Advances in the Field of Thermal Procedures in Direct Combination with Thin-Layer Chromatography

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During the last 15 years thin-layer chromatography (TLC) has proved of immense value to scientists in many fields of work. This useful procedure, however, is no more than a simple and rapid separation technique. The preparation of a suitable test solution can involve pulverization, solvent extraction, concentration, and application by means of a pipet, and often requires more time than the actual TLC. Less complicated, so-called on-line procedures, to transfer the components of a sample without solvent extraction directly to the thin layer, have been searched for. Microgram level distillation and sublimation in a stream of carrier gas offer one solution to this problem.

For several years we have been investigating thermal procedures and their direct coupling with TLC, and as a result we developed the so-called TAS procedure (T = thermomicro and transfer, A = application, S = substance) and thermofractography (TFG).[1-3] Figure 1 summarizes existing and future possibilities and clearly demonstrates that a gap has been filled by the TAS procedure and TFG.

The basic equipment of the TAS procedure is an electrically heated oven block with a drilled hole. The oven is preheated to a given temperature. A small amount of sample is placed in a special glass cartridge and introduced into the oven. One end of the cartridge is sealed by a silicone disk; the other end is drawn out to form a capillary which projects from the oven and points to the starting point on the layer. The thin-layer chromatographic plate is positioned 0.5 mm from the tip and can be moved easily. The volatile components pass through the capillary directly onto the thin layer and form the starting point for the chromatogram. The transfer of the volatile components from the sample to the layer takes in most cases 60–90 sec, and the TLC plate is subsequently developed under standard conditions.

The basic equipment for thermofractography was developed from the TAS oven and is called Tasomat. Contrary to the TAS procedure, the glass cartridge containing the sample is introduced into the oven block at room temperature. The oven is then heated linearly up to 450°C at a preselected heating rate. At the same time a motor moves the TLC plate slowly from right to left. This movement effects fractionated collection of the volatile components along the starting line of the TLC plate. The substances are fractionated according to their boiling or sublimation ranges. The plate is developed under standard conditons and the chromatogram obtained is called a "thermofractogram". This thermofractogram shows the substances separated by their boiling, sublimation, or decomposition range on the abscissa, and separated by their chromatographic behavior on the ordinate. The compounds distilled and sublimed from the starting mixture as well as those yielded thermolytically are registered on a single chromatogram.

The TAS procedure can be compared to the solid sample introduction in GC, but it has the advantages of an all-glass system, rapid and easy exchange of sample, and immediate transfer of the volatile substances. The advantages of the TAS procedure also apply to TFG, but TFG itself can be compared only to a limited extent to pyrolysis GC, since temperatures extend only up to 450°C. To help in better understanding of the procedures, Figure 2 illustrates the transport mechanisms.[4]

With our own interest focusing on the analysis of natural substances, drugs, and foodstuffs, the TAS procedure has been applied chiefly in these fields. Table I shows how the TAS procedure and especially TFG opens up numerous new possibilities in other diversified fields of microanalysis as well, e.g., forensic analysis, toxicological diagnosis of poisoning, criminology, chemical analysis in the field of archeometry, environmental analysis, and metabolic studies (see ref. 6–40). The extensive use of TLC

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Table I
New Applications of Thermal Separation and Application Procedures, Coupled with TLC

