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A Thermo Micro Procedure for Rapid Extraction and Direct Application in Thin-layer Chromatography

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A simple thermo micro procedure is described for the separation of many substances from solid materials and the direct transfer of these substances to the starting point of a thin-layer chromatographic plate. The sample is introduced into a glass cartridge with a conical tip, and heated rapidly for a short time at a pre-set temperature. The emerging vapours are deposited as a spot on the thin-layer chromatographic plate, which is then chromatographed in the usual way. There are numerous variations of the standard procedure, thus extending the range of application, and this is demonstrated with examples from the fields of drug research, phytochemistry, analysis of food additives and residues, and from organic and inorganic chemistry.

Many organic and some inorganic substances are volatile at high temperatures, therefore, if these substances are present in mixtures with non-volatile materials they can be separated by the application of heat, either by distillation or sublimation. The use of steam-distillation for the separation of volatile components must also be mentioned.

Some substances are thermally unstable, however, and decompose into volatile or non-volatile products, or both. This is known as pyrolysis. Such fragmentation and separations take place concurrently in dry distillation, and these thermal procedures are of interest for direct coupling with thin-layer chromatography.[1]

The attempts so far reported in the literature have been confined to gas chromatography. For example, high temperature pyrolysis followed by gas chromatography, used for the identification of polymers, is already an established procedure. Experiments have also been described in which terpenes from plant material have been introduced directly into the chromatographic column.[2,3,4] The equipment used for this purpose is unsuitable or suitable only to a limited extent for thin-layer chromatography. A disadvantage is, for example, the rapid fouling of the heating and sample application systems; the necessity to purify the pyrolysis products makes the procedure unsuitable for routine use.

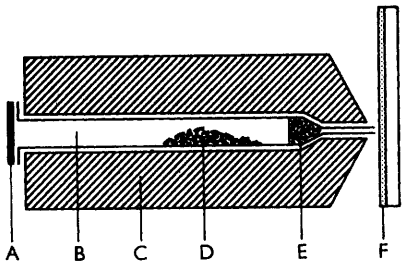
A procedure was sought that would allow a simple and inexpensive thermal extraction, which could be directly coupled with thin-layer chromatography.

General description of the thermo micro separation, transfer and application according to Stahl (TAS* procedure)—

The apparatus is shown in Fig. 1. A small amount of sample D is placed in the special glass cartridge, B, the end of which can be closed in a simple, efficient way, A. The charged cartridge is pushed into a metal-block furnace, C, adjusted to a given temperature. The tip of the glass tube projects from the furnace and points to the starting point on the layer, F. The thin-layer chromotographic plate is positioned 1 mm from the tip and can be easily moved.

The volatile materials are thus transferred from the sample directly on to the layer in the form of spots. The applied materials are then chromatographed in the usual way. Several variations of this simple standard procedure have been tested, which have led to further possibilities of application.[5,6] These procedures are compiled below, together with a description based on schematic diagrams.

* TAS: T = thermomicro and transfer; A = application; S = substance, Stahl and Saarbrücken.



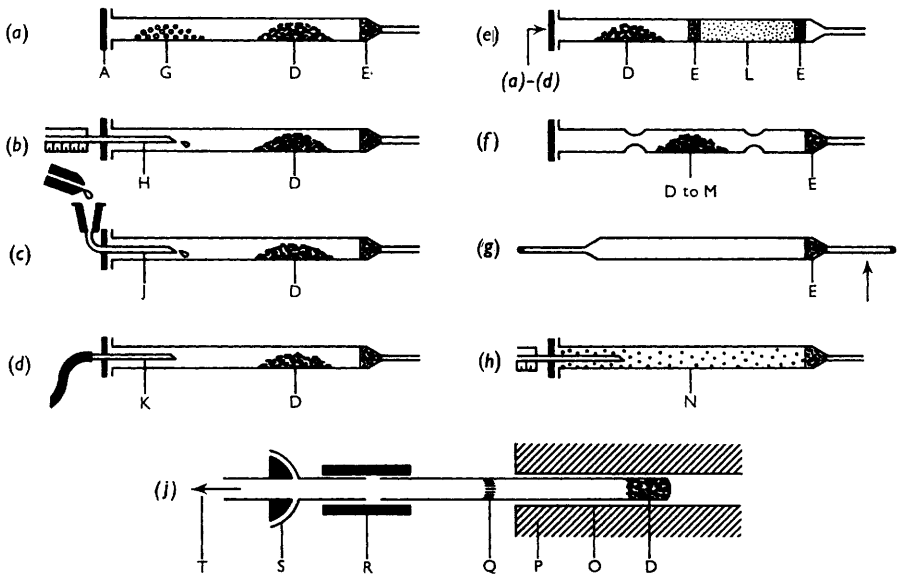
A = Seal (silicone membrane)
B = Glass cartridge
C = Heating block
D = Sample
E = Glass-wool
F = Thin-layer chromatographic layer

