DETERMINATION OF CARBAMATE HERBICIDE ASULAM IN PEACHES FOLLOWING FLUORESCAMINE FLUORIGENIC LABELLING

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Summary—Because of the primary aminic character of asulam, it is readily labelled with fluorescamine. A study of the variables affecting the derivatization reaction has been made. Two analytical approaches, that is, direct, synchronous spectrofluorimetry have been tested, and they both show good analytical performances. The linear dynamic range of the synchronous approach is 0.043–0.214 µg/ml and the relative standard deviation is 1.6%. The methods have been applied to the determination of asulam residues in peaches with recoveries of 85–106%.

Asulam (methyl 4-aminobenzenesulphonylcarbamate) is a translocation herbicide, absorbed by leaves and roots causing slow chlorosis in susceptible plants. It interferes with cell division and expansion and is used to control the growth of grasses.

The original method for asulam residues quantification was based on a colorimetric reaction. Analysis for asulam using HPLC in wheat flour was reported, but the metabolites were not examined. Using HPLC reverse phase column, asulam was determined in peaches fortified at the 0.1 µg/ml, together with its metabolites, sulphanilamide and acetylasulam. GLC was used for determination of asulam and acetylasulam after hydrolysis to sulphanilamide. Similarly asulam is analysed in forage crops and animal tissue by HPLC after derivatization. Determination of asulam in soils by isotachophoresis and liquid chromatography, using UV and conductivity detection has also been reported. Thin-layer chromatography using fluorescamine as labelling reagent has been used to analyse soil samples for the herbicide.

Because Asulam does not show native fluorescence, the technique of spectrofluorimetry has not been previously applied to its determination. Udenfriend et al. introduce fluorescamine as a labelling reagent to determine primary amines; this is superior to dansyl chloride because both the reagent and its hydrolysis products are nonfluorescent and permit homogeneous fluorogenic labelling. Such an approach has proved its usefulness in numerous analytical applications for some 30 years.

This paper describes the use of the synchronous derivative technique to determine the fluorescent derivative of asulam herbicide. This method may be used for the quantitative analysis of asulam residues in peaches fortified at levels of ng/ml.

EXPERIMENTAL

Apparatus

Emission measurements were done with a luminescence spectrometer, Perkin–Elmer LS-5, equipped with a xenon discharge lamp (9.9 W) pulsed at line frequency, monochromators F/3 Monk-Gillieson type, and 1 x 1-cm quartz cells. The spectrophuorometer was operated in the computer-controlled mode via the RS232C serial interface by a Perkin–Elmer Model 3600 data station microcomputer. Instrumental control and data collection were achieved by using the commercially available Perkin–Elmer computerized luminescence software (PECLSII). For graphical recording, an Epson FX-85 printer-plotter was connected to the spectrophuorometer. All fluorescence spectra are uncorrected because no significant wavelength shifts are observed when comparing with corrected spectra.

UV absorption spectra were recorded with a Shimadzu UV-240 Graphicord recording spectrophotometer. A rotary vacuum evaporator.
Reagents

Stock solutions of asulam (99% pure, Dr. S. Ehrenstofer), were prepared in ethanol at concentrations of 1.0 mg/ml. Working solutions at 100 µg/ml were prepared in ethanol.

The analytical-reagent-grade Fluorescamine (4-phenylspiro[furan-2(3H),1'-phtalan]-3,3'-dione) was obtained from Aldrich and was dissolved in acetone (1 mg/ml).

All solvents used were of analytical reagent grade (Merck) and only demineralized water was used in this work.

Procedures

General procedure. To a 10-ml standard flask add the volume of stock solution needed to obtain a final concentration of asulam between 0.042 and 2.14 µg/ml. Add 0.5 or 2.5 ml (low or high concentration range) of fluorescamine (3.59 x 10^{-3}M) and 2 ml of buffer solution (phthalate/HCl, pH 3) and dilute to the mark with demineralized water. For normal spectrofluorimetry, measure fluorescence intensity at 498 nm, with excitation at 402 nm, against a reagent blank.

For synchronous spectrofluorimetry, prepare the samples exactly as above, record the spectrum at Δλ = 96 nm (corresponding to the Stokes shift) at a time constant of 2 sec and scan speed of 240 nm/min.

Extraction procedure. To chopped peaches (50 g), add 60 µl of standard ethanolic solution of asulam 1170 µg/ml, and blend at high speed for 5 min in 50 ml of acetone. The blended sample was filtered through a fritted glass Büchner (coarse porosity) under reduced pressure. Add to the filtrate 25 ml of saturated sodium chloride and 50 ml of methylene chloride, make up to 250 ml with demineralized water and transfer to a 500-ml separating funnel. The organic phase was concentrated just to dryness by using a rotary evaporator and the residue diluted with demineralized water to a final volume of 25 ml. Aliquots of this solution were then analysed according to the proposed analytical procedure.

RESULTS AND DISCUSSION

Optimization of the derivatization reaction

Fluorescamine (FC), itself non-fluorescent, reacts rapidly with primary aliphatic or aromatic amines to give highly fluorescent pyrroline derivatives and non-fluorescent hydrolysis products.