Separated and Chromatographed			
Group[a]	Substances	Samples	Literature
A	Analgetics, sulfonamides	Tablets	6, 7
A	Methandiene + hexachlorophene, chlorobozoic acid + salicylic acid	Ointments	6
A	Allobarbitone + aminopyrine, triple sulfonamides	Suppositories	6
A, B	Capsaicine, and others	Adhesive plasters, liniments, drugs	8
A	Phenothiazines	Various dosage forms	9
B	Various plants active substances	Drug material	10
B	Alkaloids and fragments	Plant material and pure substances	11, 12, 13
B	Anthraquinones	Plant material and pure substances	12, 14
B	Components of essential oils	Fruits, blossoms, and leaf drugs	1, 10, 15
B	Essential oils of mentha hybrids	Plant material	12, 16
B	Essential oil of satureia	Plant material	17
B	Azulene, essential oil	Chamomile flowers	18
B	Aromatics after dehydration	Sesquiterpenes, diterpenes	19
B	Fragrances	Fungi	20
B	Glycoside cleavage, picrocrocin → safranal	Saffron	21, 22
B	Naphthoquinone derivatives	Plant material	23
B	Fragmentation to phenol derivatives	Wood, lignin	24, 25
B, C	Fragmentation to phenol derivatives	Tanning drugs, leather	26
B	α-Pyrone derivatives	Plant material	22, 23, 27
B, A	Narcotics, addictive drugs	Plant material, pure substances	28, 29
C	Antioxydants (MBT, PBN)	Rubber samples	30
C	Acetylacetonates	Pure substances	31
C	Morphactines	Soil samples	32
C	Optical brighteners	Textile fabrics, detergents	33
C	Pyrolysis products of synthetic material	Synthetics	33, 34, 35
D, C	Antioxidants	Fatty oils and preparations	33, 36
C	Aromas	Toothpastes, soaps	33
D	Caffeine, and others	Foodstuffs	10, 37
D	Gaseous compounds	Carbohydrates	38
D	Additives, preservatives	Foodstuffs	39
D, C	Pesticides (DDT, HCH, pyrethrines, 2,4-D etc.)	Foodstuffs	39
D, C	Plasticisers	Synthetic materials	3, 33
D	Sugar thermolysis products	Pure substances	40

[a] Key: A, pharmaceutical preparations; B, plant drugs; C, auxiliary agents; D, foodstuffs and others

suggests that the TAS procedure may become more widely applicable than the corresponding methods of GC.

During the investigation of numerous single samples, the idea of simultaneous transfer of the volatile compounds of several samples to the TLC layer was conceived. As a result we developed the so-called multi-TAS oven, coupling 18 single ovens directly next to each other. As early as 1971 we described a prototype,[33] which in the meantime has been considerably improved. Evidently without knowing this, Šita, Chmelová-Hlavata, and Chmel[12] recently described an "instrument for the simultaneous TFG-analysis of sample and standard or the simultaneous TAS-analysis of several samples and standards". With this instrument we have already investigated thousands of individual fruits of Umbelliferae plants and discovered that the composition of the volatile components can vary from fruit to fruit—even within one and the same umbel—quantitatively and even qualitatively.[27] We also discovered new "chemical races". A further development is a large TAS oven for the preparative isolation of volatile compounds or thermolysis products. Whereas TAS cartridges used

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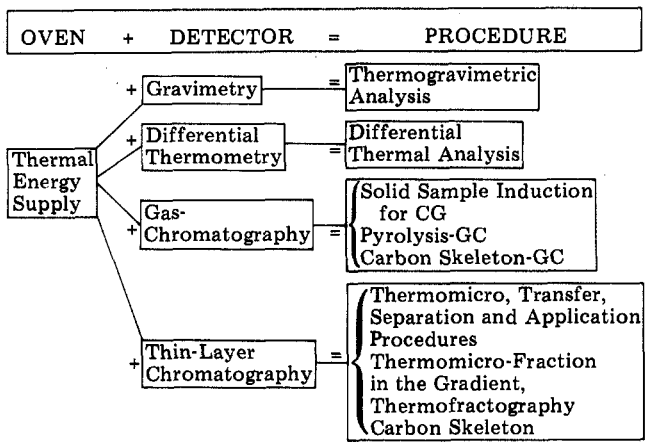

Figure 1. Thermal coupling procedures.

for analytical purposes have a volume of 2 ml, the preparative version has a volume of 20 or 2000 ml. The products evolved are retained in a special cooling trap system.[5]

We have applied TFG to lower molecular weight natural substances, such as alkaloids, amino acids, triglycerides, and other lipids. Summarizing our results, we can state that the steam-volatile tobacco, areca, and hemlock alkaloids and similar compounds can be transferred from the natural drug to the TLC layer without decomposition. The tropane alkaloids also can be transferred directly from the drug to TLC. Cinchona alkaloids may be tranferred without decomposition, but only as isolated substances and not from the natural matrix, since they are seemingly bound so strongly to acids and tanning substances that influence of heat produces only cleavage products. These fluoresce light blue like the initial alkaloids and can therefore be used as fingerprints. The substances in question are 6-methoxyquinoline and 6-methoxylepidine, derived from quinine and quinidine.