Fig. 1. Cross-section of the major components of the TAS oven

FURTHER TECHNIQUES AND VARIATIONS OF THE TAS PROCEDURE—

The application and technical details of the diagrams shown in Fig. 2 are as follows.

(*a*) A solid, G, is added to sample D in the tube, which at elevated temperatures releases a part of its weight in the form of vapours. These solids may be organic or, preferably, inorganic in nature. For this "ultramicro steam-distillation," *e.g.*, at 220° C, 100 mg of corn starch, wetted "Bluegel" (pink) containing 20 per cent. of water, or calcium sulphate, are used, and $CaCl_2.8NH_3$ can be used if basic vapours are required.



A to F. See Fig. 1
G = Solid with volatile components, e.g., crystal water
H = Micro-injection of solvent
J = Bent syringe needle for solvent introduction
K = Needle for the introduction of gas
L = Reactive layer
M = Reactive layer
N = Glass bead packing
O = Test-tube, diameter as B
P = Edge of heating block
Q = Condensation zone
R = Tubing connection
S = Rotatable ball-joint connection
T = Vacuum

Fig. 2. Various techniques and cartridges for the TAS procedure (explanations of (*a*) to (*j*) in the text)

(*b*) and (*c*) In this technique a small amount (5 to 20 μl) of a solvent is introduced into the hot tube. This is carried out with a microsyringe, H, in procedure (*b*). In procedure (*c*) the solvent is introduced with a micropipette into the bent syringe needle, J. The spontaneously developed vapour stream obtained in procedures (*b*) and (*c*) is not as advantageous as the "steam evolution" described in (*a*), because the organic solvent is vaporised in a few seconds, which results in too fast a stream of vapour, whereas in (*a*) a more continuous vapour stream lasting for between 30 to 120 seconds is formed.

(*d*) The purging is achieved here with a slow stream of gas. This procedure is used in preparative work, for the application of bands.

(*e*) In this procedure the vapours must pass through a reactive layer, L, for example, a catalyst. The purging can be accelerated by procedures (*a*), (*b*), (*c*) and (*d*).

(*f*) In this procedure the sample is mixed with a substance, for example, potassium hydroxide or zinc powder, or with several reactants. To prevent flowing of a melt, the reaction chamber is confined by two constrictions of the glass tube.

(*g*) Reactions under vacuum or pressure can be carried out in a suitable tube. After cooling the tip is broken off at the spot indicated by the arrow and the products applied directly to the thin-layer chromatographic layer.

(*h*) The tube is packed loosely with small glass beads or coarse quartz sand. The liquid sample, *e.g.*, 100 μl, is injected and the solvent distilled off in the furnace at low temperature. The residue is then volatilised by increasing the furnace temperature.

(*j*) In a preliminary experiment, a test-tube, O, of appropriate size, containing the sample D, is inserted into the TAS furnace, P, and the appearance of a condensate ring, Q, on the protruding cold part of the test-tube observed; this is a separation without thin-layer chromatography. Fig. 2 shows that this procedure can be applied *in vacuo* and, by using a ball-joint, with simultaneous rotation of the test-tube (micro-spherical tube distillation, vacuum sublimation).

EQUIPMENT FOR TAS PROCEDURE—

The three important features in the development of the apparatus were as follows: the form of the glass tube (cartridge) and the sealing of the inlet end; provision of a micro furnace that could be controlled over the temperature range 50° to 450° C; suitable positioning of the thin-layer chromatographic plate in front of the exit end of the glass tube.

As in other procedures, several different forms are possible and some of the equipment tested is listed and explained as follows.

