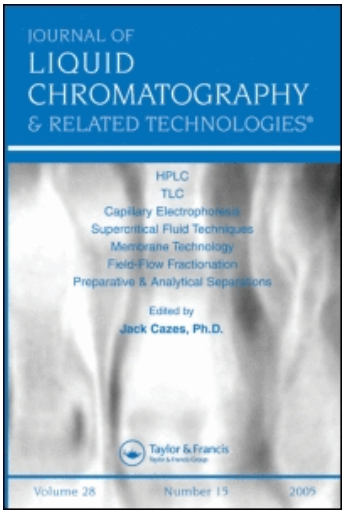

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High-Performance Liquid Chromatographic Determination of Fusilade Using a Fluorescence Reagent, 4-Bromomethyl-7-Methoxycoumarin

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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF FUSILADE USING A FLUORESCENCE REAGENT, 4-BROMOMETHYL-7-METHOXYCOUMARIN

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ABSTRACT

A high-performance liquid chromatography method for Fusilade has been developed which utilizes 4-bromomethyl-7-methoxycoumarin to provide derivative for high-sensitivity detection. The reagent reacts with Fusilade to form a fluorescent derivative which is separated on a RP-18 column (5 um) anddetected at ex: 325 nm, em: 395 nm. Fusilade can be determined in the range of at least 1 nmol, with the detection limit at 0.5 ng.

INTRODUCTION

Fluazifop-butyl,(RS) butyl 2-[4-(5-trifluoromethyl-2-pyridyloxy)phenoxy]-propionate is the active ingredient of the herbicide Fusilade and the structure is presented in Fig. 1a. Fusilade is a highly selective systemic post emergence grass herbicide for use in broadleaf crops (1,2,3). It is rapidly hydrolyzed in the environment to the corresponding acid: 2-[4-(5-trifluromethyl-2-pyridyloxy) phenoxy]propionic acid (Fig. 1b) or Fluazifob.

Most of the methods for determination of Fluazifop-butyl and Fluazifop have been developed for vegetables and formulations. Gas liquid chromatography (GLC) and

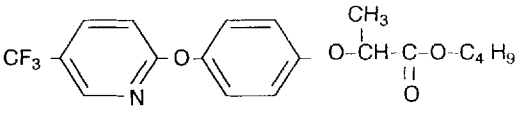


Fig. 1a Fluazifop–Butyl

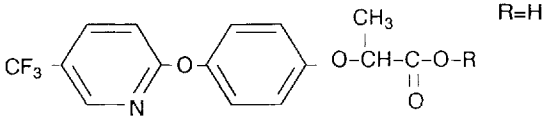
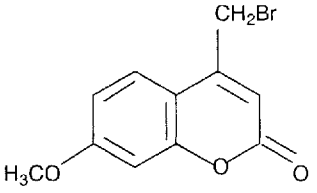


Fig. 1b Fluazifop Fusilade



Bromo Methyl Coumarin (BrMmc)

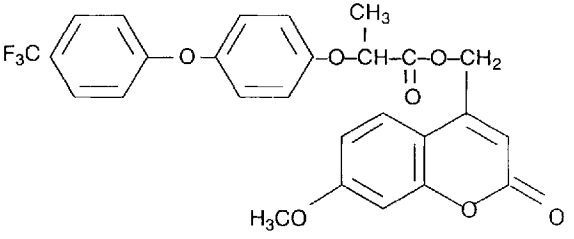


Fig. 1c Fluazifop–Methyl Coumarin ester

high performance liquid chromatography (HPLC) are the most commonly used method. Several methods have been reported for the HPLC determination of Fluazifop-butyl or Fluazifop-butyl and Fluazifop plus conjugated esters through hydrolysis of all compounds to Fluazifop. These procedures do not require derivatization and an UV detector can be used. Formation of various fluorescent derivatives for liquid chromatography are known for tagging acids, primary amines and carbonyl compounds (8,9).

The present paper deals with the development of a derivatization method using 4-bromomethyl-7-methoxycoumarin as a fluorescent agent for the detection of Fusilade. The derivatization of the carboxilic acid (Fusilade acid) has to be carried out in water-free aprotic solvent such acetone to which 18-crown-6 ether and potassium hydrogen carbonate have been added to increase the reaction rate (10,11,12).