Quinoline and lepidine, derived from cinchonine and cinchonidine, have a dark blue fluorescence. A classical identity test in which the drug is pyrolyzed in a test tube to yield a red condensate of characteristic light blue fluorescence was elucidated by our investigations.[13] Studies on additional alkaloid drugs are being continued.

Simple amino acids, such as glycine, alanine, valine, leucine, and proline, are transferred without decomposition to the TLC layer in a stream of nitrogen at 220°C. Fragmentation occurs only if additional functional groups are present. These reactions are presently being investigated.[5] Preservatives, pesticides, and fragrances have been separated from lipid mixtures in the temperature range of 200–250°C by the TAS procedure,[33] but TFG has made it possible to transfer the triglycerides also without decomposition in the 325–400°C range. TFG analysis of fatty

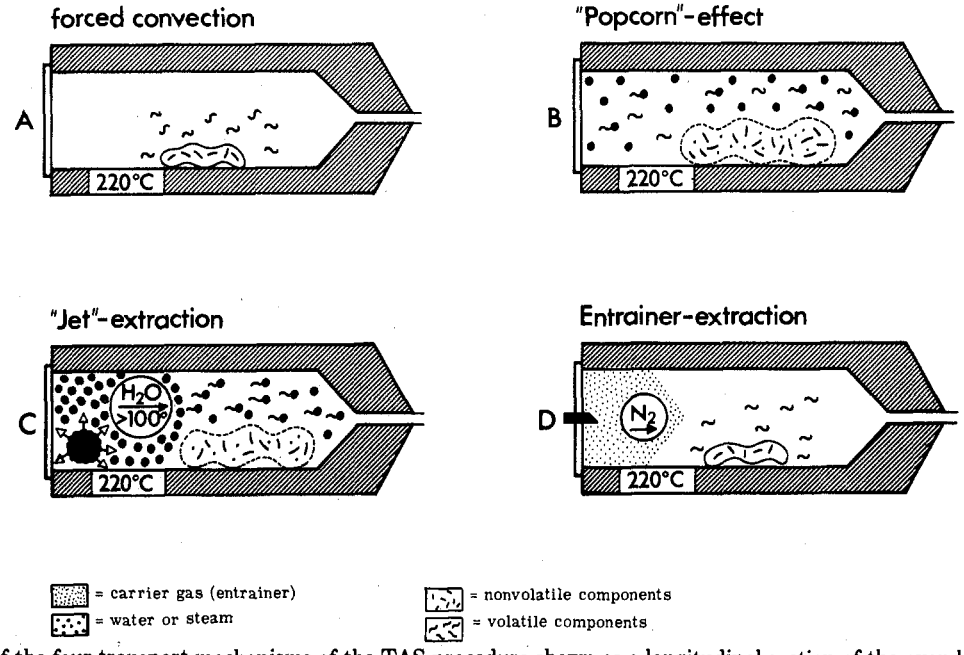

Figure 2. Diagram of the four transport mechanisms of the TAS procedure shown as a longitudinal section of the oven block with loaded cartridge. (A) The transport of the components of water- and solvent-free samples is achieved by the increase of their vapor pressure and by the expansion of the air volume by, e.g., 50% at 220°C in the cartridge. Both effects of this "forced convection" cause only a very small stream of vapor to be directed onto the TLC layer, and thus "thermoextraction" is incomplete and the yields are low. (B) Yields increase if the sample contains water, e.g., 10% in natural drugs. When heating rapidly, the sample puffs, the surface becomes enlarged and porous (popcorn effect), and the vapor entrains the volatile components, thus considerably improving the transport to the start point of the TLC layer. The process can be compared to a "steam distillation", even though the water content is mostly too low and the evaporation too fast. (C) Yields are increased further by adding so-called "propellants". While in the oven they release water which heats up on the hot walls of the TAS cartridge, effecting so-called "distillation with superheated steam". Thermogravimetric investigations proved that a spherical 4-Å molecular sieve charged with 25% of water yields the optimal amount of vapor. Suitable basic and acid propellants were also tested. Hexaamminenickel(II) chloride releases 6 mol of ammonia in two steps up to 350°C, oxalic acid up to 210°C releases a stream of CO_2, CO, and water vapor, while malonic acid up to 210°C develops a stream of CO_2–acetic acid. (D) The transport of the molecules evaporated from the sample can also be accomplished by means of a gas stream admitted from the outside. Nitrogen or preferably helium is used as an inert gas. This carrier gas distillation or sublimation is the most suitable procedure in TFG. Using this, even substances with a high boiling range, e.g., fatty oils, can be transferred without decomposition to the TLC layer. The effect is comparable to that of a vacuum distillation at below 0.1 Torr.[5]

