

TABLE II

ANALYSIS OF COMMERCIAL STEROID OIL SOLUTIONS

Preparation No.	Progesterone 10 mg labelled	Testosterone propionate 15 mg labelled
	mg found	mg found
1	9.45	14.23
2	9.47	14.90
3	9.53	14.75

Work on the quantification of other steroid mixtures after thin-layer chromatographic separation is being continued in this laboratory.

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Institute for the Control of Drugs,
Zagreb (Yugoslavia)*

T. BIČAN-FIŠTER

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* Director: V. KUŠEVIĆ, Ph. D.

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A sensitive non-specific localization test for thin-layer chromatography

During the chromatography of unknown mixtures considerable time and effort can be saved if the total number of components and their R_F values can be determined immediately.

This requires a spotting or localization agent capable of reacting with a large variety of compounds of different chemical structure.

Once the number and R_F values of all the constituents are determined, more specific localization and identification tests can be applied.

Among the spotting agents of wide applicability, the sulfuric acid test, the chlorine-*p*-toluidine test (ref. 1, Test No. 32) and the iodine vapor test (ref. 1, Test No. 72 and No. 73) are the most widely used.

The sulfuric acid test requires a highly corrosive spray and if used as a 50% aqueous solution as usually recommended, will often give a very non-uniform droplet size thus capable of damaging the thin layer of adsorbent.

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We find, furthermore, that with certain classes of compounds both tests exhibit poor sensitivity. The iodine test is sensitive but the spots fade very rapidly.

Convenient and sensitive detection is accomplished by exposing the dried and developed chromatogram to an atmosphere of ozone at periods up to 30 min, driving off excess ozone with a gentle current of air and then detecting the oxidation products (hydroperoxides, aldehydes, ketones, etc.) by well-known spotting sprays. Among

TABLE I

SENSITIVITY OF VARIOUS ORGANIC COMPOUNDS TO OZONE TEST

Spotting agent: aqueous 2,4-dinitrophenylhydrazine

Compound	2.5 γ^*	5 γ^*	10 γ^*	Notes
<i>Aromatics</i>				
<i>n</i> -Decylbenzene	+	++	++	
Chlorobenzene	—	—	—	
Anthracene	+	++	+++	
Naphthalene	+	++	+++	
2,4-Dichlorobenzene	—	—	+—	
<i>Alcohols</i>				
<i>n</i> -Decyl alcohol	+—	+—	+	
2-Methylcyclohexanol	+—	+	+	
Pentaerythritol	+	+	+	
Glycerol	+—	+	++	
<i>Amines</i>				
Aniline	+	++	+++	
Cyclohexylamine	+	++	+++	
Pyridine	+—	++	+++	
Dodecylamine	+	++	+++	
<i>Olefins</i>				
Styrene	+	+	++	
Oleic acid	+	+	++	
<i>Saturated hydrocarbons</i>				
Tetradecane	—	—	+—	Ozone treated at 55°: + at 20 γ
Pentadecane	—	—	+—	
Decalin	—	—	+—	
Stearic acid	—	—	+—	
<i>Ether, polyether</i>				
Dipropylene glycol	—	—	+—	+ at 20 γ
1,2-Diethoxyethane	—	—	+—	
Ethoxylated alfol (59.5% ethylene oxide)	—	—	+—	
<i>Amides</i>				
RCONH-CH ₂ CH ₂ OH	+—	+	+	++ at 20 γ
<i>Oxazolines</i>				
$ \begin{array}{c} \text{N}-\text{CH}_2 \\ \parallel \quad \\ \text{R}-\text{C} \quad \text{CH}_2 \\ \quad \quad \quad \\ \quad \quad \quad \text{O} \end{array} $	+	++	++	+++ at 20 γ

* +— = perceptible; + = clear spot; ++ = strong spot; +++ = very strong spot.

these an acid aqueous solution of 2,4-dinitrophenylhydrazine or an ethanolic solution of phosphomolybdic acid (ref. 1, Test No. 120) gives very satisfactory results.

Very inert compounds such as paraffinic hydrocarbons or aliphatic amides are exposed to the ozone atmosphere at 55-90° instead of room temperature and will be easily detected.

A large variety of chemical classes were tested by this procedure and approximate sensitivities determined. The data are presented in Table I.

Of particular interest is the high sensitivity to aromatic compounds and this should find many applications in the field of air and water pollution.