MATERIALS AND METHODS

Materials

Fusilade acid (99% purity) was a gift from ICI, Chipman; (Stoney Creek, Ont., Canada). 4-Bromomethyl-7-Methoxycoumarin, 16-Crown-6 ether were purchased from Aldrich Chem. Co. (Milwaukee, WI, U.S.A.). Potassium hydrogen Carbonate reagent grade was purchased from J.T. Baker (Ottawa, Ont., Canada). Deionized and distilled water was used. Acetone, hexane, ethylacetate acetonitrile were supplied by J.T. Baker (Ottawa, Ont., Canada), iso-octane was supplied by Caledon Laboratories (Georgetown, Ontario, Canada). All solvent used for HPLC are HPLC grade, or analytical-reagent grade.

Filter tip: cone TM syringe, 0.45 um Nylon 66-Filter (CSC-Montreal-Canada).

Instruments

A Perkin-Elmer Series 4 Liquid Chromatograph Microprocessor-Controlled solvent Delivery System and ISS-Sampling System were employed. The system was equipped with a guard Column (RP-18 packed) along with an RP-18 HPLC Column

(25 cm, 5 um), a Perkin-Elmer LS-4 programmable fluorescence detector and an integrator/recorder Spectra Physics SP 4270. Excitation wavelength was set at 325 nm and emission at 395 nm.

Thermospray Liquid Chromatographic - Mass Spectrometry was used to identify the derivatized standard. The HPLC was interfaced with a Finnigan MAT thermospray probe to MAT-90 magnetic sector (B/E) mass spectrometer (Finnigan MAT, San Jose, CA). The mass spectrometer was operated at resolution of 1000 and 5000 for both magnetic and electric scanning and for multiple ion detection (MID). The electron multiplier was either operated at 1.7 or 1.8 KV. Source temperature was initially set at 240 °C for the first direct loop studies but all column and sample work were carried out at 250 °C.

Preparation of the Stock Solutions

In a sealed amber-coloured flask 4-Bromomethyl-7-Methoxycoumarin (BrMmC) reagent was added to acetone at level of 5 mg/mL (19 mM) and heated at ca. 50 °C for 5 min. to completely dissolve the compound. The 18-crown-6-ether stable at room temperature was dissolved in acetone at a concentration of 3 mg/mL (11.6 mM). The fusilade solution in acetone was prepared at a concentration of 5 mM. All solutions were stored at 4 °C. The amount of Potassium hydrogen Carbonate 2,5 to 5.0 mg was selected in this procedure.

Derivatization Procedure

10 µL Volume of Fusilade solution was added to a small screw cap chromatographic vial (1.5 mL) containing 915 µL of acetone. The vial was wrapped to allow the reaction to proceed in the dark. To this was successively added 30 µL of the 18-crown ether solution, 5 mg of potassium hydrogen carbonate and 40 µL of BrMmc. The reaction mixture was stirred thoroughly after each addition. The vial was tightly capped and heated at 70 °C for 20 min. The reaction was stopped by placing the reaction mixture in dry ice.

Purification

a) Partition: To the cooled reaction mixture, 2 mL of distilled water was added and the mixture was partitioned with 2 X 1 mL of iso-octane. The iso-octane phases were combined, evaporated to 1 mL volume that was either injected into the HPLC after filtration (tip filter) or purified further with SPE column.

b) Diol-SPE Extraction column

The iso-octane phase containing the derivatized product was transfered into a diol-SPE column which was previously conditioned with: 5 mL HCCl3/MeOH (70+30), 5 mL of iso-octane and, layered with anhydrous sodium sulfate. An additional 3 mL of iso-octane was added to the column, followed by 14 mL of ethylacetate/hexane (95+5), each fraction was discarded. The product of derivatization was then eluted from the column using 5 X 2 mL ethylacetate/hexane (75+25), which was collected, evaporated to dryness, and the residue dissolved in 1 mL of mobile phase.

HPLC Separation

The mobile phase consisted of methanol/water (75+25). A pre-column packed with the same material was used as the guard column. The flow rate was set at 1 mL/min and 10 μL of the sample was injected. The derivatized Fusilade was well separated from the reagent peaks at a retention time of 12.0 min. All chromatographic operations were carried out under ambient conditions.

RESULTS AND DISCUSSION

The separation of the bromomethyl -7-methoxy-coumarin derivative of Fusilade (Fig. 1c) was studied on the reverse-phase column using methanol and water or their mixtures as mobile phase. The best separation of the derivative was achieved on a RP-18 column with an isocratic elution using methanol/water (75+25). The change in concentration of methanol had no effect on the fluorescence excitation (325 nm) and emission (395 nm).

