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**Note**

# Amperometric high-performance liquid chromatographic method for narcotic alkaloids

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Phenanthrene alkaloids of opium have structural similarities to the catecholamines (Fig. 1). Since the catecholamines have been shown to participate in an oxidation reaction in a low potential field, thus affording their detection by amperometric means, we investigated the same method for quantitation of the alkaloid opiates: morphine, oxymorphone and codeine as well as the closely related narcotic antagonists: naloxone, naltrexone, nalorphine and pentazocine.

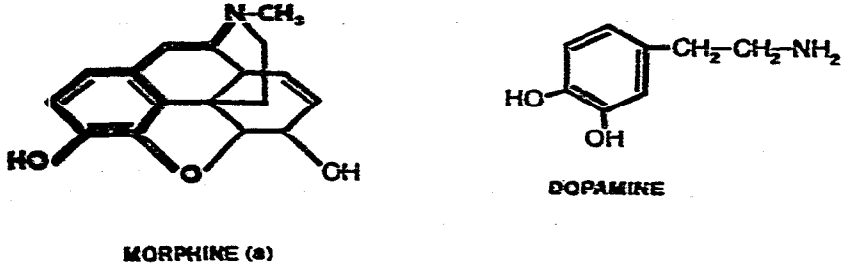


Fig. 1. Similarity between the opiate alkaloids and catecholamines. Heavy lines superimposed on the morphine structure coincide with the general structure of the catecholamine shown on the right.

We have established conditions whereby a rapid reversed-phase chromatographic separation of several of these substances can be made and found that amperometry detected only those alkaloids with a "catechol" structure.

## METHODS

A Waters Assoc. (Milford, Mass., U.S.A.) high-pressure liquid chromatograph consisting of a Model 6000 pump, U6K loop injector and a Model 450 ultraviolet detector (254 nm) in series with an amperometric detector (Model LC-2A electronic controller, Model TL-3 electrochemical cell; Bioanalytic Systems, West Lafayette, Ind., U.S.A.) afforded separation using a reversed-phase μBondapak $C_{18}$ column (Waters Assoc.) with dimensions of 30 cm × 4 mm. The column eluent was methanol–water (20:80) containing 50 m*M* tetramethylammonium hydroxide and pH adjusted to 6.1 with $H_3PO_4$.

An isocratic elution at 2.0 ml/min in ambient room temperature resulted in a back pressure of 13.8 MPa (2000 p.s.i.). Standards of morphine, codeine, nalorphine (Applied Science Labs., State College, Pa., U.S.A.), oxymorphone, naltrexone and naloxone (Endo Labs, Garden City, N.Y., U.S.A.) and pentazocine (Sterling Winthrop Labs., New York, N.Y., U.S.A.) were made by dissolving the hydrochloride salts in methanol.

The amperometric method was compared with a gas chromatographic procedure. Samples were prepared by adding known amounts of morphine to human plasma. To 1.0-ml aliquots were added 20 μg nalorphine as an internal standard, 500 μl carbonate buffer (1 *M*, pH 9) and 5 ml benzene. After extraction with shaking, the samples were centrifuged. The organic layer was removed and evaporated to dryness. A 20-μg amount of Meth Elute® (Supelco, Bellefonte, Pa., U.S.A.) was added, the sample vortexed and again evaporated to dryness. The residue was taken up in 25 μl methanol, of which 2–4 μl were injected into the gas chromatograph (Perkin-Elmer Sigma 3, with a nitrogen–phosphorus detector). The column (6 ft. × 1/4 in.) was packed with 3% OV-17, operated at 250°.

The same samples were assayed amperometrically, using an injection volume of 1 μl. Both assays were run in duplicate.

