

## OPTIMIZATION OF HPLC CONDITIONS FOR SIMULTANEOUS DETERMINATION OF TERBUTHYLAZINE AND TERBUMETON

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**Abstract:** One of the most used herbicides on farming land – vineyards, apple and pear trees, is the herbicide commercial called "Karagard kombi A-50 VP" produced by AD "OHIS" Skopje. The substances terbuthylazine and terbumeton are the active compounds in this pesticide formulation. The aim of this work is to optimize the experimental conditions for simultaneous HPLC determination of these two compounds. This method can be applied for further determination of their residue in different matrix. The best results for terbuthylazine and terbumeton are obtained with: analytical column Lichrosorb RP 18, 200 x 4.6 mm, 5  $\mu$ m, 20  $\mu$ l loop, ternary gradient pump, flow rate 1 ml/min, isocratic elution by mobile phase CH<sub>3</sub>CN-H<sub>2</sub>O / 70-30 (v/v). Detection was carried out at 220 nm by polychrom diode array detector. The detection limit was determined and for their quantitative determination the calibration curves are constructed.

**Key words:** terbuthylazine, terbumeton, HPLC, determination

### 1. Introduction

Triazines are still widely used as herbicides in agriculture. During and after the herbicide application onto farm land, triazines may be transported to the ground water [1], to the surface water [2] and into the atmosphere [3]. Therefore, their determination is of great importance in environmental studies. Clearly there is a need for analytical methods that allow simultaneous determination of pesticides and their metabolites.

The methods actually used for the determination of trace amounts of triazines include gas chromatography (GC) [4,5] and high performance liquid chromatography (HPLC) [6-8].

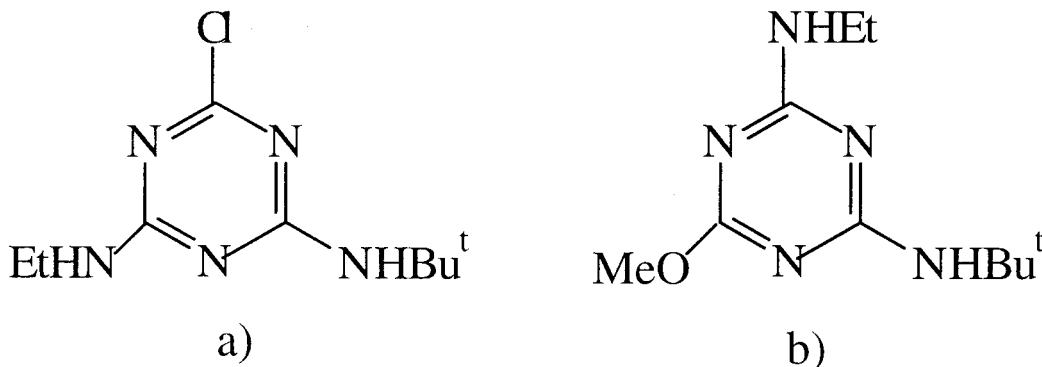
A disadvantage of gas chromatography is that it is limited to volatile chlorotriazines. The hydroxy derivatives cannot be analyzed without derivatization. High performance liquid chromatography is directly applicable to triazines and their degradation products.

In this paper, we introduce optimization of HPLC conditions for simultaneous determination of terbuthylazine and terbumeton, at relatively low levels, less than 5 ppb.

### 2. Experimental

#### 2.1. Materials

The compounds analyzed are shown in Fig.1. Acetonitrile (HPLC grade) was from Sigma – Aldrich (Germany). Methanol (for liquid chromatography) and water (for chromatography) were from Merck (Germany).



**Figure 1.** Molecular structures of: terbuthylazine a) and terbumeton b)

## 2.2. Standard solutions

Stock solutions of 1000 ppm terbuthylazine and terbumeton were prepared by dissolving 10 mg of the respective triazine in 10 ml of methanol. Stock solutions were used to prepare standard mixtures with different triazine concentrations in methanol / water (50/50, v/v). These mixtures were used for external calibration as well as for the preparation of artificial samples. All solutions were stored at 4°C.