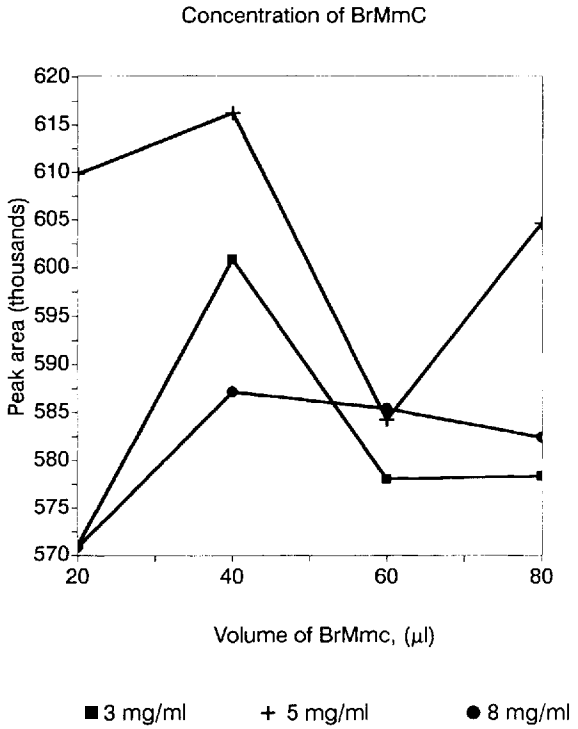


FIGURE 2 Effect of 4-Bromethyl-7-methoxy coumarin on the reaction rate of Fusilade Ester (4.1 ng injected)

The derivatization reaction has been studied with respect to the concentration variation of the reagents namely: BrMmc; crown-ether; potassium hydrogen carbonate, and other parameters such as: solvents media; temperature and reaction time.

The derivatization reaction was carried out in different solvents including acetone, acetonitrile, dichloromethane and benzene. The reaction yield was very good in acetone or acetonitrile; but in acetone the reaction yield was slightly higher. Very poor yield was obtained with the non polar solvents: dichloromethane and benzene.

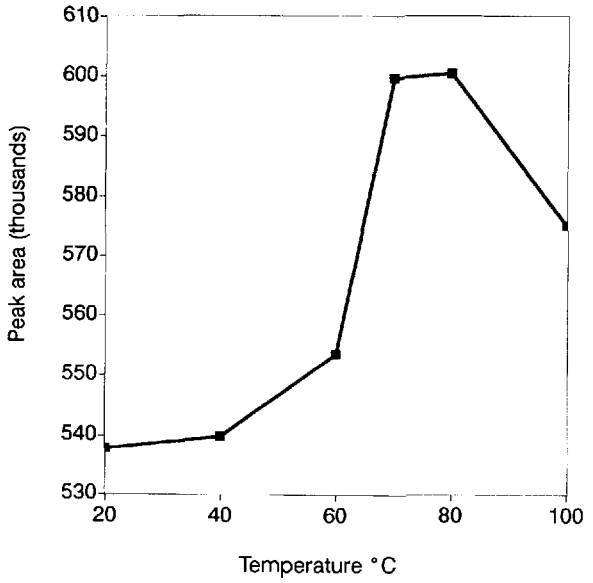


FIGURE 3 Effect of temperature on the reaction yield of Fusilade Ester.

The solubility of BrMmc in acetone at room temperature was ca. 2.5 mg/mL. In this experiment, concentration of 5 mg/mL gave the most intense peaks (Fig. 2), but at that concentration it was necessary to heat to ensure solubility at room temperature, the solution is slightly saturated. 18-Crown-6 ether and potassium carbonate have been used to facilitate the derivatization of carboxilic acid (ref 10), but constant peak height and a stable derivatized product were obtained with potassium hydrogen carbonate (ref. 12). The amount of $KHCO_3$ between 2.5 - 5 mg was selected, the maximum and constant peak heights were obtained at 18-crown-6 ether between 0.26 - 0.6 mM.