SAMPLE TUBE (TAS CARTRIDGE)—

The tube can easily be made from thin-walled, high-melting glass tubing. The tube must fit exactly into the furnace to ensure good heat transfer. The constricted end requires special attention. The inner diameter of the tip, drawn into a capillary, should be 0·8 to 1 mm. The length of the tube is chosen so that the tip projects only 0·5 to 1 mm from the furnace. The other end, through which the sample is introduced, has a rim. It serves as a seat for the seal and simultaneously as a rest for the sealing clamp. The sealing is achieved by means of a silicone gum membrane, held in place with the clamp. When in use, a thin aluminium foil is placed between the glass tube and the membrane. The handles of the clamp are extended so they can be used for manipulating the tube. The dimensions of the tube have been developed through practical experience, and can be reduced as well as increased. The glass tubes can be discarded after use to save cleaning.

MICRO FURNACE (TAS OVEN)—

The best source of heat for rapid and uniform heating of the tube is an aluminium block heated by a suitable bunsen burner with the necessary controls. The block, which has a cylindrical bore to accommodate the glass cartridge, has a conical outer shape to reduce heat radiation on to the thin-layer chromatographic plate. The temperature is measured with a glass thermometer placed in the block and protected from draught.

Direct electric heating is, however, much more advantageous, as the required temperature can be obtained by adjusting a simple bi-metal control. A small thermocouple placed in the block is used for temperature measurement. It is necessary that the furnace block can be

moved a few millimetres in either direction along its main axis to allow exact adjustment of the distances between the cartridge tip and the adsorbent layer on the thin-layer chromatographic plate to be made. The equipment which we have developed and tested for routine use has been made according to these specifications (Fig. 3).

The relatively expensive electronic two-point regulators can be used if a temperature control accuracy of about 1 per cent. is required. Such a device which has also been developed in other directions, will be described in a later communication. A multiple unit for special purposes has been constructed, accommodating ten cartridges and with electrical heating. This furnace allows the application of samples to a 20 × 20-cm thin-layer chromatographic plate in one operation cycle. This presents an advantage in routine work, particularly in the detection of "chemical races" (*i.e.*, botanical taxonomic uniform plants, which differ in their main chemical substances[5]) in drug-containing plants.[7,8,9]

POSITIONING OF THE THIN-LAYER CHROMATOGRAPHIC PLATE—

The thin-layer chromatographic plate must be positioned in such a way that it can be easily moved along the starting line, *i.e.*, along a line 15 mm above the lower edge of the plate. The distance between the cartridge tip and the thin chromatographic layer should only be 1 mm and must remain the same when the plate is moved.

In the standard procedure the thin-layer chromatographic plate is shifted 15 to 20 mm in one direction after each direct application. A uniform movement of the plate allows application of samples in band form. Both operations can be mechanised and controlled, as will be shown in a paper given at a symposium held at Birmingham in July.

SOME APPLICATIONS OF THE TAS PROCEDURE

Only a few examples, from the many applications already tested, can be discussed here.

DRUG RESEARCH—

It is not widely known that many drugs are volatile at higher temperatures and, therefore, amenable to the TAS procedure. Well known drugs, *e.g.*, aspirin, pyramidone, librium, veronal, butazolidine, and many others, can be directly transferred from mixtures to the thin-layer chromatographic plate. Furthermore, all components from known drug combinations, such as saridon, quadronal, allional and irgapyrine, can, at 200° C, be transferred directly to the starting point in less than 2 minutes. The required amount of these mixtures, usually in the form of tablets, is small (maximum 0·1 mg). Fig. 4 compares direct application with application of a solution after extraction.

ANALYTICAL PHYTOCHEMISTRY AND PHARMACOGNOSY—

Many interesting constituents of drug-containing plants can, after use of the TAS procedure, be easily analysed by thin-layer chromatography. We have been using the technique for more than a year on a routine basis in research work as well as with students. The preferred fields of application are all plant parts containing essential oils,[6] for example, aniseed (Fig. 5), peppermint leaves, camomile flowers, ginger rhizome and cinnamon bark. Depending on the amount present, 5 to 25 mg of the powdered drug are introduced into the cartridge and the direct application is carried out by heating to 250° C for 90 seconds (Fig. 5). Similarly, coumarin derivatives, lactones such as santonin, some anthraquinones and phloroglucinol derivatives can be isolated directly from the drug. Alternatively, the substance can be sublimed directly on to a glass slide, and the crystal formed examined with a microscope, before carrying out spot tests.