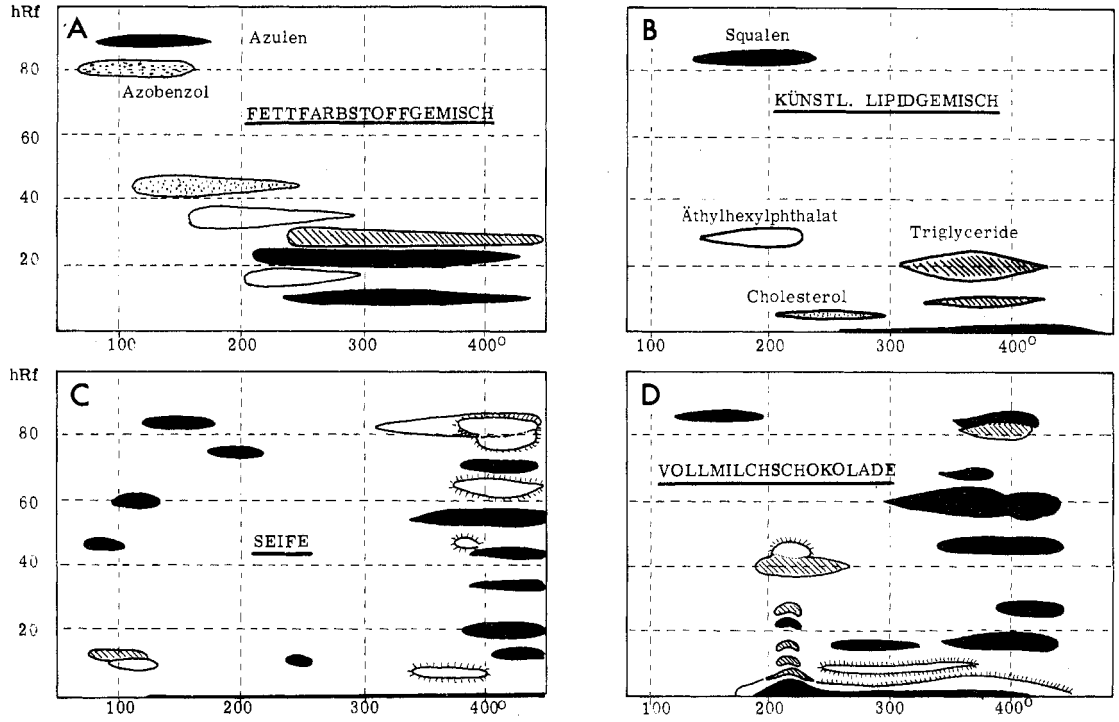

Figure 3. (A) Mixture of fat dyes (azobenzene, azulene); (B) artificial mixture of lipids (squalene, ethyl hexyl phthalate, cholesterol, triglycerides); (C) soap; (D) whole milk chocolate.

oils showed that fractional distillation is possible also in the ultramicro range. Squalene, cholesterol, plasticizers, and a series of nonidentifiable products can be distinguished in this way, thus opening up new possibilities for the analysis of soaps, chocolates, detergents, and numerous other lipid preparations.[3,5] Figure 3 shows the corresponding thermofractograms.

