfuron determination was checked by injecting of the standard solution of these ingredients in concentrations of 0.16 and 0.12 mg/ml of mesotrione and nicosulfuron five times, respectively. The repeatability of the method expressed as relative standard deviations was 0.33% and 0.18%.

The precision of the method is defined by repeatability. In order to check the repeatability of determination 1  $\mu$ l of investigated sample with 0.369 mg/ml of mesotrione and 0.147 mg/ml of nicosulfuron were injected 5 times. The obtained RSDs values for mesotrione and

nicosulfuron were 0.38% and 0.99% respectively. This result suggests good precision of the method for determination of mesotrione and nicosulfuron [2].

According to SANCO 3030/99, the assessment of the method precision is based on Horwitz's limit. Horwitz's equations aroused from numerous inter-laboratory studies conducted by AOAC during several years lasting period, and represent exponential dependence between in laboratory determined relative standard deviation  $RSD_r$  and concentration  $C$ .

The value of Horwitz's limit is calculated by the equation:

$$\%RSD_r = 2^{(1-0.5 \log C)} \times 0.67$$

The obtained relative standard deviation for the repeatability of mesotrione and nicosulfuron determination for the peak areas (1.97% and 5.32%) were significantly under modified values of Horwitz's limit for inter-laboratory repeatability of formulated products determination with active ingredient content of 7.56% and 3.24%, respectively. These results suggest good reproducibility of mesotrione and nicosulfuron determination.

The recoveries of the three fortification levels for mesotrione and nicosulfuron were 100.5-102.9% and 99.3-103.9% respectively. The acceptable values of accuracy for the active ingredients determination in pesticide formulations with a content of less than 10% [2], as well as high accordance between the values obtained in the process and the actual value (102.7% and 102.2%), confirm accuracy of the applied method for determination of mesotrione and nicosulfuron in products of OD formulation.

### **Conclusion**

The method established in this study enables new, simple, selective and accurate routine HPLC analysis of the herbicides mesotrione and nicosulfuron in the OD (oil dispersion) pesticide formulation. This method fully meets the standards of HPLC pesticide analysis in formulations, according to IAEA-TECDOC-1612. Finally, developed method was applied for the determination of mesotrione and nicosulfuron content in a pesticide product. The obtained results of active ingredients are within the permissible deviations [3].

### **References**

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