Fig. 4 also shows that it is possible to sublime purines, *e.g.*, caffeine, directly from the drug on to the thin-layer chromatographic plate and then to separate them chromatographically. The identification is carried out directly on the plate by recording an ultraviolet spectrum with the new Zeiss spectrophotometer.[10] Of particular interest is the rapid identification of small amounts of narcotics, directly from drugs, or from cigarette or pipe residues. Examples of this are the identification of marijuana constituents, of mescaline from Mexican narcotic fungi (Fig. 6), and of indole derivatives from drugs. The pyrolysis products of opium can also be identified by this method.[11] (A separate communication will deal with further applications, especially in the alkaloid field.)

Fig. 3. Frontal view of the equipment for the micro application of substances (TAS oven): *centre:* heating block with thermometer and cartridge; *left:* temperature control; *right:* filled cartridges; *in the background:* a 20 × 20-cm thin-layer chromatographic plate, positioned on a rail

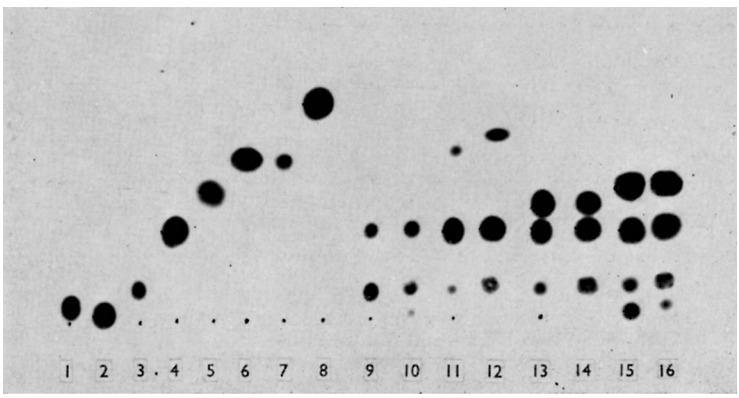


Fig. 4. Chromatogram of well known drugs. Comparison of TAS procedure (10, 12, 14, 16) with usual application of solutions (9, 11, 13, 15). Standard substances in solution: 1, librium; 2, pyramidone; 3, caffeine; 4, phenacetin; 5, salicylamide; 6, aspirin; 7, luminal; 8, butazolidine. Drugs: 9, quadronal solution (10 μg of substance); 10, 10 mg of a mixture of quadronal with 1000 times its weight of Kieselguhr; 11, thomapyrin solution; 12, analogous to 10 with thomapyrin; 13, saridon solution; 14, analogous to 10 with saridon; 15, antineuralgicum MBK solution; 16, analogous to 10 with antineuralgicum. TAS conditions: 200° C; 90 seconds; 100 mg of maize starch added to provide propellent. Thin-layer chromatographic conditions: layer, silica gel GF_{254}; solvent, benzene - ether - acetic acid - methanol (60 + 30 + 9·5 + 0·5); detection, short-wave ultraviolet light

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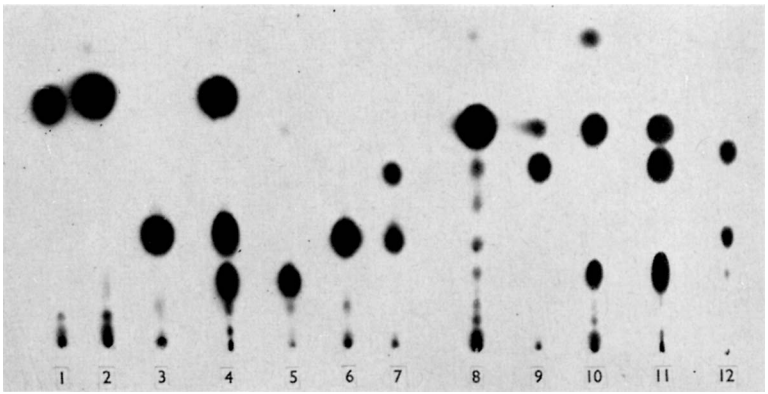

Fig. 5. Chromatogram following TAS procedure on volatile constituents of Umbelliferae fruits: **1**, fennel; **2**, aniseed; **3**, caraway; **4**, test mixture anethole (R_F 0·6), carvone (R_F 0·3), linalol (R_F 0·2); **5**, coriander; **6**, dill seed, European; **7**, dill seed, Indian; **8**, parsley, myristicin race; **9**, parsley, apiol race; **10**, parsley, allyltetramethoxybenzene (atmob), myristicin race; **11**, test mixture myristicin (R_F 0·55), apiol (R_F 0·45), atmob (R_F 0·2); **12**, test solution Desaga