## RESULTS

Fig. 2 shows the chromatographic separation with detection by amperometry of a standard mixture of oxymorphone (OXY), morphine (MOR), naltrexone (NT), naloxone (NX) and nalorphine (NL). The substances were present in equimolar quantities of 0.3 nmoles with the exception of oxymorphone which was present at 0.15 nmoles. Morphine was observed to give the best signal at a potential across the electrochemical cell of +0.8 V. Table I gives the peak height ratios of each of these substances as compared to morphine. An example of the lower limits for detection of morphine and naloxone is shown in Fig. 3. Increased sensitivities of the detector (5–20 nA/V) indicated an ability to detect morphine down to 100 pg and naloxone down to 1000 pg. As shown on the right in Fig. 3, the signal-to-noise ratio of morphine deteriorates below 1 ng. Nevertheless, the ability to quantitate morphine to 1 ng and naloxone to 5 ng is demonstrated by the linear response when peak height is plotted *versus* nanograms injected in Figs. 4 and 5.

The comparison of the high-performance liquid chromatographic (HPLC)–amperometric method for morphine to the gas chromatographic method is shown in Fig. 6. As can be seen, the comparison resulted in a line giving a correlation coefficient of 0.994 (slope, 14.7; and *y*-intercept, −0.044).

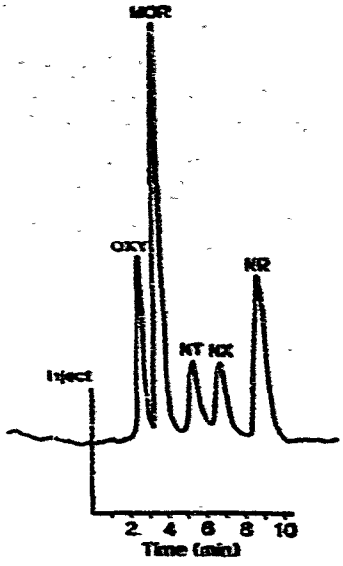


Fig. 2. Chromatographic separation of oxymorphone (OXY), morphine (MOR), naltrexone (NT), naloxone (NX) and nalorphine (NR) under conditions given in text. Detection is by amperometry.

TABLE I

COMPARISON OF AMPEROMETRIC RESPONSE OF OTHER ALKALOIDS TO MORPHINE

| *Alkaloids* | *Ratio (A:M)* |
|---|---|
| Morphine | 1 |
| Oxymorphone | 0.70 |
| Nalorphine | 0.41 |
| Naltrexone | 0.28 |
| Naloxone | 0.26 |

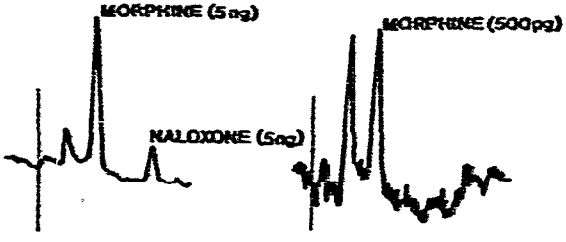


Fig. 3. Amperometric detection of standards of morphine and naloxone. Numbers in parentheses indicate quantities injected.

We next investigated the necessity for a catechol-like structure of the alkaloid for the appearance of an amperometric response. The measured response to codeine by both ultraviolet (UV) absorption and amperometry (both detectors in series following the reversed-phase column) is shown in Fig. 7. A 200-ng amount of codeine

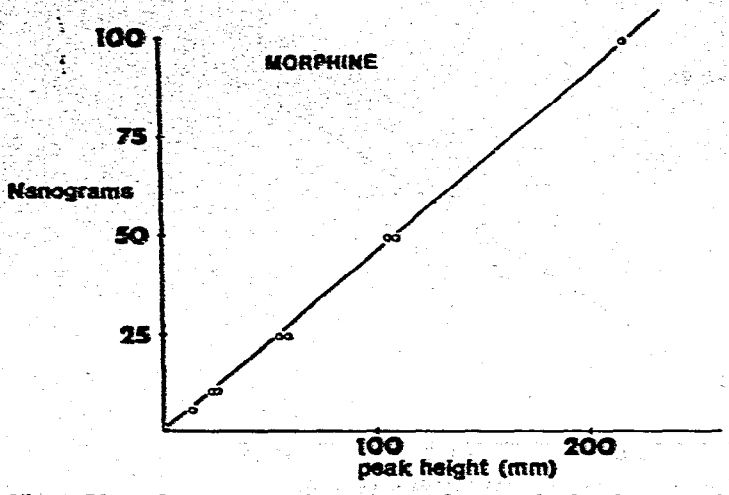


Fig. 4. Plot of amperometric response for standard solutions of morphine. Ordinate represents quantity injected.

