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Note

High-performance liquid chromatographic separation of apomorphine and its O-methyl metabolites

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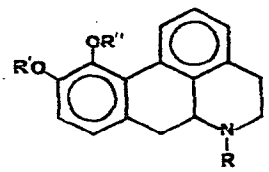
As part of on-going studies of the metabolism of apomorphine and other potential antiparkinsonian aporphines[1–4], we became interested in high-performance liquid chromatographic (HPLC) systems for the development and separation of apomorphine (I) and its mono-O-methyl metabolites, apocodeine (II) and isoapocodeine (III). The apomorphine homolog, N-*n*-propylapomorphine (IV) was included in these studies because of its potential use as an internal standard for the analysis of compounds I–III.



(I) Apomorphine: R = CH_3; R′ = R″ = H.
(II) Apocodeine: R = R′ = CH_3; R″ = H.
(III) Isoapocodeine: R = R″ = CH_3; R′ = H.
(IV) N-*n*-Propylnorapomorphine: R = $CH_2CH_2CH_3$; R′ = R″ = H.

A number of analytical methods are available for the determination of the aporphines of interest[1,2,5–10]. However, it was felt that an HPLC procedure might be advantageous because of its potential for high resolution and sensitive detection without the formation of derivatives. Several HPLC systems including reversed-phase columns and a variety of mobile phases were investigated for the analysis of compounds I–IV.

A system using acetonitrile–methanol–buffer mobile phase containing dodecyl sulfate counter ions and a μBondapak phenyl column was ultimately developed to provide baseline resolution of compounds I–IV.

MATERIALS AND METHODS

Apomorphine hydrochloride hemihydrate was purchased from McFarland-Smith (Edinburgh, Great Britain) and determined to have a purity of 98% based on

ultraviolet spectroscopy. Apocodeine was prepared as previously described[1]. Isoapocodeine was prepared via a microbial O-demethylation of the dimethyl ether of apomorphine[11]. N-*n*-Propylnorapomorphine hydrochloride was obtained from Sterling Winthrop Research Institute (Rensselaer, N.Y. U.S.A.). Water was double distilled in glass. Organic solvents were obtained from Burdick and Jackson Labs. (Muskegon, Mich., U.S.A.). All other reagents were of analytical reagent grade. Mobile phases were filtered through glass fiber pads (Whatman GF/F glass fiber paper) prior to use. HPLC columns (μBondapak C_{18} and phenyl, particle size = 10 μm; 30 cm × 4 mm I.D.) were obtained from Waters Assoc. (Milford, Mass., U.S.A.).

Standard solutions

For initial HPLC work, solutions of 1 mg/ml of aporphines or aporphine hydrohalides were prepared in methanol. In later studies, more dilute solutions were used. Standard solutions were prepared fresh daily.

HPLC system

A Tracor 995 pump and 970 A variable wavelength detector were used throughout. Gradients were generated by a Tracor 980 A solvent programmer. Samples were introduced into the system through a Rheodyne loop injector or a Waters U6K injector. The detector was set to monitor the absorbance at 273 nm. Flow-rates were normally kept at 1 ml/min.

Separations were developed by first running a linear gradient from 0 to 100% organic solvent (rate of change 5%/min). The position of the eluted apomorphine peak was used to calculate approximate isocratic mobile phases. Various closely related isocratic mixtures were then tried until a satisfactory retention of apormorphine was found ($k' \approx 2$). The other standards were then chromatographed in that system to determine degree of separation. If resolutions were not adequate, variations were systematically made in mobile phase compositions.

RESULTS AND DISCUSSION

The best separation devised for compounds I–IV is shown in Fig. 1. The μBondapak phenyl column provided complete resolution of apomorphine and the potential internal standard, N-*n*-propylnorapomorphine (IV), with most of the methanol– or acetonitrile–buffer combinations (containing dodecyl sulfate) tested. However, the O-methyl metabolites co-chromatographed with IV when either methanol or acetonitrile was used as the sole organic modifier. Various combinations of methanol and acetonitrile were evaluated until the mobile phase methanol–acetonitrile–buffer [0.02 *M* Na_2HPO_4 + 0.03 *M* citric acid (pH = 3.25), containing 0.001 *M* sodium dodecyl sulfate] (36:9:55) was developed and found to provide the most satisfactory separation.

When the counter ion, dodecyl sulfate, was omited from the described mobile phase, resolutions were poorer and considerable evidence of tailing was observed. Inadequate development and resolution were also noted when dodecyl sulfate was substituted by heptanesulfonic acid. This is of theoretical interest and may indicate the importance of having sufficiently lipoidal counter-ion for the reversed-phase development of compounds similar to those studied in this work.

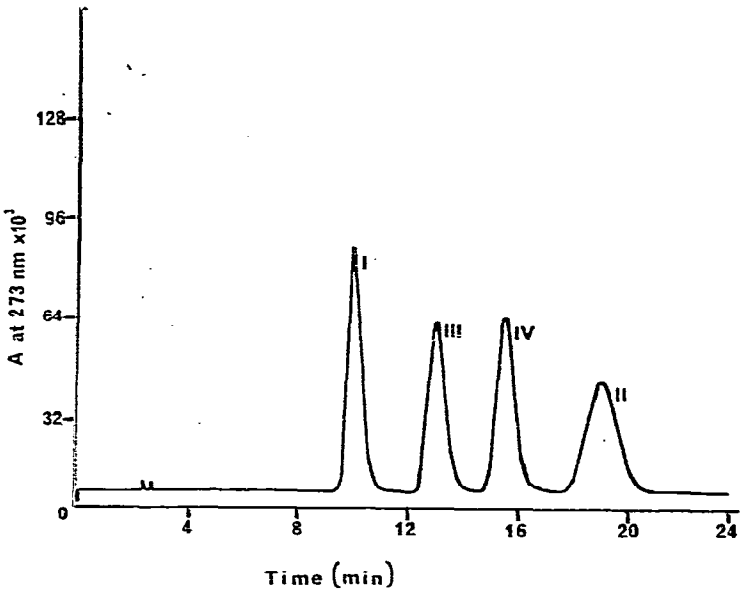


Fig. 1. HPLC separation of aporphine compounds I–IV (approx. 1 μg each) on a 30 cm × 4 mm I.D. μBondapak phenyl column eluted at 1 ml/min with methanol–acetonitrile–buffer [0.02 *M* Na_2HPO_4 + 0.03 *M* citric acid (pH = 3.25) containing 0.001 *M* sodium dodecyl sulfate] (36:9:55). UV detector set at 273 nm, 0.32 a.u.f.s.

Resolutions of the compounds studied were never as good on the C_{18} column as on the phenyl column. Furthermore, when low levels of aporphines were injected, adsorption seemed to occur during the separation process as indicated by extensive tailing. Additionally, no linear relationship between amount of compound I injected and peak height was observed below 1 μg. Other C_{18} columns may be more useful than the one tested because of their heavier loading[12].

The HPLC system employing the phenyl column provided an excellent linear relationship ($r = 0.999$) for peak height ratio (I/IV) *vs.* amount of I injected over the range 20 to 200 ng of apomorphine. The described HPLC system (Fig. 1) is currently being used to develop methods for determination of compounds I, II, and III in biological fluids. Results from these investigations will be the subject of a future report.

ACKNOWLEDGEMENTS

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