

CHROM. 3487

Thin layer chromatography of aldehydes

During the past 10 years much work has been done on the isolation, separation and identification of volatile mono-, di- and oxycarbonyl compounds. There is a real need for a specific and objective method of determining the carbonyls produced as a result of the decomposition of fat and fatty tissue. A number of investigators have reported a method of identification of carbonyls, mostly as DNPH derivatives. There is column chromatography employing different adsorbents, such as polyethylene¹ or magnesia², but also column partition systems are described in which the stationary phase is ethanolamine³ or acetonitrile⁴. DE VRIES used silver nitrate on silica gel as adsorbent⁵. Only SCHWARTZ³ separating keto aldehydes and 2,3-diketones mentioned the different colours (blue-grey-violet).

Paper chromatography is described by RICE⁶ employing normal filter paper and after development and drying, sprayed with a 10% solution of potassium hydroxide in water. Filter paper impregnated with propylene glycol was introduced by ELLIS⁷⁻⁹. In addition there are investigators working on thin layer chromatography. Silica gel impregnated with 3% polyvinyl alcohol¹⁰; alumina with silver nitrate¹¹; a mixture of magnesia and Celite¹²; activated alumina¹³; and silica gel impregnated with 2-phenoxyethanol or *n*-undecane¹⁴ are claimed to give the best results. Besides the method of MOOKHERJEE¹⁵ employing a magnesia-Celite column to separate mixtures into 4 classes of carbonyl compounds, each with a different colour, there is not one method described in which specific and different colours are developed indicating single compounds.

A very good comprehensive review: "Identifying and estimating of aldehydes and ketones" was published by the British Drug Houses Ltd., leaflet DPH/9/6501.

Materials

- Benzene (Merck 1781)
- Heptane (Merck 4365)
- 1,2-Dichloroethane (Merck 955)
- Sodium hydroxide (Merck 6498)
- Silica Gel G (Merck 7731)
- Carbowax 4000 (Merck 9727)
- 2,4-Dinitrophenylhydrazine (BDH 28234)
- Tinopal WG (Geigy)

Experimental

Using a home-made thin-layer applicator, glass plates are coated with a layer (thickness 0.3 mm) of a well stirred air-free suspension of 10 g Silica Gel G and a solution of 3 g Carbowax 4000 and 35 mg Tinopal WG in 15 ml distilled water and 5 ml 1 *N* NaOH. The plates were dried at 80° for 30 min. On preparing the 2,4-DNPH compounds, we found the method described by JONES¹⁶, using 2 *N* hydrochloric acid as solvent, very useful. The hydrazones were dissolved in 1,2-dichloroethane and applied with a micropipette on the starting points, 1½ cm from the end of the plates.

The plate was then placed on its side in the chamber for development. The solvent system was a mixture of 65 ml benzene and 35 ml heptane.

Usually 40 min were required for the solvent front to cover a distance of 12 cm above the start line. Then the plates were air dried. The compounds showed up as coloured areas on a white background, some of these change colour within a few hours. Very small quantities can be detected under U.V. light using a Blacklight lamp, Philips type HPW.

The nature of the different colours and the discolouration is not known. However it is thought to be due to the combination polyglycol-'OH-silica gel. Discolouration by alkaline reagents such as ammonia vapour or alcoholic solutions of potassium hydroxide is already known: the yellow spots become red, or blueish in the case of keto aldehydes. Omitting one of the compounds results in lack of colour formation. The colour formation is surprisingly specific for single compounds; for instance compare the dialdehydes, or the hydroxy aldehydes or the cyclic aldehydes within a homologous series there are different colours. We have investigated our combination very extensively; one may alter the quantity of each compound within wide ranges, also the molecular weight of the polyglycol need not be fixed and may be chosen between 350 and 10 000. There is only a very slight influence on the colours but the R_F values change completely. Our combination seems to give optimum results. In addition, the origin of the polyglycol and its purity have no influence on the results obtained. Technical polyglycol (ex Union Carbide) gives nearly equal R_F values and colours.

The temperature of drying and activation is very important. Higher temperatures increase the R_F values but decrease the separation of the successive compounds. Higher temperatures also have a remarkable effect on the colours, for instance:

TABLE I
 R_F VALUES AND COLOURS OF CARBONYL COMPOUNDS

Compounds	R_F	Immediate colour	Colour after 6 hours	Sensitivity γ
Formaldehyde	0.37	yellow	dark reddish brown	0.2
Acetaldehyde	0.52	yellow	ochre	1.0
Propionaldehyde	0.63	yellow	ochre	0.5
Butyraldehyde	0.69	yellow	brown	0.5
Valeraldehyde	0.75	yellow	brown	0.5
Caproaldehyde	0.81	yellow	brown	0.5
Oenenthal	0.85	yellow	brown	0.5
Acrolein dimer	0.36	yellow	reddish brown	0.2
Crotonaldehyde	0.57	yellow	greyish brown	0.2
Glyoxal	0.02	pink	purple	2.0
Malonaldehyde	0.29	very faint yellow	vivid orange-brown	1.0
Glutaraldehyde	0.07-0.14	yellow	dark green	0.2
Glyceraldehyde	0.10	blue	lilac	5.0
Pyruvaldehyde	0.08; 0.15; 0.36	red; blue; red	red; yellow; pink	2.0
2-Hydroxyadipaldehyde	0.00	brown	dark brown	0.5
Salicylaldehyde	0.04	brick-red	faint brown	0.5
Terephthalaldehyde	0.15	faint brown	purple	1.0
Cinnamaldehyde	0.46; 0.66	yellow	yellow	5.0
Furfural	0.25; 0.49	purple	dark red	0.5
Acetone	0.73	blue	yellow	2.0

at 150° formaldehyde turns green and at 80° yellow-brown; at 150° crotonaldehyde turns orange-brown and at 80° greyish brown.

The R_F value combined with the colour is absolutely specific for each single compound. Compounds with nearly equal R_F values can now be differentiated in one single run. Compare glutaraldehyde-glyceraldehyde and ethanal-crotonaldehyde and many others.

We used this method for determining aldehydes in leather in which only formaldehyde or glutaraldehyde are used on a commercial scale. But of scientific interest are malonaldehyde, glyoxal, glyceraldehyde, acrolein, crotonaldehyde and furfural; some of them are claimed as tanning agents (U.S. Patents 2009255, 2976111 and 3093440) or are formed *in situ* during tanning¹⁷⁻¹⁹.

The R_F values, colours and the sensitivity of the compounds investigated by us are listed in Table I.

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