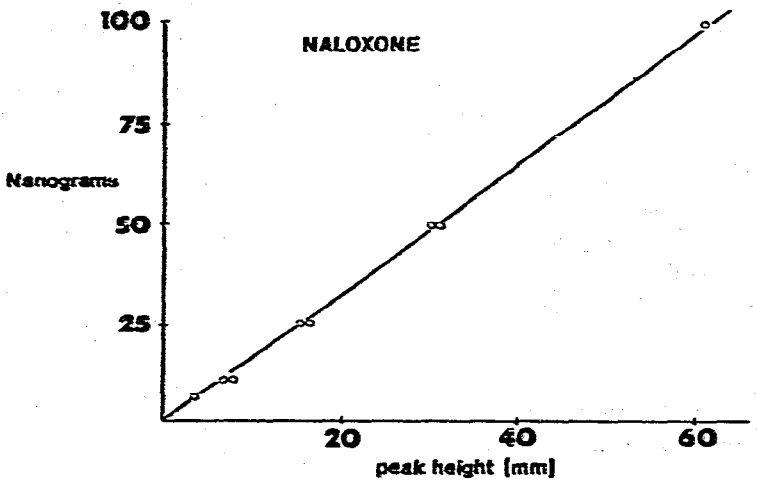


Fig. 5. Plot of amperometric response for standard solutions of naloxone. Ordinate represents quantity injected.

was injected in order to detect the material by UV absorption. The same sensitivity of the amperometric detector as allowed detection of 500 pg of morphine was used. As can be seen, there was no response by amperometry to codeine.

One further compound investigated was pentazocine, the structure and response shown in Fig. 8. Here, once again, there was no measured response by amperometry.

## DISCUSSION

Standard methods for narcotics and narcotic antagonists have been reported using gas chromatography with a nitrogen or an electron capture detector[1–4]. In

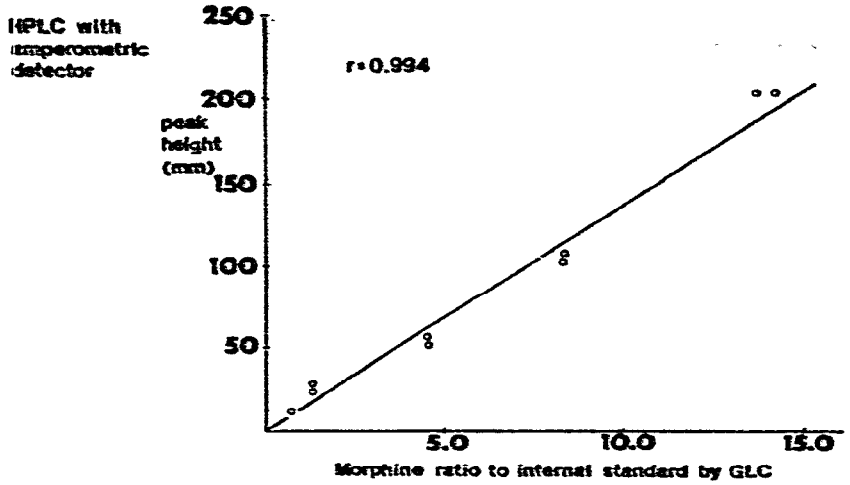


Fig. 6. Comparison of HPLC–amperometric method for morphine to a standard gas chromatographic (GLC) procedure.

