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## Technique for the transfer of alkaloids from thin-layer plates to potassium bromide discs for spectroscopy

A number of methods have been devised for the determination of the infrared spectra of substances separated on thin layers.

McCOY AND FIEBIG[1] used a modified Pasteur pipette to collect the eluted compound which was transferred to a liquid medium for spectroscopy. RICE[2] preferred the use of potassium bromide discs and developed a method for the direct removal of test compound from the thin layer to a small amount of potassium bromide by washing the separated spots with an appropriate solvent. KLEIN[3] improved the method using a wall of potassium bromide to absorb the eluted compound. The use of a porous potassium bromide wedge was suggested by KROHMER AND KEMMER[4], the unknown compound being eluted from the support into the tip of the wedge which was used to form potassium bromide discs. GARNER AND PACKER[5] preferred a pressed potassium bromide triangle supported on a Wick-Stick. The solvent, climbing the Wick-Stick by capillary action, is preferentially evaporated from the apex of the potassium bromide triangle which is free of silica because the adsorbent is effectively filtered in the lower part of the triangle. STAHL AND SCHILD[6] employed a narrow column packed with potassium bromide into which the adsorbed compound was introduced by ascending elution. AMOS[7] commenting on earlier methods, referred to problems of contamination due to unclean apparatus and silica and proposed an alternative method employing a syringe, the eluting solvent being forced through the adsorbent and a layer of potassium bromide before incorporation in the final potassium bromide for disc preparation. This method minimised the contamination of the final disc with silica gel fines. GOODMAN[8] used twin plates of silica gel and potassium bromide, the test substance being transferred from the silica gel layer to the potassium bromide layer from which the discs were prepared.

The load of substances separated from plant extracts on thin-layer plates is necessarily low and it is essential to effect maximal extraction of the substances from the adsorbent and to employ microdiscs of potassium bromide for infrared spectroscopy. If reproducible results are to be obtained, such discs must be silica-free and, in our experience, any method involving removal by scraping from glass surfaces produces silica contamination.

Having failed to obtain satisfactory results by the available earlier methods, we have devised an alternative method which has enabled us to recover *Rauwolfia* alkaloids from thin layers and to produce satisfactory infrared spectrograms.

### *Method*

Approximately 100-$\mu$g load of alkaloids was applied to the baseline of the silica gel thin-layer chromatographic plate as a row of spots (1–5 $\mu$l volume). After development and drying of the plate, the separated spots were removed using the micro-vacuum cleaner technique[9].

A drawn glass tube, A, 15 cm length and 0.5 cm diameter (Fig. 1), was plugged at the tapering end with methanol-washed glass wool. The adsorbent and adsorbed alkaloid collected in the micro-vacuum cleaner was transferred to the drawn tube using a

small glass funnel. The tube was closed with a further plug of methanol-washed glass wool.

Dry powdered potassium bromide (5.0 mg) was placed in the ignition tube, B, 5 cm length and 0.5 cm I.D. 0.5 ml of chloroform was dropped on to the plugged upper end of the drawn tube and allowed to percolate through the adsorbent and into the ignition tube.

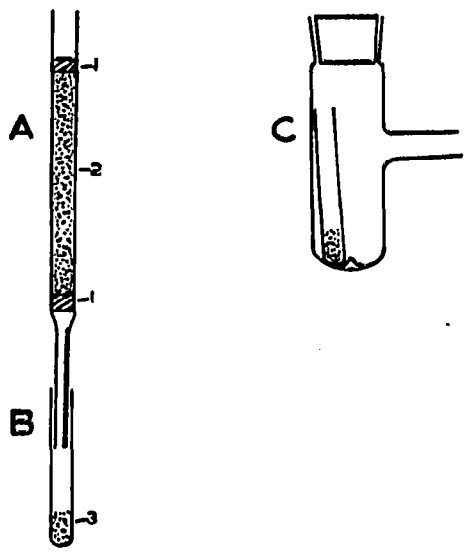


Fig. 1. Apparatus. A = Packed column; B = ignition tube with potassium bromide; C = apparatus for spinning and drying; 1 = glass-wool plug; 2 = column packed with silica gel and adsorbed alkaloid; 3 = powdered potassium bromide.

After preliminary drying in a vacuum oven at 40°, the ignition tube was transferred to the chamber, C (6 × 2 cm I. D.) and the apparatus connected to a reduced pressure line. An air leak was induced by slightly raising the glass stopper, causing rapid revolution of the tube around the basal glass protuberance. After 1-min spinning and drying, the tube was removed and the powdered potassium bromide with alkaloid, which did not adhere to the walls of the tube, was transferred to a Perkin-Elmer ultra-micro-die apparatus.

For efficient production of micro-discs of 1.0 mm diameter, it is essential that the potassium bromide powder is maintained dry by storage in a desiccator except when in actual use and the compression apparatus must be absolutely clean and dry. Under these conditions the powdered potassium bromide flowed freely through the polished metal funnel of the micro-die apparatus, was pressed down using a manual plunger and then satisfactory micro-discs were prepared using a minimum of 0.5 mg of dried powder and a pressure of 80 kg for 3 min.

Infrared characteristics were determined using a Unicam SP 200 spectrophotometer with beam condensing unit and an attenuator to compensate the reference beam. The method was tested using twelve *Rauwolfia* alkaloids.

The advantages of this method are:

(1) limited manipulation of unstable substances: (2) rapid removal of solvent at room temperature limiting decomposition; (3) minimal contamination with silica.

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