Because of their extremely high polarity, numerous high molecular weight natural and synthetic substances cannot be analyzed by chromatography. However, by reproducible degradation to defined lower molecular weight compounds, the starting material can be identified by a "structural unit analysis". The best procedures are thermolysis (up to 500°C) and pyrolysis (500–1200°C).[38] Thermolysis combined with TLC is the most suitable procedure since only very small amounts of gaseous products and large amounts of condensable products are yielded in the lower temperature range. Of the numerous substances considered here, the groups of natural tanning agents, lignins, and the presently most important plastics have been selected for TFG. In TFG the free di- and triphenols are transferred without decomposition to the TLC layer while the tanning agents based on them are fragmented in a reproducible and hence characteristic way.[26] Tanning agents of the gallotannin type stand out through an almost complete absence of the catechol and resorcinol zones. These two are the main zones in catechin tanning agents. The generally strongly defined pyrogallol zone is a common characteristic of both tanning agent groups. Based on these chromatographically identifiable structural units, classification of the kind of linkage can be made. Further indications are that hydrogen bonds may be present. Thermofractograms of tanning agents provided significant information about the "phlobaphenes" and their structure when compared to the extracts and extracted drugs. A type of "fine structure analysis" of the TFG of different extracts from tanning agents shows relatively large differences in the structure of the tanning agents. Experiments with model untreated and treated leathers proved it possible to detect and identify a vegetable tanning agent through typical phenol zones, even if the leather was pretanned, pretreated, and dyed with chromium salts.

The auxiliaries of the preparation also appear in the thermofractogram, and sample amounts of only 2–5 mg are needed for analysis. The method should thus be useful for the rapid analysis and criminological comparison of leather samples as well as for the food chemist in investigating consumer leather goods.

Lignins are divided into three major groups:[24] softwood lignins consisting mainly of guaiacylpropane units; hardwood lignins containing both guaiacyl- and syringylpropane units, the amount of the latter varying from species to species; and grass lignins containing all three, guaiacyl-, syringyl-, and *p*-hydroxyphenylpropane units. The analytical procedures which have hitherto been used for characterization and classification are time consuming, require larger amounts of sample, and are only of limited diagnostic value. Preliminary experiments with model substances from the Freudenberg school indicated that thermal fragmentation in TFG leads to defined substances. The chromatogram of the lignins investigated showed as common structural units (fingerprint substances) guaiacol, 4-vinylguaiacol, vanillin, coniferylaldehyde, and coniferyl alcohol.

Additional syringyl derivatives, e.g., 4-vinylsyringyl, sinapinaldehyde, and sinapin alcohol, are observed in the thermofractograms of angiosperm-lignins, and possibilities of a further differentiation are interesting. The TFG procedure allowed us to clarify the controversial question whether the semiparasite, mistletoe, synthesizes the lignin of the host tree or its

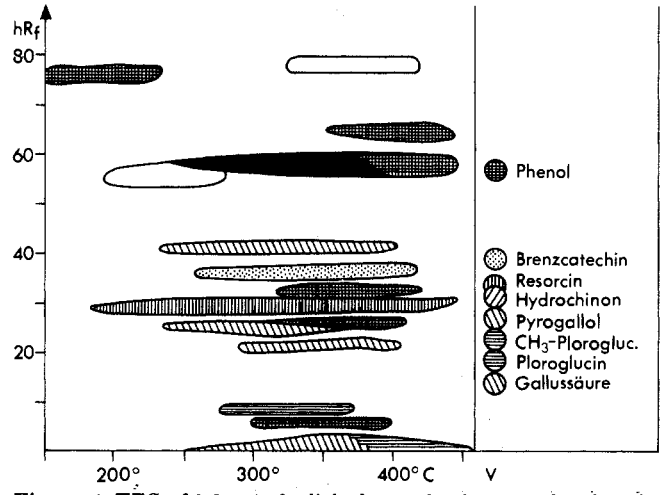

Figure 4. TFG of 5.2 mg of a light brown leather sample: phenol, catechol, resorcinol, hydroquinone, pyrogallol, methylphloroglucinol, phloroglucinol, and gallic acid (from top to bottom).