The derivatization reaction of Fusilade occurs at low temperature but higher temperature allowed the fluorescence to develop rapidly (Fig. 3). However at 80°C the peak area decreased rapidly at heating times of 10 min. or longer. At 70°C the peak area of fusilade derivative was maximal after heating

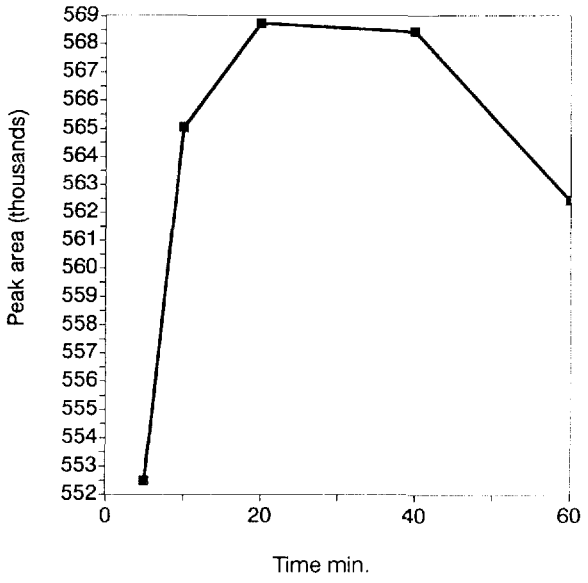


FIGURE 4 Effect of time on the Reaction yield of Fusilade Ester.

for 20 min. or longer. Thus, heating for 20 min. at 70°C was employed in subsequent work (Fig. 4). Fusilade concentration as low as 50 nM could be derivatized by the procedure described above. Fig. 5a shows a chromatogram of derivatized fusilade ester, at 4.1 ng injected.

Partitioning of the reaction media (acetone) with iso-octane gave 95% recovery. The Fusilade (ester) is a less polar compound than acid form, and as such is more soluble in iso-octane (partition). Furthermore some of the by-products of the reaction are insoluble in iso-octane, and partitioning resulted in partial purification as shown in (fig. 5b).

By the use of the SPE Diol column most of the reagent peaks were eliminated (Fig. 5c). The recovery of the ester is 85%.

The ester in the mobile phase is stable at room temperature over a period of 24 hours.

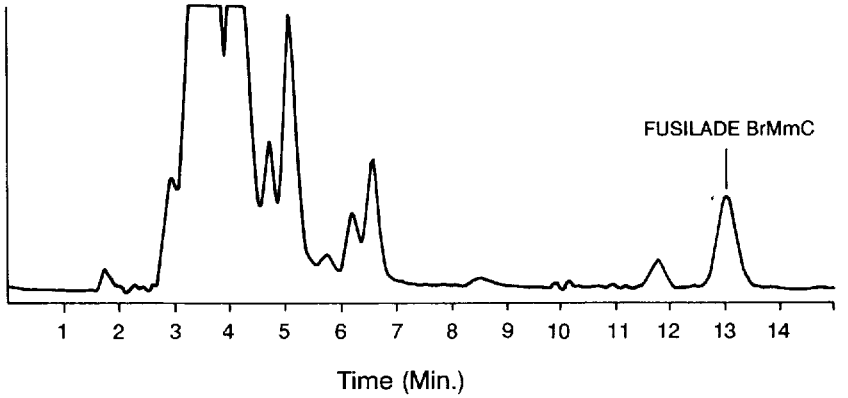


FIGURE 5a HPLC Separation of Fusilade ester derivative of BrMmC, HPLC conditions: column RP 18, 5 µm, (25 X 4.6 cm I.D.); mobile phase, Methanol-water 75:25 isocratic flow rate, 1 mL/min. Temperature ambient; detection, excitation and emission 325 um and 395 nm respectively.

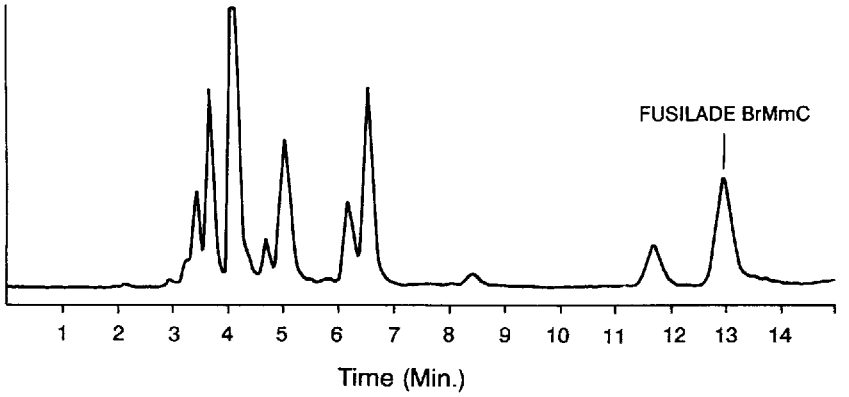


FIGURE 5b Separation of Fusilade Ester after partition with isotane (see text for details). Same chromatographic conditions as in fig.