The fluorescence spectra obtained when the reaction takes place in an aqueous solution of 2 µg/ml at pH 3 exhibit two excitation maxima at 325 and 402 nm and an emission band at 498 nm. In order to check that the excess of fluorescamine is hydrolysed and gives no fluorescence signal, a blank test was performed without asulam. No significant blank signal was found.

We examined the effect of the pH on derivative formation in the media and the relative fluorescence intensity of the asulam derivative. In the range pH 2.8–3.1, the variations were minimum, and pH values greater than 10 produce very little fluorescence. To maintain the pH, a set of buffers was assayed and significant differences were found. Figure 1 shows the results obtained from a 10-ml solution containing 3.6 x 10^{-4}M of FC and 2 µg/ml of asulam. Two sets of data points are shown, one obtained by adjusting the pH by little additions of diluted solutions of sodium hydroxide or hydrochloric acid to the phthalate buffer of pH 3, and the other by using 2-ml aliquots of different buffer solutions.

To keep the optimum pH selected to carry out the derivatization reaction constant, several buffers of different composition may be possible. Experiments to test the influence of the nature of buffer in fluorescence signal show that it is a critical variable, since phthalate based
buffer gives twice the signal of glycine or citrate based buffer.

Also, buffer concentration affects the relative fluorescence intensity. A 1-ml portion of 0.05M phthalate buffer in an over-all volume of 10 ml produces a constant and maximum signal; higher concentrations of buffer depress the signal. This effect is due, probably, to changes in ionic strength and in the thermodynamic hydrolysis constant of FC. No changes have been found in the derivatization reaction on changing the addition order of the reactants, if we kept in mind that Asulam must be in the flask when buffer and FC are added.

The amount of FC used was found to influence the results. Approximately a 20-fold reagent excess is required to complete derivative formation.

The possibility of enhancing the sensitivity of the FC derivative by using different organic solvents to extract or dissolve the fluorophor was investigated. Derivative fluorescence was found to be strongest in the aqueous medium.

**Synchronous derivative spectrofluorimetry**

The most appropriate parameters to register synchronous derivative spectra for the procedure were selected. The contribution of this approach to the analytical methodology when the problem is the determination of an isolated compound, not a multicomponent fluorescent compound sample, is the improved sensitivity that may be attained. In this case, the limiting factor in the estimation of the detection limit is the blank signal and the associated standard deviation, and this figure can be significantly reduced since less contribution of the blank signals are observed, especially when the broad band blank spectra are obtained. In addition, the band narrowing effect that synchronous spectrofluorimetry applies, together with the sensitivity of the first derivative to narrowest spectral profiles, gives a gain in analytical sensitivity of the method. Also, in general, in the synchronous scanning the signal is stabilized and is less affected by random fluctuations, thus better precision in measurements can be obtained.

For simple analysis, the recommended scanning interval is the one that provides the highest intensity and the minimum peak half-width, which often corresponds to the Stokes shift. The derivative amplitude is normally taken as the vertical distance from peak to trough.

In this work, the best results were \( \Delta \lambda = 96 \, \text{nm} \). The reduction of the peak half-width and the position of the synchronous maxima were calculated. A reduction of \( \Delta \lambda \) from 72 to 45 nm was observed. It is important to note that the effectiveness of derivative spectroscopy is a function of the bandwidth of the normal spectrum.

**Analytical parameters**

The calibration curves were recorded for both the direct and synchronous techniques. The linear dynamic ranges were from 0.043 to 2.14 \( \mu \text{g/ml} \), were identical, that is while the correlation coefficients were 0.996 and 0.999, respectively (\( n = 5 \)).

The precision of the methods determined by analyzing seven replicate of 64.2 ng/ml, was 6.2 and 1.6%, respectively.

**Residue analysis of asulam in peaches**

To check the usefulness and to compare two fluorimetric procedures proposed in the present work, known amounts of asulam were added to peach extracts and the samples were analysed as indicated under the general procedure.

Fruits were bought from the local market and their origin and previous treatment were unknown. The fruits were spiked with the herbicide at 0.14 \( \mu \text{g/ml} \).

The results obtained for the analysis of the herbicide in peaches using direct and synchronous spectrofluorimetry are given in Table 1.

Recoveries obtained show that the synchronous technique fits the results with almost 100% efficiency with better precision than the direct approach. Because of its simplicity, the

<table>
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<tr>
<th>[Asulam] Added, ( \mu\text{g/ml} )</th>
<th>Synchronous</th>
<th>Direct</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Found, ( \mu\text{g/ml} )</td>
<td>Recovery, %</td>
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<tr>
<td>0.140</td>
<td>0.120</td>
<td>85.6</td>
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<tr>
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<td>0.141</td>
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<tr>
<td>0.140</td>
<td>0.149</td>
<td>106.0</td>
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<tr>
<td>( \bar{x} )</td>
<td>0.137 ± 0.015</td>
<td>97.4 ± 10.5</td>
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method might find use as a rapid screening technique for pesticides.

REFERENCES