POLISH JOURNAL OF FOOD AND NUTRITION SCIENCES

Pol. J. Food Nutr. Sci., Vol. 1/42, No. 3 September 1992

SHORT REPORT

METHOD FOR OBTAINING VERBASCOSE STANDARD

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Key words: column chromatography, faba bean, HPLC, TLC, verbacose

INTRODUCTION

Verbascose is a major oligosaccharide of faba bean, lupine, field pea, black gram and broad bean seeds [8]. High performance liquid chromatography HPLC has been commonly applied for determining of this compound [3, 7, 9]. Since well known chemical companies do not offer a verbascose standard it is neceassary to prepare it before setting up analyses. For this purpuse Muzquiz et al. [7] applied TLC and Quemener [9] preparative HPLC column with Lichroprep NH_2 support/packing. The aim of the study was to prepare a method of obtaining verbascose for analytical purposes applying column chromatography on silica gel.

MATERIAL AND METHODS

Oligosaccharides were extracted from ground and hexane defatted seeds of the Dino faba bean variety acc. to Macrae et al. [6]. Suspension containing 20 g of flour in 400 cm^3 of 60% methanol was heated at 92°C for 2h under a reflux condenser, cooled and centrifuged at 3000 rpm for 5 min. Methanol wasremoved from extract in a rotation evaporator at temperature not exceeding 50°C. Thus obtained and cooled aqueous solution of saccharides was purified through extraction in water-n-butanol (1:1) solution [1]. Water phase was next lyophilised.

In order to select an optimal washing system a preliminary TLC analysis was carried out using column chromatography. Silica gel 60G (Merck) plates and the following developers were applied: n-butanol-acetic acid-water 12:3:5 acc. to Muzquiza et al. [7], n-butanol-acetone-water 75:75:25 acc. to Dini et al. [3] and n-butanol-acetone-water 75:50:50 (our own formula). Apart from

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oligosaccharide extract from faba bean seeds also saccharose, rafinose and stachiose standards (Sigma) were stained on the plate. Oligosaccharides were visualised by spraying the plate with anilin-difenyloamin-phosphoric acid acc. to Bailey at al. [2].

Column chromatography on silica gel was performed to isolate and purify verbascose from the oligosacchride mixture. Obtained lyophilisate (1.05g) was dissolved in 10 cm^3 60% methanol and transferred to a column (1 m long and 2 cm in diameter) supported with Silica gel 60G (Merck). Oligosaccharides were washed from the column with n-butanol-acetone-water 75:50:50. Fractions (20 cm^3) were collected with a fraction collector. The presence of sugars was indicated with colour reaction [4]. Oligosaccharides in each fraction were monitored with TLC method. Those containing only verbascose were combined and concentrated in rotation evaporator. The purity of thus obtained verbascose standard was checked with HPLC method applying the following parameters: chromatograph Shimadzu (pump LC 6A, C-R4A chromatopac, RID-6A detector, CTO 6A Column oven), column 10 μm Nucleosil NH_2 250 $\times$4.6 mm, 20 μl loop, oven temperature 30°C, flow rate 1 ml/min, 65% CH_3CN phase.

RESULTS

Data from Table 1 and diagram of TLC separation in Figure 1 indicate that the most advantageous oligosaccharide separation with TLC method took place at the suggested by us system n-butanol-acetone-water 75:50:50.