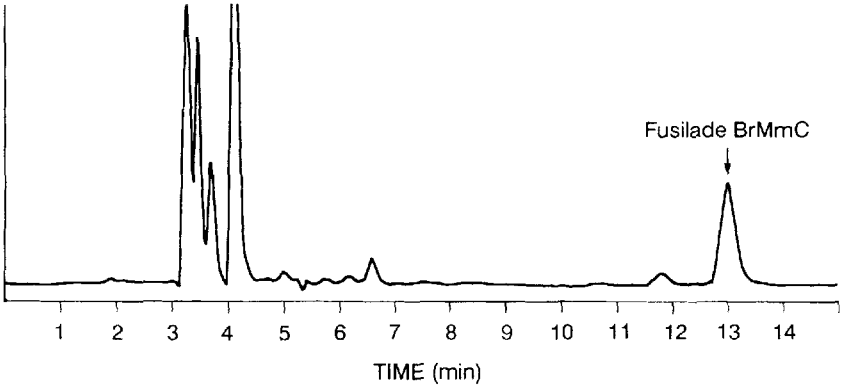


FIGURE 5c Fusilade Ester after purification through SPE Diol column. (See text for details). Same chromatographic conditions as fig.

TABLE 1

CALIBRATION CURVE DATA FOR FUSILADE ESTER

Fusilade conc. µM in React.	Peak Area	Coefficient of Variation %
0.13	17673	5.42
0.25	27462	5.13
0.50	63148	5.06
1.25	148399	6.11
2.50	306510	3.69
5.00	577941	5.53

Each value is expressed as the mean of 4 replicates

TABLE 2

STABILITY OF FUSILADE ESTER IN ACETONE AND 4°C

Time min.	Peak Area	% Recovery
0	216829	100
48	195934	90
72	227163	100.4

Stability of the Derivative

The stability of the reaction product in acetone was investigated by keeping it at 4°C over a period of 72 hours. The recovery is very high (table 2).

Confirmation of the Ester

Identification of the ester was achieved by thermospray - mass spectrometry using EI (electron impact ionisation) since it is more energetic and causes more fragmentations, thermospray ionisation is like CI (chemical ionisation), but it is a soft ionisation method which produces predominant molecular ion plus an adduct ion (NH_4^+) if ammonium acetate has been used as ionic reagent.
A major fragment molecular ion was obtained at 516 ($M+H$) which corresponds to the ester plus the molecular hydrogen ion and a very weak molecular fragment of the fusilade acid was detected at 328.

Precision Calibration and Detection Limit

The precision was established by repeated determination using standard in the range of 0.125 µM - 2.5 µM. The coefficient of variation did not exceed

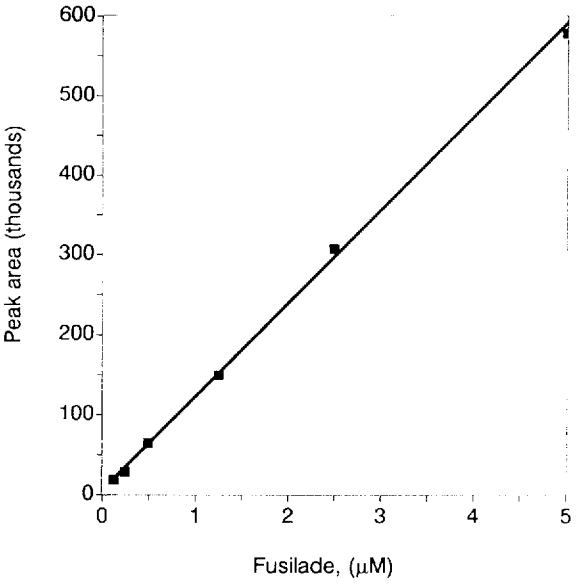


FIGURE 6 Standard Curve for Fusilade Ester. Fusilade concentration in the derivatized product (μM), 10 μL injected.

5.5% in each case (n=4) (table 1). The relationships between the peak areas and the amount of Fusilade is linear from .125 umol to at least 2.5 μmol per injection volume (10 μL) (Fig. 6).

To ascertain the sensitivity, a standard amount of fusilade was treated according to the procedure described earlier and the reaction mixture was subjected to HPLC. Fig. 7 shows a chromatogram in which the derivative peak corresponds to 0.125 μM mole of fusilade in the reaction. It indicates the detection limit for Fusilade ester is 1.25 nmol (0.4 ng injected) at a signal-to-noise ratio of 4.