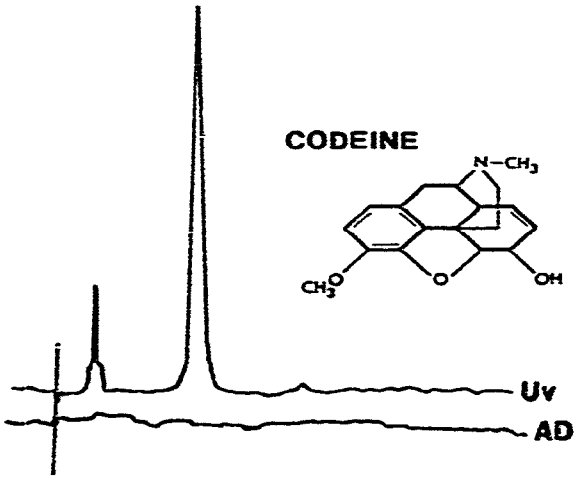


Fig. 7. Structure–response relationship for codeine and amperometry. Position of codeine in column elution demonstrated by ultraviolet (Uv) peak. No amperometric response was observed.

addition, radioimmunoassay methods have been reported for morphine[5,6] and for naloxone[7]. We have utilized the structural similarities between the alkaloid opiates and the catecholamines to allow a more rapid analytical method not requiring the derivitizations necessary for electron capture gas chromatography nor the long incubation and counting times for radioimmunoassay. The method allows separation of complex mixtures of this class of compounds and detection limits which are comparable to the alternate methods discussed above.

Evaluation of the analyses of various alkaloids did show striking differences in the amperometric response based upon structural alterations as compared to morphine, the compound showing the best signal-to-noise ratio (Fig. 2, Table I). Sub-

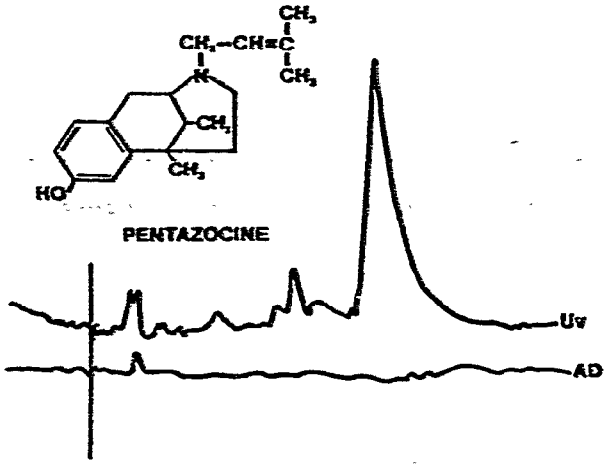


Fig. 8. Structure–response relationship for pentazocine. Broad ultraviolet (Uv) peak not seen by amperometry.

stitutions of various alkyl groups on the alkaloid ring nitrogen did decrease the amperometric signal as could be seen with naloxone, naltrexone and nalorphine, but not with oxymorphone, a compound with the same N-methyl structure as morphine. Alteration of the structure by incorporation of a hydroxyl group on the 14 position of morphine as is the case in oxymorphone did not have a large effect upon the amperometric signal, and therefore, is probably not related to the low response seen for naloxone and naltrexone, the other alkaloid's with this structural feature.

Very marked alterations in response was seen for those compounds where the "catechol" structure was altered. No signal was seen for codeine (3-methoxymorphine) and this most likely reflects the necessity for the 3-hydroxyl group of the alkaloid opiates for oxidation in the electrochemical field. In addition, the necessity for the second "catechol" oxygen between the 4 and 5 positions of morphine was demonstrated by analysis of pentazocine, the other compound without an amperometric response. It must be noted that there is a bulky substitution of the analogous nitrogen in pentazocine which could have lowered the signal, but such a large excess of the substance was injected in order to obtain the UV response that there appeared to be a total loss of the response by amperometry.

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