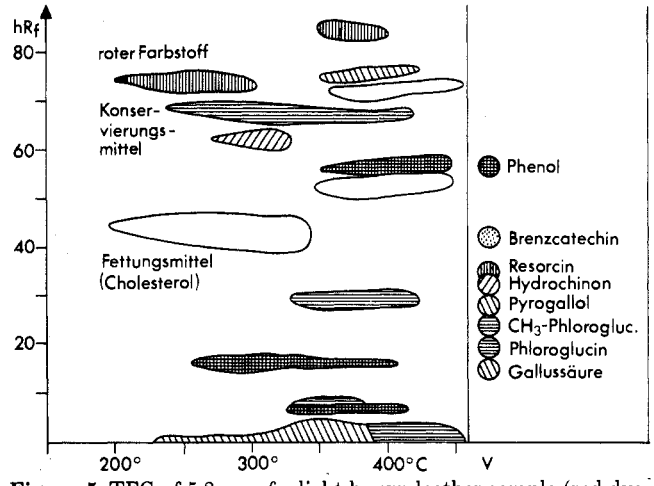

Figure 5. TFG of 5.2 mg of a light brown leather sample (red dye, preservative, and fat liquor (cholesterol) listed in descending order on the left; phenol, catechol, resorcinol, hydroquinone, pyrogallol, methylphloroglucinol, phloroglucinol, and gallic acid on the right).

own. The latter is the case. This new kind of rapid lignin analysis will also facilitate investigation of the dissemination of lignins and discover new types of lignins throughout nature.

The question arose whether the applied conditions of thermolysis lead to secondary reactions as in mass spectrometry. The problem was examined in an additional experiment[25] on the formation of coniferylaldehyde and coniferyl alcohol. TFG of defined model substances (dilignols and artificial polymer mixtures) and spruce lignin as well as supplementary experiments demonstrated that the free and/or preformed aldehyde and alcohol groups of the structural units of lignin remain practically unchanged under the conditions of TFG. The procedure has the advantage that the fragmentation products yielded are diluted immediately with the inert gas, transferred to the cool TLC layer, and fixed there until separation. Experiments on the TFG of carbohydrates and the identification of their pyrolysis products[3,5,38] have found practical application in the investigation of mixed textile fabrics.

A TFG is carried out for preliminary orientation, i.e., group analysis of polycondensates.[35] Based on this thermofractogram it can be determined whether the polycondensate belongs to the group of the polyamides of the nylon or perlon type, or whether a polyester or polyurethane is present. For "fine structure analysis" an alkali fusion in a nickel boat is carried out in the TAS cartridge, and the corresponding bases and neutral compounds are chromatographed following the direct transfer. To analyze the salt-forming compounds, the sample is acidified and the free glycols and acids are carried over and chromatographed. The different polycondensates can thus be analytically distinguished. Further, minor modifications in the polymer chains may be recognized, something which has hitherto not been possible by means of chemical analysis.

Possibilities of rapid analysis of phenolic resins and vinyl polymers (polystyrenes, acrylates) by TFG are at present under investigation. We can already state that a differentiated analysis with very small amounts of sample is possible. Figure 6 shows the thermofractogram of 5 mg of a technical grade 4-*tert*-

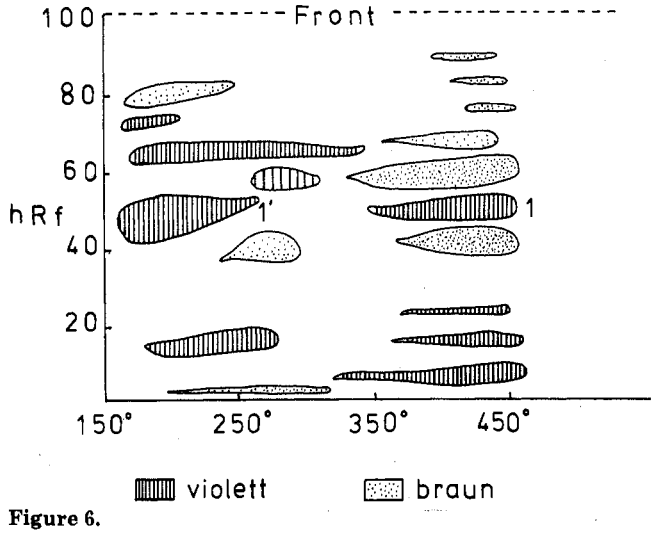

Figure 6.

butylphenol–formaldehyde resin. The free phenols appear in the range of 200–300°C and the fragmentation products of the condensate (1 and 1′ = 4-*tert*-butylphenol) in the 350–450°C range. Fast blue salt was used as detecting agent. The results in our opinion are much more conclusive than those obtained with the thermal analytical procedures hitherto used.