## 2.3. HPLC analysis

A HPLC system (Varian) equipped with a ternary gradient pump (9012), 20 $\mu$ l loop (Rheodyne) and polychrom diode array detector (Varian-9065) was used for the HPLC analysis. Separation was performed using analytical column Lichrosorb RP18, 200 x 4.6 mm, 5  $\mu$ m (Hewlett – Packard).

## 3. Results and discussion

The best composition of mobile phase, the flow rate as well as the detection limit were determined. Two mobile phases methanol/water and acetonitrile/water were used for separation to compounds of interest – terbuthylazine and terbumeton. For best separation and symmetrical peak shape, several isocratic and gradient programs for the two mobile phases were evaluated. Separation with methanol/water was not satisfied, but with acetonitrile/water the mobile phase was very good. It is found that the best mobile phase is acetonitrile-water / 70-30 isocratic, so that mobile phase was used for further investigations. Acetonitrile/water was used instead of methanol/water mobile phase because acetonitrile has advantages over methanol: lower operating pressure and applicability for detection in the very low UV range [9].

For best separation, several flow rates of mobile phase ( 0.5 – 1.5 ml/min.) are evaluated. It was found out that the best flow rate is 1.0 ml/min. Retention times of terbuthylazine and terbumeton are 4.8 and 8.9 min, respectively. The flow rates down to 1.0 ml/min give no symmetrical peak shape, and those up to 1.0 ml/min give peaks that are very close ( low resolution).

Separation under above mentioned HPLC conditions is shown in Fig.2.

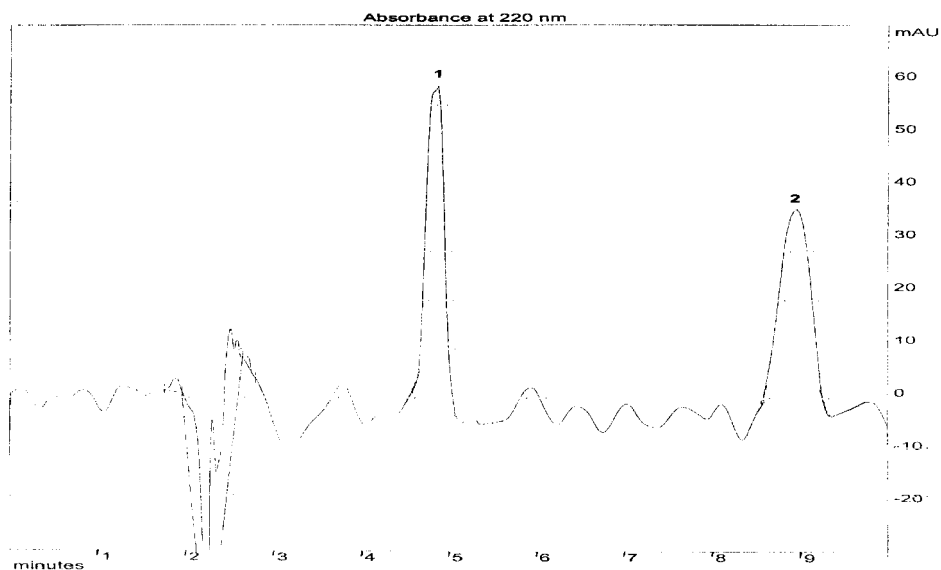


Figure 2. HPLC separation of terbuthylazine (1) and terbumeton (2)

Absorbance was measured continuously in the range 190-350 nm by diode array detection following solute separation on the HPLC column. The peaks were measured at a wavelength of 220 nm, where the compounds of interest have an absorption maximum (Fig.3).