The only prior reference to the use of ozone for spot localization is that of KAUFMANN AND NITSCH² where it was used to oxidize primary alcohols to acids followed by identification of the latter by the copper salt-potassium ferricyanide test.

Limitations of the method

Since ozone will oxidize almost any organic compound, it is extremely important to drive off the developing solvent before the ozone exposure, so as to avoid a colored background after the hydrazine or phosphomolybdic acid spray.

Since some ozone remains adsorbed, a better background is obtained if the carbonyl detection agent (such as the hydrazine) is used in aqueous solution instead of an ethanolic solution. Since ethanol will be oxidized by the adsorbed ozone to acetaldehyde, a yellow background will obscure the spots of the compounds to be detected. This is less important with the phosphomolybdic acid spray since the blue spots are easily detected on a white or yellow background.

The ozone test can be combined with other more specific tests, such as the ninhydrin test for amines or amino acids, by first carrying out the specific test and marking R_F and spot size of the detected compounds, followed by the usual procedure for the ozone exposure. The areas known to contain the less reactive compounds have to be protected from the ninhydrin by covering it with a sheet of aluminum foil or plastic.

Sensitivities

Sensitivities vary with the structure of the compound to be detected.

Within a class of compounds itself sensitivity can also vary, though generally on a somewhat lower scale. Thus aromatic compounds are easily detected with polycyclics being more sensitive than alkylbenzenes and these in turn are more sensitive than halogenated aromatics. This is most probably due to the fact that the color is produced by ozonide formation and a larger concentration of ozonide could be achieved with the former compounds.

The best sensitivities are obtained with aromatics and amines (0.5-5 μg) followed by olefins (2.5 μg), alcohols (2.5-5 μg), polyether alcohols (10-20 μg) and saturated hydrocarbons (10-20 μg).

These sensitivities refer to the 2,4-dinitrophenylhydrazine test. From the limited amount of data on the phosphomolybdic acid test it appears that the latter is much more sensitive: alcohols and ethoxylated alcohols can be detected to within 1 μg .

Experimental

A 0.25 mm Silica Gel G (Desaga) layer applied with the Desaga template and

spreader* is used in all experiments. 10 mm³ solution of the appropriate concentration is applied to provide spots containing from 0.5 to 50 µg of material to the plate. (The plates were previously activated for 1 h at 110° and stored in a desiccator over P₂O₅.)

The plates are then exposed to an atmosphere of ozone (2-4 % ozone in air) for 30 min at the required temperature. A rectangular developing tank (30 × 26 × 6 cm) with a ground glass top is provided with an aluminum cover-plate. This metal plate is provided with an ozone inlet tube extending to the tank bottom and an exit tube leading to a water bubbler. Ozone is generated from a commercial instrument (Welshbach, Model T-23). All lines are made of Tygon. The unreacted ozone is led away from the bubbler into the hood exhaust chimney. Another convenient container is available commercially*.

When a higher reaction temperature is desired the tank is immersed in a thermostated oil bath.

After the ozone exposure the plate is placed under a gentle air stream, then sprayed with a 0.4 % of 2,4-dinitrophenylhydrazine (0.4 % aqueous solution in 2 N HCl) or a 5 % ethanolic solution of phosphomolybdic acid. The first reagent gives yellow or orange spots on a white or light yellow background while the second reagent gives blue-black spots on a light yellow background.

The sensitivities thus determined are somewhat better than those obtained after actual development but will serve as a very good guide in the choice of concentrations required for practical separations.

Allied Chemical Corporation, Central Research Laboratory,
Morristown, N.J. (U.S.A.)

R. FUHRMANN
D. JERALOMON

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H. P. KAUFMANN AND H. G. KOHLMAYER, *Fette, Seifen, Anstrichmittel*, 57 (1955) 231.

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* Desaga standard developing tank with controlled atmosphere cover. Brinkmann Instruments Catalogues Nos. 25-10-20 and 25-10-29, respectively. Brinkmann Instruments, Inc., 115 Cutter Mill Road, Great Neck, Long Island, N.Y.

An apparatus for quantitative application of samples as streaks in thin-layer chromatography

The use of thin-layer chromatography for quantitative analysis of chemical mixtures has been described recently and the merits of various techniques in sample application discussed¹. One problem has been the transfer of enough material to the chromatoplate to allow analysis of the separated components. This has been overcome