

A NOTE ON THE PAPER CHROMATOGRAPHIC SEPARATION OF CODEINE, MORPHINE AND NALORPHINE

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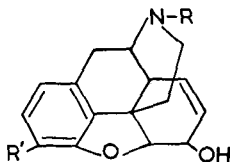
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Codeine has been separated from a mixture of codeine, morphine and nalorphine on Whatman ET20 ion-exchange paper, in 5 min. A mixture of morphine and nalorphine has been separated in 90 min. by horizontal circular reversed phase paper chromatography. Using ascending chromatography at 86° on tributyrin-treated paper, an unequivocal resolution of a mixture of morphine, nalorphine and codeine has been achieved within 20 min.

IN the treatment of acute morphine poisoning, nalorphine is often used as a specific antidote. Curry (1959) isolated morphine and nalorphine from the intestinal contents of an 18 months child who died after the ingestion of 10 mg. of morphine sulphate. A total of 20 mg. of nalorphine was given during treatment.

Furthermore, according to Stewart and Stolman (1960) some 5-17 per cent of a 30 mg. injected dose of codeine is excreted in the urine of man as morphine. It follows that the toxicologist may occasionally be faced with the problem of differentiating these three closely related compounds.



Morphine	R = Me; R' = OH
Codeine	R = Me; R' = OCH ₃
Nalorphine	R = CH ₂ CH:CH ₂ ; R' = OH

In an excellent chapter on Alkaloids, Farmilo and Genest (1961) give a list of twelve solvent systems for the paper chromatographic separation of morphine and codeine. Nalorphine is mentioned in two of these systems, but in neither instance is it separated from codeine. This I have set out to do, in the first instance using the pure alkaloids in admixture.

With ascending paper chromatography on modified cellulose anion-exchange paper, development with ammonia solution and the three pure crystalline alkaloids separately and in admixture I have been able to separate codeine from the mixture in 5 min.

Morphine and nalorphine are not resolved by this procedure but by horizontal circular paper chromatography on paper treated with glycerol monoricinoleate and dried, and development with a phosphate buffer, nalorphine could be separated from codeine or morphine within 90 min.

A clear resolution of all three compounds in admixture was effected

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using the ascending technique, and paper treated with tributyrin, dried, and developed with the phosphate buffer at a temperature of 86° for 20 min. Individual alkaloids were run on the same paper for comparison.

An iodoplatinate reagent was used to identify the alkaloids in the latter two procedures, but could not be used with anion exchange paper with which it reacted. Therefore the spots in the ion-exchange paper were examined under light of a selected wavelength.

EXPERIMENTAL

Ascending ion-exchange chromatography. Whatman ECTEOLA (ET20) modified cellulose anion-exchange paper sheets were cut into 4 in. × 4½ in. rectangles. The alkaloids were applied in 100 µg. amounts and the sheets were made into a cylinder and chromatographed in a suitable cylindrical glass jar using freshly prepared ammonia solution (0.2N). After 5 min. the paper was examined in light of wavelength 254 mµ in which the alkaloids show up as dark areas on a white faintly fluorescent background. Light blue fluorescent spots may also be seen, but these should be ignored as they are due to decomposition products of the alkaloids. The R_F value for codeine was 0.84. The other alkaloids remained at the origin.

Horizontal circular paper chromatography. Whatman No. 1 slotted (26.5 cm. diam.) papers were used with the apparatus described by Kawerau (1956). The papers were treated with glycerol monoricinoleate (10 per cent in acetone) and air dried. The alkaloids were applied in 100 µg. amounts and the papers developed with M/15 phosphate buffer, pH 7.4, for 90 min. The papers were dried in warm air and then dipped into iodoplatinate reagent prepared by mixing together 10 per cent platinum chloride (1 ml.) and 4 per cent potassium iodide (25 ml.) and diluting to 50 ml. with distilled water. The alkaloids appear as purplish black spots on a brown background. R_F values were: codeine 0.58, morphine 0.57, nalorphine 0.41.

Ascending reversed phase paper chromatography. Whatman No. 1 papers were treated with tributyrin (10 per cent in acetone) and air dried. The alkaloids separately and in admixture in amounts of 100 µg. were applied to the paper, which was then chromatographed in a suitable cylindrical jar in an incubator at 86° using the M/15 phosphate buffer for development. Resolution of the three alkaloids was complete in 15 min. but an even better separation is obtained after a further 5 min. The paper was dipped in the iodoplatinate reagent and the alkaloids identified as described in the previous paragraph. R_F values were: codeine 0.62, morphine 0.80, nalorphine 0.38.

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