TAS conditions: 250° C; 90 seconds; 10 mg of powdered drug without propellent

Thin-layer chromatographic conditions: layer, silica gel GF_{254}; solvent, benzene - chloroform (50 + 50); detection, molybdophosphoric acid (20 per cent. in ethanol) and heating

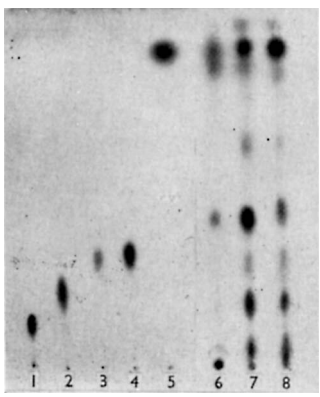

Fig. 6. Constituents of Mexican narcotic fungi following TAS procedure and thin-layer chromatography: **1**, gramine; **2**, serotonin; **3**, mescaline; **4**, tryptamine; **5**, indole; **6**, 25 mg of powdered *Lophophora williamsii*, **7** to 25 mg of powdered *lewinii*, **8** to 25 mg of powdered *lewinii* spec.

TAS conditions: 250° C, 90 seconds, without propellent

Thin-layer chromatographic conditions: layer, silica gel GF_{254}; solvent, acetone - concentrated ammonia solution (99 + 1), detection, anisaldehyde - sulphuric acid combined with molybdophosphoric acid and heating

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FOOD ADDITIVES AND RESIDUES—

Much work is being carried out at present on the analysis of residues in food, and the TAS procedure has proved useful in this field.[12] It is possible, for example, to sublime biphenyl directly from a piece of lemon peel or from wrapping paper on to the thin-layer chromatographic plate without solvent extraction, and then to identify it. Other preservatives can also be determined, *e.g.*, hexamethylenetetramine, benzoic acid, salicylic acid, boric acid, and *p*-hydroxybenzoic acid esters (Nipagin, methylparaben; Nipasol, propylparaben). Flavouring additives can be separated directly from the preparations by sublimation, for example, vanillin and coumarin from blancmange powder. Traces of insecticides, such as DDT, and herbicides, such as 2,4-dichlorophenoxyacetic acid, can be determined by this procedure. Another wide field of application is the analysis of polymers and of the plasticisers usually present in them. Valuable information is obtained by using a temperature programme from 100° to 450° C, with simultaneous stepwise movement of the thin-layer chromatographic plate. The procedure is also applicable to the detection of wood preservatives and other artificial or natural wood impregnations.

ORGANIC CHEMISTRY (PURIFICATION, IDENTIFICATION, SYNTHESIS AND STRUCTURE ELUCIDATION)—

Distillation and sublimation are simple, frequently applied purifying operations. On a micro scale they are frequently the last steps before the identification by physical methods. The apparatus described is useful in this field. It is possible, for example, to sublime the substance in the form of a uniform layer directly on to a potassium bromide disc or a quartz plate, and to obtain a spectrum.

In preliminary studies for a synthesis, thermal re-arrangements, condensations and fragmentations can be carried out on a micro scale and the products formed separated by chromatography directly following the pyrolysis. In structural elucidation the basic problem is the establishment of the skeleton and, in many instances, only the classical methods can be applied. Zinc powder distillation, alkali fusion, sulphur and selenium dehydrogenation are such classical methods which lead to the establishment of the basic skeleton. They can be carried out on the micro and ultramicro scale with the procedure described [Fig. 2 (*f*) and (g)].

INORGANIC CHEMISTRY AND GEOCHEMISTRY—

Relatively little use is made of thin-layer chromatography in geochemistry and inorganic chemistry. The TAS procedure offers new possibilities for the analysis of samples containing volatile elements of compounds. For example, the determination of elemental sulphur in rocks can be accomplished with this procedure, as well as the sublimation of sulphur from medicament powders or spraying solutions. Iodine, mercury, phosphorus, arsenic and other elements can be sublimed as can numerous salts, *e.g.*, ammonium chloride and mercury (II)chloride. The procedure can be used to advantage in geochemistry for micro determination of various organic materials, primarily for the analysis of coal, oil shale and bitumen.

These examples show that the given procedure, as well as thin-layer chromatography, already represents a versatile analytical aid in the laboratory. The TAS procedure is outstanding because of its startling simplicity and wide range of application. It will prove to be indispensable in research as well as in routine work.

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