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Note

High-performance liquid chromatographic determination of colchicine and colchicoside in colchicum (*Colchicum autumnale* L.) seeds on a home-made stationary phase

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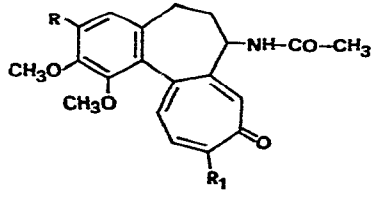
Colchicine has long been included in many pharmacopoeias and is used in the treatment of gout. However, the colchicum corm and its preparations have been described in very few pharmacopoeias[1,2], which generally provide gravimetric methods for the determination of the total alkaloid content. More recently, colorimetric and spectrophotometric assays[3–7] have become popular; previous methods assayed not only colchicine but also other related alkaloids that are present in the corm, and are therefore not specific. Polarography[8] has not been employed widely and chromatographic methods[9–11] are not very precise. The previous methods can also be applied to colchicum seeds.

Both colchicine and colchicoside are obtained industrially from the seeds of *Colchicum autumnale* L.; thiocolchicoside, a semi-synthetic derivative of colchicoside, has been shown to have a decontractant effect and anti-inflammatory and analgesic activity.

The structures of these compounds are shown below:



Colchicine	$R=OCH_3$	$R_1=OCH_3$
Colchicoside	$R=OC_6H_{11}O_5$	$R_1=OCH_3$
Thiocolchicoside	$R=OC_6H_{11}O_5$	$R_1=SCH_3$

The purpose of this investigation was to develop a method for the high-performance liquid chromatographic determination of both alkaloids in colchicum seeds. The time required to carry out an analysis is short because purification and separation steps are not necessary. We obtained a useful separation on a silanized silica gel column (which versatility has been reported[12–16]).

EXPERIMENTAL AND RESULTS

Chromatographic system

A Hewlett-Packard Mod. HP 1010A liquid chromatograph equipped with solvent flow controller and gradient elution was used. The column was a stainless-steel tube (50 cm × 3 mm I.D.) packed with the absorbent by a dry packing procedure. The flow-rate of the eluent was 2.0 ml/min. After the sample injection (2 μl) without interrupting the solvent flow by means of a 5-μl SGE syringe the analysis was carried out at room temperature using gradient elution from a 10% solution of acetonitrile in water (eluent B) to a mixture of 0.6 ml of acetonitrile (eluent A) plus 1.4 ml of eluent B, according to the scheme shown in Fig. 1. A UV monitor at 254 nm was used as the detector.

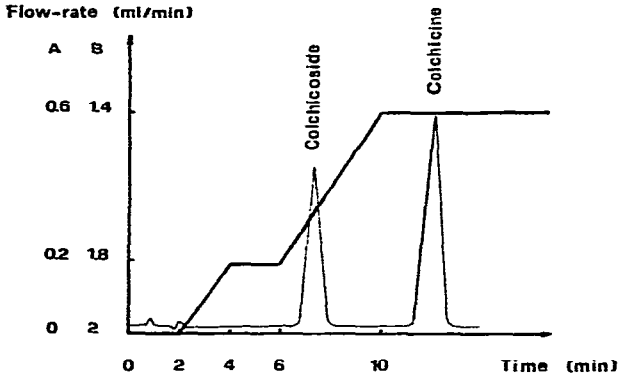


Fig. 1. Liquid chromatogram of colchicum seed extract.

Absorbent

A 5-g amount of LiChrosorb SI 60, particle size 30 μm, was refluxed with concentrated hydrochloric acid (100 ml) for 4 h. The hydrochloric acid was filtered off and the silica was washed with water until the washings were no longer acidic; finally it was washed with acetone (100 ml) and methanol (100 ml) and dried overnight at 65° at a pressure not exceeding 50 mm Hg.

The silica was placed in a round-bottomed flask and 5 ml of chloromethyldimethylchlorosilane in 40 ml of tetrahydrofuran were added. Reaction was carried out for 3 h at 60° followed by 48 h at room temperature. The silanized silica was filtered off, washed several times with methanol and acetone and transferred into a glass gas chromatographic column. The column was connected to a gas chromatograph and maintained overnight at 100° with a flow-rate of helium of 15 ml/min, after periodic injections of Sylil 8.

Sample preparation

Colchicum seeds (10 g), in coarse powder, was mixed with 0.15 g of calcium carbonate and the mixture transferred to an apparatus for continuous extraction.

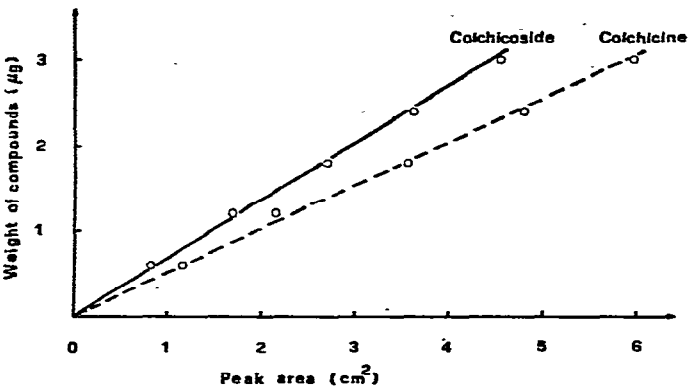


Fig. 2. Calibration graph for colchicine and colchicoside

The extraction was performed with 150 ml of 90% (v/v) methanol for 6 h. The extract was cooled and evaporated to dryness at reduced pressure and the residue was dissolved in 100.0 ml of methanol.

Quantification

The peak areas were measured as the product of the peak height and the width at half-height. The peak areas allowed the calculation of the contents of colchicine and colchicoside in the sample preparation by using calibration graphs of where average peak areas were plotted against the concentration of the pure compound.

Combined liquid chromatography–mass spectrometry

The specificity of the proposed chromatographic system was demonstrated by combined liquid chromatography–mass spectrometry. The peaks corresponding to colchicine and colchicoside were collected separately, evaporated and the mass spectra obtained. The characteristic fragments of each substance were identical with those obtained from pure compounds.

TABLE I

RESULTS OF PRECISION STUDY

Run No.	*Colchicine (%, w/w)*	*Colchicoside (%, w/w)*
1	0.686	0.320
2	0.665	0.329
3	0.703	0.312
4	0.668	0.305
5	0.638	0.296
6	0.623	0.296
7	0.643	0.305
8	0.652	0.329
9	0.675	0.321
10	0.688	0.338
Average	0.6641	0.3151
Standard deviation	25.08	14.58
Coefficient of variation	3.77	4.63

Linear range

The calibration graph is shown in Fig. 2. It has a zero intercept and is linear from 0.6 to 3 μg (injected amounts) of each compound. Each point shown is the average of three determinations.

Precision

The precision of the method was determined by repeated analysis of a single sample of colchicum seeds. The results are shown in Table I and indicate a good repeatability with coefficients of variation for colchicine and colchicoside of 3.77 and 4.63, respectively.

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