A very rapid and stable reaction product is formed between 4-bromomethyl-7-methoxy coumarin and Fusilade acid. Advantage is taken of the fluorescent nature of the Fusilade ester for determination by HPLC. The method is very sensitive and can be applied to determine low level of fusilade in samples. The

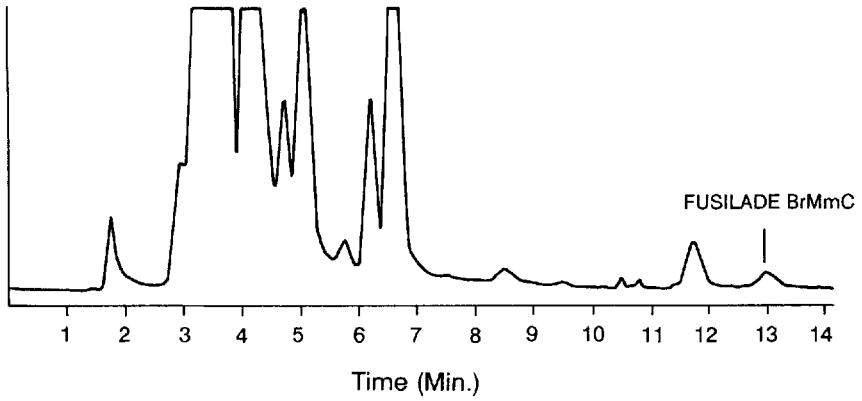


FIGURE 7 Fusilade Ester at detection limit of 1.25 nmole (0.4 ng injected) same chromatographic conditions as fig. 5a.

peak of interest is well separated from reagent peaks and therefore would not interfere. Furthermore, the purification system would be sufficient for sample clean up and the detection of minute quantity of fusilade ester.

REFERENCES

1. Higgins J.M., Mc Carty L.B., Whitwell T., Miller L.C., Bentgrass and Bermudagrass putting green turf tolerance to post-emergence herbicides, Hortscience, 22,248.

2. Gilreath P.J., Gilreath J.P., Réponse of 17 species of container-grown woody landscape and foliage plants to four post-emergence herbicides, J.

3. Environn. Hort., 4,52 (1986).
Herellou A., Painparay G., Morand P, Qu'est-ce que le "Fluazifop-butyl", La Défense des Végétaux, 218,251 (1982).

4. Clegg S., Gas chromatographic analysis of Fluazifop-butyl (Fusilade) in potatoes, soybeans, and soil., J. Agric., Food Chem., 35,269 (1987).

5. Patumi M., Marucchini C., BusinelliM., and Vischetti C., An Analytical method for the determination of Fluazifop-butyl and Fluazifop residues in soil, Pest. Sci., 21,193 (1987).

6. Worobey B., Shield J., Determination of LFluazifop-butyl and Fluazifop acid in soybeans and soybean oil using liquid chromatography with oxidative amperometric detection., J. Assoc. Off. Anal. Chem., 72,368.

7. Negre M., Gennari M., Cignetti A., High-performance liquid chromatographic determination of Fluazifop-butyl and Fluazifop in soil and water, J. Chromatogr. 387,541 (1987).

8. Lawrence J.F., Frei R.W., Chemical Derivatization in liquid chromatography, Plenum Press-N.Y. (1982).

9. Kindberg C.G., Slavick M., Riley C.M. and Stobaugh J.F., High-performance liquid chromatography of 5-fluoracil after derivatization with 4-bromomethyl-7-methoxycoumarin. Characterization of the derivative and the use of column switching for improvement of resolution and the enhancement of the sensitivity, J. Pharm. & Biomed., Anal., 7,459 (1989).

10. Van Der Horst F., Post M., Holithuis J.J.M., Study of the influence of aqueous micellar systems on the derivatization of undecylenic acid with 4-bromomethyl-7-methoxycoumarin, J. Chromatogr., 456,201, (1988).

11. Tsuchiya H., Hayashi T., Naruse H., and Tagaki N., Sensitive high-performance liquid chromatographic method for prostaglandins using a fluorescence reagent, 4-bromomethyl-7-acetoxycoumarin, J. Chromatogr., 231,247 (1982).

12. Yamagushi M., Fukuda K., Hara S., and Nakamura M., Fluorometric high-performance liquid chromatography of prostaglandins and its application to their determination in human seminal fluid, J. Chromatogr., 380,257, (1986).