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Note

Determination of the alkaloid perloline in grasses by high-performance liquid chromatography

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Perloline is the predominant alkaloid of some fodder grasses (*Festuca arundinacea* Schreb., *Lolium perenne* L.). It has been implicated in causing impaired performance in ruminants[1]. For this reason, breeding research is directed to grasses with a low alkaloid content.

Colorimetric[2], fluorometric[3,4] and thin-layer chromatographic (TLC)[4,5] methods have been developed for the analysis of perloline in grasses. However, these procedures incorporate time-consuming clean-up steps and therefore they are not suitable to analyze a large number of samples routinely. This paper describes a fast and efficient method for determination of perloline in grass species using high-performance liquid chromatography (HPLC).

EXPERIMENTAL

Materials and reagents

All reagents were of analytical reagent grade, unless otherwise stated: glass-distilled water; chloroform; methanol; ethanol; hydrochloric acid; ammonium hydroxide; anhydrous sodium sulphate.

Perloline hydrochloride was prepared in our laboratory according to the literature method[6]. The purity of perloline was confirmed by TLC, HPLC and UV spectrometry in different solvents and by melting point determination. A stock solution (30 μg/ml) was made up in 0.2 *M* hydrochloric acid containing 1% ethanol. Other solutions were obtained by dilution.

Apparatus

HPLC was performed using a Hewlett-Packard Model 1010 B liquid chromatograph equipped with a HP 1030 B variable-wavelength detector. A plotting integrator HP 3380 A was used to calculate peak areas. Separations were achieved with a stainless-steel column (25 × 0.46 cm I.D.) containing LiChrosorb Si 100 (particle size 10 μm) at an elution rate of 1 ml/min. Perloline was detected at 254 nm with the absorbance detector at sensitivities of 0.04–0.2 absorbance units full scale. Samples were injected onto the column by means of a six-port loop injector (Rheodyne, Model 7010).

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Elution solvent system

An elution system consisting of chloroform–methanol–ammonium hydroxide (90:9.5:0.5, v/v) was used. The solvent was filtered and degassed before use.

Extraction and clean-up procedure

The dried and finely ground plant tissue (0.5 g) was refluxed for 30 min with 50 ml of 0.2 *M* hydrochloric acid. The hot suspension was filtered through a wet filter-paper and the residue was washed three times with 10 ml of 0.2 *M* hydrochloric acid. The combined aqueous extracts were transferred to a 100-ml volumetric flask and brought to volume with 0.2 *M* hydrochloric acid. An aliquot of 50 ml was basified by adding 5 ml of 10% aqueous ammonium hydroxide and extracted with 25 ml of chloroform. Phase separation was achieved by centrifugation. The chloroform layer was collected over anhydrous sodium sulphate and can be used directly after filtration for subsequent injection onto the HPLC column.

Standard solutions for HPLC analysis were prepared by adjusting an appropriate standard solution in hydrochloric acid with ammonium hydroxide to pH 9.7 and extraction with chloroform. The chloroform extract was dried over anhydrous sodium sulphate, filtered and 50-μl aliquots were analyzed by HPLC.

RESULTS AND DISCUSSION

Several combinations of solvent mixture and column packing were tested in the HPLC analysis of perloline. The best results were achieved using a LiChrosorb Si 100 (particle size 10 μm) column with an elution solvent of chloroform–methanol–ammonium hydroxide (90:9.5:0.5, v/v). Under these experimental conditions, perloline was eluted as a sharp peak within 4.5 min. The use of small amounts of ammonium hydroxide in the mobile phase was found to be helpful in suppressing tailing of the perloline peak. The alkaline eluent sometimes gave rise to a slight instability of the column, but the resulting flow-rate changes were not dramatic. Twenty-eight injections of a perloline standard solution over a 3-day period gave a mean retention time of 266 sec with a coefficient of variation of 1.18% (Table I). No decrease in column efficiency was noted over 2 weeks of daily injections.

The relationship between peak heights and areas and the quantity of perloline injected was linear over a range of 50–500 ng. Contrary to previously described procedures[2–5], the clean-up of sample extracts comprised only a single partitioning of the alkaline aqueous solution against chloroform. Fig. 1 shows that the extracts obtained

TABLE I

REPRODUCIBILITY OF RETENTION TIME FOR PERLOLINE

Parameter	
No. of injections	28
Retention time (sec): range	258–271
mean	266
Standard deviation (sec)	3.15
Coefficient of variation (%)	1.18

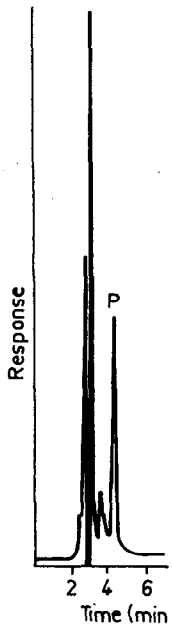

Fig. 1. Typical HPLC separation of a natural contaminated *Festuca arundinacea* sample on a LiChrosorb Si 100 (particle size 10 μm) column at a flow-rate of 1 ml/min with chloroform–methanol–ammonium hydroxide (90:9.5:0.5, v/v) as eluent and UV detection at 254 nm. P = Perloline.

TABLE II

REPRODUCIBILITY OF THE HPLC METHOD FOR THE ANALYSIS OF PERLOLINE IN GRASSES

The x_i values are given in mg/kg of dry matter. Each value represents the mean of values from duplicate HPLC analyses.

Sample	x_i	$\bar{x}$	*S.D.*	*C.V. (%)*
1	900 860	880	28.3	3.22
2	862 842	852	14.1	1.65
3	820 800	810	14.1	1.74
4	900 940	920	28.3	3.08
5	675 670	673	3.5	0.52
6	670 650	660	14.1	2.14
7	440 464	452	17.0	3.76
8	310 304	307	4.2	1.37

are pure enough for direct HPLC analysis. Perloline was well separated from other peaks in the chromatogram and accurate quantitation is possible.

The average recovery of added perloline from grasses was 97.7% over a range of 300–1000 mg/kg of dry matter (D.M.). The reproducibility of the procedure was determined by independent analysis of several grass samples containing different amounts of perloline. The results of these experiments given in Table II show the good precision at the levels examined. The standard deviation of the mean was less than 4% for all samples analyzed.

The HPLC procedure described is simple and rapid, the analysis time being 5–6 min. It allows direct analysis of grass extracts after a simple one-step clean-up and is well-suited for field monitoring of perloline levels in grasses.

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