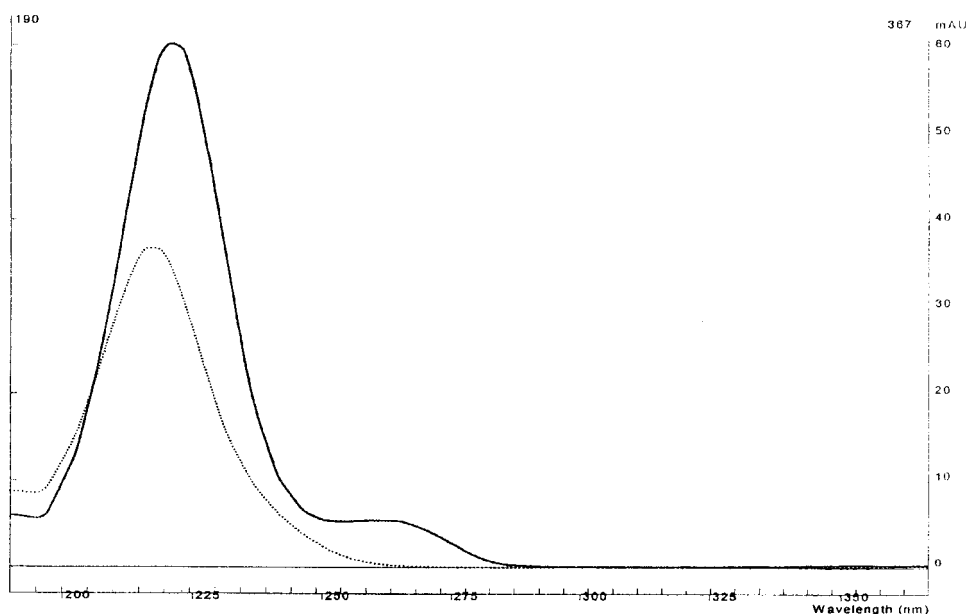


Figure 3. Absorption spectra of terbuthylazine (\_\_\_\_\_) and terbumeton (.....)

The calibration was carried out by injecting standard solutions onto the HPLC column (external calibration). The calibration curves had a correlation coefficient  $r^2 > 0.995$  for both measured triazines.

#### 4. Conclusion

The developed HPLC procedure is suitable for the detection and the determination of terbuthylazine and terbumeton. The optimal HPLC conditions are: mobile phase acetonitrile-water / 70-30 isocratic, flow rate 1.0 ml/min, detection at wavelength of 220 nm, analytical column Lichrosorb RP18, 200x4.6 mm, 5  $\mu$ m.

#### Literature

- [1] Pionke, H.B; Glotfelty, D.E; Lucas, A.D; Urban, J.B. *J. Environ. Qual.*, **17**, 76, (1988).
- [2] Thurman, E. M; Goolsby, D. A; Meyer, M.T; Mills, M. S; Pomes, M. L; Kolpin, D. W. *Environ. Sci. Technol.*, **26**, 2440, (1992).
- [3] Richards, R. P; Kramer, J. W; Baker, D. B; Kreiger, K. A. *Nature*, **327**, 129, (1987).
- [4] J. C. Molto, Y. Pico, G. Font and J. Manes, *J. Chromatogr.*, **555**, 137, (1991).
- [5] H. Bagheri, J. J. Vreuls, R. T. Ghijsen and U. A. Th. Brinkman, *Chromatographia*, **34**, 5, (1992).
- [6] D. Barcelo, *Chromatographia*, **25**, 9928, (1988).
- [7] G. Durand and D. Barcelo, *J. Chromatogr.*, **502**, 275, (1990).
- [8] W. Schussler, *Chromatographia*, **27**, 431, (1989).
- [9] L. R. Snyder, J. G. Glajch and J. J. Kirkland, *Practical HPLC method development*, Wiley, New York, 1988.

#### Апстракт.

Еден од најчесто применуваните хербициди за сузбивање на плевели во насади на винова лоза, јаболки и круши е хербицидот со комерцијално име "Карагард комби а-50 ВП" производ на АД "ОХИС" Скопје. Како активни компоненти во оваа формулација на пестицид се јавуваат компонентите тербутилазин и тербуметон.

Целта на оваа работа е да се оптимизираат експерименталните услови за симултано HPLC определување на овие две компоненти. Најдобри резултати при HPLC определување на тербутилазин и тербуметон се добиени со примена на: аналитичка колона Lichrosorb RP 18, 200 x 4,6 mm, 5  $\mu$ m, тернарна градиентна пумпа, луп од 20  $\mu$ l, брзина на проток на мобилната фаза 1,0 ml/min, изократно елуирање со мобилна фаза CH<sub>3</sub>CN-H<sub>2</sub>O / 70-30 (v/v). Детекцијата е извршена на 220 nm со polychrom diode array детектор. Определена е границата на детекција и конструирани се калибрациони криви за квантитативно определување на испитуваните компоненти.