

A NEW METHOD FOR THE IDENTIFICATION OF SMALL QUANTITIES OF NICOTINIC ACID AND ITS ESTERS

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The colour reaction of nicotinic acid and its esters with epichlorhydrin is developed as a method for identification of these bodies.

THE PRINCIPAL method for the identification of nicotinic acid has hitherto been based upon the cyanogen bromide reaction of König.¹ In this, nicotinic acid and cyanogen bromide react to give glutaconaldehyde, which gives coloured complexes with aromatic amines. This method has been applied by Huebner² for the separation and identification of nicotinic acid by paper chromatography in quantities down to 5 μ g. This author's method has recently been applied in this laboratory to identify successfully both nicotinic acid and its tetrahydrofurfuryl ester in the presence of each other.

The highly toxic and unstable character of cyanogen bromide, however, prompted a search for a simple, stable and innocuous reagent specific for nicotinic acid and its esters. This report records the successful application of epichlorhydrin



for this purpose. Melzer³ employed this reagent for the identification of nicotine itself. Prolonged boiling of the alkaloid in dilute aqueous solution with epichlorhydrin gave an orange-yellow solution with as little as 0.25 mg. of alkaloid.

The present work shows, however, that nicotinic acid and certain of its esters will also react with this reagent, on strong heating, to give a lemon-yellow colour in ordinary light. Such traces, when absorbed on filter paper, will give well defined spots of brilliant blue-green fluorescence under ultra-violet light.

Chromatograms were therefore prepared in the following way. Spots of alcoholic solutions of nicotinic acid and some of its esters were applied to sheets of Whatman No. 1 chromatographic paper and allowed to dry in air at room temperature. The sheets were then developed by upward displacement with *n*-butanol saturated with 1.5 *N* aqueous ammonia (Huebner, loc. cit.) for 1–2 hours. They were then dried in air at room temperature and sprayed with a solution of 1 volume epichlorhydrin in 4 volumes acetone. Finally, they were dried in air over a hotplate at maximum temperature for 3–5 minutes. Subsequent irradiation by ultra-violet light shows that 0.5 μ g. of nicotinic acid may readily be detected by this method, and quantities down to 5 μ g. of tetrahydrofurfuryl-, methyl- and *n*-heptyl nicotines have

also been located. R_F values for these esters using this solvent, however, are very close together, and work continues in search of a suitable solvent for their wider separation. The R_F values for nicotinic acid and tetrahydrofurfuryl nicotinate obtained by this method are compared below with those obtained by the cyanogen bromide/benzidine method of Huebner.

	<i>Huebner Method.</i>	<i>Present Method.</i>
Nicotinic Acid	0.19	0.23
Tetrahydrofurfuryl Nicotinate	0.95	0.94

It may be noted that such chromatograms of nicotinic acid continue to exhibit fluorescence in ultra-violet light even 20 days after their preparation.

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