Table 1. R_f values for oligosaccharides obtained at various developing systems

Developing system	Saccharose	Rafinose	Stachiose	Verbascose
n-butanol-acetic acid-water 12:3:5	0.12	0.06	0.04	0.03
n-butanol-acetone-water 75:75:25	0.33	0.12	0.03	0.00
n-butanol-acetone-water 75:50:50	0.43	0.27	0.20	0.12

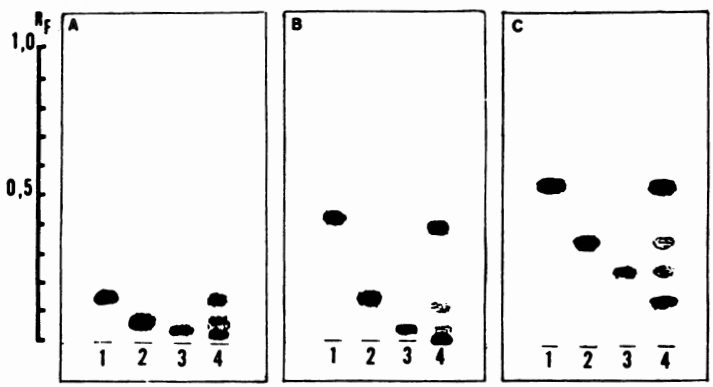

Fig. 1. Oligosaccharides separation with TLC depending on the applied developing system. *A:* *n*-butanol-acetic acid-water 12:3:5; *B:* *n*-butanol-acetone-water 75:75:25; *C:* *n*-butanol-acetone-water 75:50:50; *1*-saccharose, *2*-rafinose, *3*-stachiose, *4*-faba bean extract

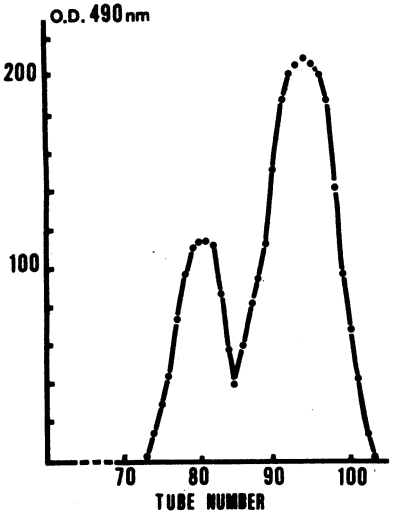

Fig. 2. Oligosaccharides separation on silica gel column

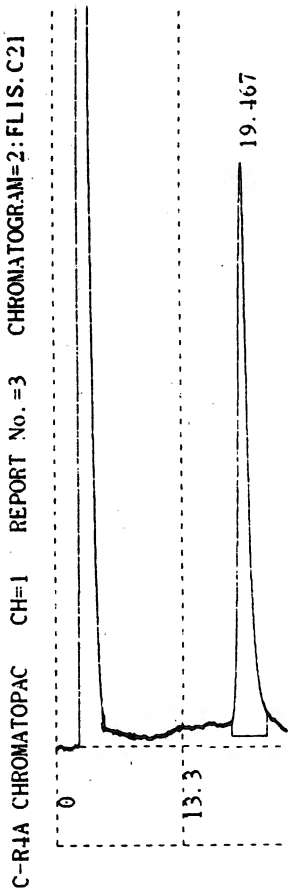

Fig. 3. HPLC chromatogram of the obtained verbascose standard

Applying this system there was observed the best separation of verbascose (R_f 0.12) from stachiose (R_f 0.20). The system is also more advantageous from ethyl-acetic acid-methanol-water 60:15:15:10 and n-propanol-water 85:15 systems proposed by Gasparic and Churacek [5] for oligosaccharides analysis. The value of R_f for those systems was 0.04 and 0.13 respectively (authors do not report data for stachiose and verbascose).

TLC analysis of particular fractions washed from the column combined with results of colour reaction against sugars (Fig. 2) demonstrated a good separation of stachiose from verbascose on silica gel supported column. Verbascose was observed from fraction 82 onwards; it was accompanied by stachiose until faction 88. Starting from fraction 89 verbascose was the only oligosaccharide washed from the column.

Only one clear verbascose peak obtained on the chromatogram (Fig. 3) permits to conclude that the suggested analytic course ensures obtaining verbascose standard with purity required for oligosaccharides in plant material with HPLC method.

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Received March 1992. Revision received April 1992, and accepted May 1992.