Natural products chemists still utilize the classical thermal methods for establishing the skeleton in structural elucidation of natural substances. However, the small amounts of sample available often prevent application of these procedures. The idea of combining classical thermal procedures directly with TLC was conceived when first testing the TAS procedure.[2] A parallel in GC is the "carbon skeleton chromatography" of Beroza et al.,[41] which we realized with our own instruments using the TAS cartridge as reaction vessel and the TAS oven as source of heat. Detailed investigations showed that the zinc dust distillation with 20–200 μg of starting material can be rapidly and easily performed under controlled conditions, and can be coupled with TLC. The sample is heated in the TAS cartridge on copper-activated zinc

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Table II
Directive Values for "Thermal Reactions and Dehydrogenations" Coupled with TLC[a]

	Sample, μg	Reaction partner or catalyst	Temp, °C
Zinc dust distillation	5–50	200–300 mg of Cu-activated zinc dust	350–450
Sulfur dehydrogenation	50–100	10 mg of S-dehydrogenation mixture	160–220
Selenium dehydrogenation	100–200	20–30 mg of Se-dehydrogenation mixture	250–320
Catalytic dehydrogenation	20–100	25 mg of Pd–$BaSO_4$ (10%)	250–350

[a] The optimal conditions in the given ranges vary from substance to substance.

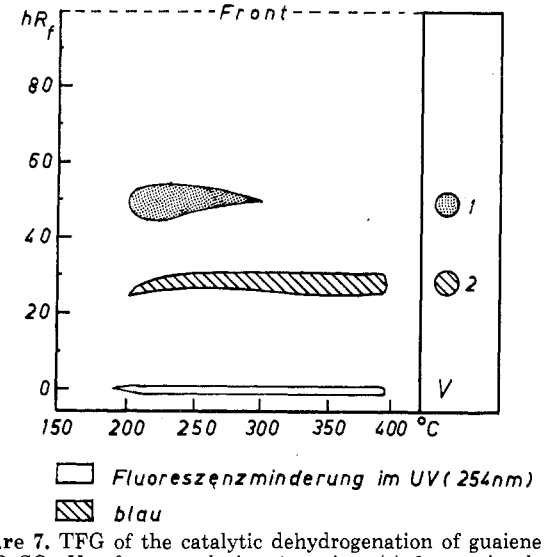

Figure 7. TFG of the catalytic dehydrogenation of guaiene with Pd–$BaSO_4$: V, reference solution; 1, guaiene (s); 2, *s*-guaiazulene

for several minutes up to 350–450°C and the evolving oxygen-free aromatic or stable heterocyclic components are carried by a stream of nitgrogen directly on to the starting point of a TLC plate. The reaction products are then identified after chromatography. The optimal temperature ranges were determined by thermofractography, and they depend on the compound class.

The procedure was tested on naphthalene, anthracene, phenanthrene, tetracene, and indole derivatives.[14] Further, it has been shown that a coupled dehydration and sulfur or selenium dehydrogenation can be carried out with 50–300 μg of starting material in direct combination with TLC. The sample together with a potassium hydrogen sulfate, sulfur, or selenium mixture is heated up to the preselected temperature of between 160 and 400°C for several minutes in the TAS cartridge. The resulting aromatic compounds are transferred by a stream of nitrogen to the starting point of the TLC plate. The reaction products are identified and in part quantitatively determined after chromatography. The procedure was tested on samples of the sesquiterpene, diterpene, and steroid groups.[19]

We also investigated catalytic dehydrogenation in the microgram range. Palladium on barium sulfate or calcium carbonate was found to be especially useful as catalyst. The reaction takes place in the gas phase. In contrast to the previously mentioned procedures the highest yields are gained if the dissolved substance is squirted in very small portions on to the catalyst.[5,42] A special attachment is needed to inject the solution of the compound to be dehydrogenated in batches into the TAS cartridge. Results obtained are much more precise than those obtained with the sulfur or selenium dehydrogenation.

Investigation of the gaseous products of thermal separation and thermolysis procedures proved the amounts to be relatively small compared to the condensable products. Details and results compared to those of thermogravimetric analysis and the inductor–GC system are described and listed in the original paper.[38] Additional experiments with natural and synthetic substances have begun, and are expected to bring conclusive information.

(42) E. Stahl and Th. K. B. Müller, *Z. Anal. Chem.*, **274**, 257 (1975).