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A simple apparatus for derivatizing amino acids to their corresponding N-trifluoroacetyl *n*-butyl esters*

The direct esterification and subsequent acylation of amino acids[1,2] to their corresponding N-trifluoroacetyl *n*-butyl esters have been an important milestone to achieve volatility in gas-liquid chromatographic (GLC) quantitative analysis of protein hydrolysates and free amino acids. With the aid of a nitrogen detector equipped on the gas chromatograph, it has been possible to eliminate the elaborate sample clean-up prior to derivatization. A complete derivatization so far requires heating and drying devices such as sand or oil bath providing temperature up to 150°. Physically, the manipulation of each microvial (30 mm × 17 mm)[2] through a series of open–close, re-open–re-close, and final open operation (Table I) prior to GC injection presents some difficulties such as oily vials which are hard to close and to open and danger of introducing sand into the sample vial.

TABLE I

THE PROCESSES AND CONDITIONS FOR DERIVATIZATION

Abbreviations: H_1 = 100° oil or sand bath; H_2 = 150° oil or sand bath; V_o = micro-vial open; V_c = micro-vial close.

Step I	Sample drying (H_1, V_o)	→ Cooling (V_o)	→ Ultrasonic mixing (V_c)	
Step II	Esterification (H_1, V_c)	→ Cooling (V_c)	→ Ester drying (H_1, V_o)	
Step III	Acylation (H_2, V_c)	→ Cooling (V_c)	→ Dilution (V_o)	→ GLC

A solution to the problem is to carry out the heating-drying and derivatization process in the same micro-vial. These vials are accommodated in three aluminum blocks (*ca.* 4 in. × 3 in. × 5/8 in.). Into each of these blocks are drilled conical holes (0.60 in. × 3/8 in. deep tailored to the micro-vial) (Fig. 1). The bottom surface of each block is smoothed to ensure good thermal contact with the hot plate (6 in. × 6 in.) on which they are placed. The temperature settings and conditions are listed in Table II.

In our routine work, it has been found satisfactory to introduce 0.1 to 0.2 ml of deproteinized serum, protein hydrolysate[3], or amino acid extract of plant material[4] (up to 100 μg amino acid) directly into the micro-vials which sit comfortably onto each aluminum hole and safely go through the derivatization process. Vials are clean and dry. The worry of contaminating the vial or trapping debris outside the vial neck is eliminated. The latter causes the loss of sample and cracking of the vial neck during acylation.

The temperature of Block 2 and Block 3 fluctuates by no more than 1°. The exposed area of the surface of the hot plate for Block 1 may or may not be covered with asbestos board to reduce heat loss during ventilation under the fume hood.

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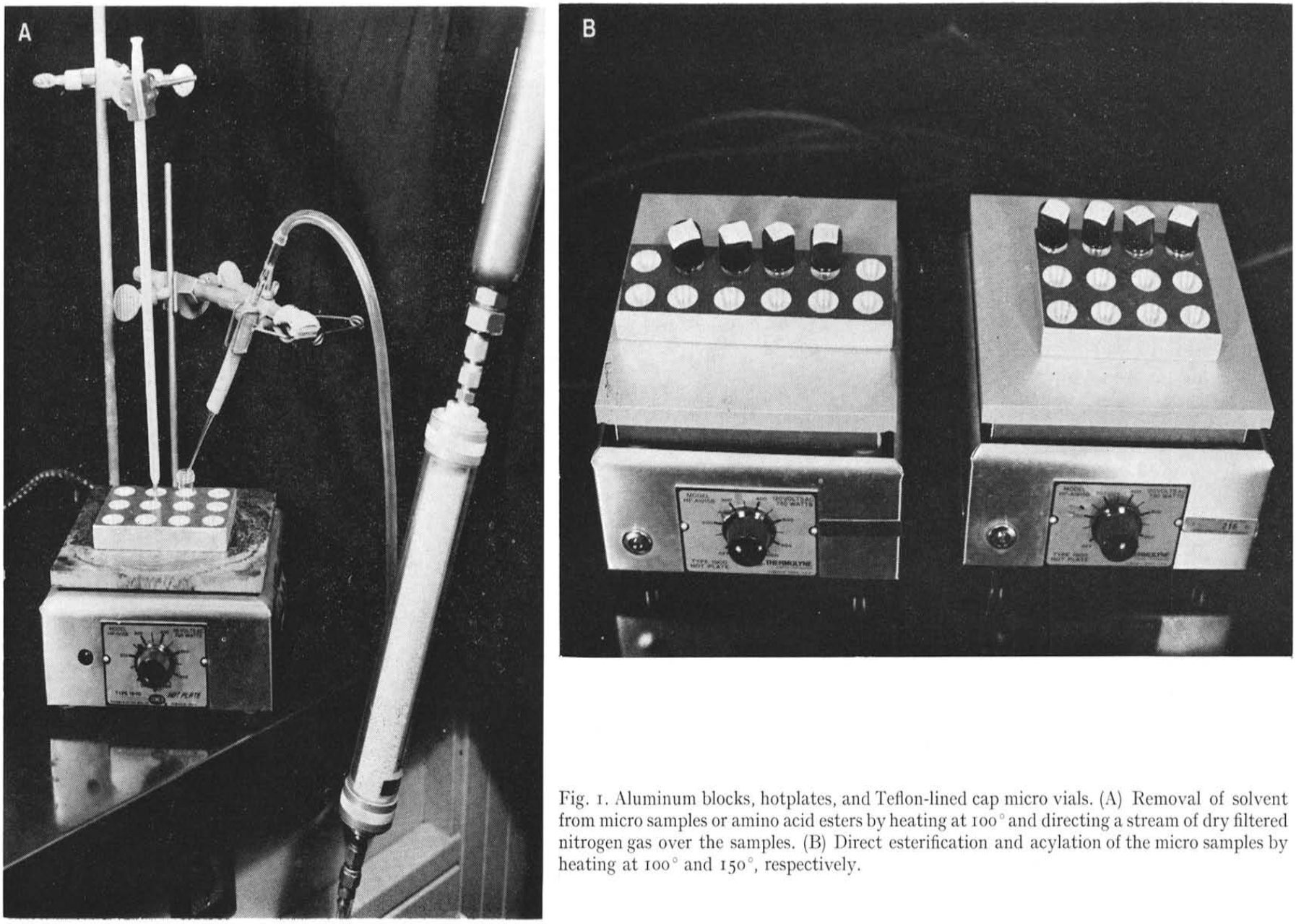

Fig. 1. Aluminum blocks, hotplates, and Teflon-lined cap micro vials. (A) Removal of solvent from micro samples or amino acid esters by heating at 100° and directing a stream of dry filtered nitrogen gas over the samples. (B) Direct esterification and acylation of the micro samples by heating at 100° and 150°, respectively.

TABLE II

TEMPERATURE AND CONDITIONS FOR ALUMINUM BLOCK

Block 1	100 °, sample drying (N_2 blow) under fume hood
Block 2	100 °, esterification
Block 3	150 °, acylation, behind safety glass shield

Apparatus needs little bench space and one can finish four derivatized samples per hour. This results in an efficient analytical procedure.

Department of Animal Sciences, University of Hawaii, Honolulu, Hawaii 96822 (U.S.A.)

JOHN M. L. MEE
COY C. BROOKS

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