

The system used proved satisfactory in most respects. Two-dimensional chromatography using the following solvent combinations could be used to separate mixtures of phenolics: A-C, B-C, D-C, and D-B. Solvent C proved to be unsatisfactory for use as the first solvent in a two-dimensional system.

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A spray reagent for methylated phenolic compounds on thin-layer plates

Phosphomolybdic acid is a nonspecific reagent which gives blue spots with phenolic compounds and their methyl ethers¹ and also with lipids² on thin-layer plates. In order to develop a specific spray reagent for some classes of methylated phenols, use has been made of the characteristic colour reaction of certain phenolic methyl ethers when these are dissolved in conc. HNO₃ (ref. 3). Examination of conc. HNO₃ as a spray reagent with various types of phenolic methyl ethers has shown its suitability as a sensitive spray reagent on thin-layer chromatograms of Silica Gel G (E. Merck). The results are detailed in Table I.

TABLE I

COLOUR DETECTION OF PHENOLIC METHYL ETHERS

The sensitivity in each case was 5 µg.

<i>Sample No.</i>	<i>Name of the compound</i>	<i>Colour produced</i>	<i>Time taken for colour development</i>
1	Phloroglucinol trimethyl ether	Deep blue	Immediately
2	Resorcinol dimethyl ether	Green	Immediately
3	Tetra-O-methyl-ellagic acid	Light blue	Immediately
4	Vanillin	Deep yellow	Immediately
5	Ferulic acid	Deep yellow	2 min
6	Vanillic acid	Yellow	5 min

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Fully methylated ethers of pyrogallol, catechol and gallic acid produce no specific colour even when present in higher concentration ($> 10 \mu\text{g}$); only faint dirty yellow spots are produced (due to nitric acid). This spray reagent does not give any colour with nonmethylated phenolic compounds.

The study of fully and partially methylated derivatives of phenolic compounds is of great importance in the determination of the structure of flavonoid compounds and condensed tannins. From the results, it appears that those compounds with a free methine ($=\text{CH}-$) group between two methoxyl groups or having an activating group such as carbonyl or else unsaturation in their structures produce characteristic colours, whereas methyl ethers of vicinal trihydroxy phenolic compounds do not produce characteristic colours. Due to a lack of authentic samples, we have not tested for reactions with methylated derivatives of flavonoids (esp. flavanones), but flavonoids containing a carbonyl group are likely to give characteristic colour reactions.

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