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# HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC–AMPEROMETRIC DETERMINATION OF NALOXONE HYDROCHLORIDE INJECTION

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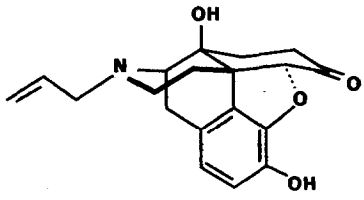
## SUMMARY

Naloxone hydrochloride has been measured in the injectable dosage form at 0.4 and 0.02 mg/ml using high-performance liquid chromatography with amperometric detection. This method was contrasted with an ultraviolet detection method at 229 nm and found to provide comparable recovery and linearity results. At the electrochemical detection limit of 0.1 ng injected a signal-to-noise ratio of 10.4 was found.

## INTRODUCTION

Naloxone hydrochloride, the potent pure narcotic antagonist of the oxymorphone series, has been previously analyzed by several sensitive and specific procedures. These include gas chromatography[1–3], thin-layer chromatography (TLC)[4,5], radioimmunoassay[6,7], and high-performance liquid chromatography (HPLC)[8–14]. Fluorescence, ultraviolet (UV) and electrochemical detection (ED) methods have been studied for HPLC determination of naloxone. A high extinction coefficient (10,140) at 280 nm was measured for naloxone although no fluorescence emission spectrum was obtained at an excitation wavelength of 290 nm[8] as would be expected from previous work[15]. UV detection has been used for naloxone measurement in injectable dosage forms at 254 nm for Narcan®, Neonatal, 0.02 mg/ml[9] and at 220 nm for Narcan at 0.02 and 0.4 mg/ml[10]. Linearity between 0.1 and 0.5 μg/ml and recoveries of 99.0% ± 2.6% standard deviation were demonstrated in the former dosage form study. The Endo workers in the latter study obtained recoveries of 100.3 and 100.8% for standards and 95.5% and 100.8% for actual samples at 0.02 and 0.4




Naloxone

mg/ml, respectively. These workers also showed linearity for standard naloxone recoveries using a papaverine internal standard between concentration ratios of 0.8–3.2, which corresponded to 16.4 μg/ml naloxone hydrochloride in the lower limit.

Naloxone detection by amperometric means following HPLC was reported early using a silica column with a methanol–ammonium nitrate buffer (pH 10.2) mobile phase[11]. Later reversed-phase HPLC–ED of naloxone utilized mobile phases containing tetramethylammonium hydroxide[12,13] or phosphate buffers[14]. Linearity of recovery from 5 to 100 ng injected[12] and from 100 to 2000 ng added to brain homogenate[13] was shown.

The purpose of the present investigation is to ascertain whether electrochemical means can be utilized for routine detection of naloxone in dosage forms where stringent statistical limitations must be met in view of the preeminence of this method in detecting phenolic compounds in biological material. Comparisons will be made between external standard methods using electrochemical and conventional UV detection methods.

## EXPERIMENTAL

### *Reagents*

Methanol was HPLC grade MCB Omnisolve, EM Scientific. Water was distilled and filtered (Millipore, 0.45 μm). Phosphoric acid, 85%, was from Mallinckrodt and 1-octanesulfonic acid, sodium salt, was from Eastman. Acetonitrile was HPLC grade and disodium EDTA was reagent grade, both from Fisher Scientific. Anhydrous monobasic potassium phosphate and sodium chloride were reagent grade from J. T. Baker. Alpha alumina, 0.3, 1.0 and 5.0 μm and polishing cloth (Texmet®) were from Buehler.

### *Compounds studied*

The compounds utilized in this study were naloxone hydrochloride (Endo), naltrexone hydrochloride (Endo), hydromorphone hydrochloride (Knoll), oxycodone hydrochloride (Endo), levorphanol tartrate (Hoffman-La Roche), cyclazocine hydrochloride (Sterling-Winthrop), 8-acetylpentazocine hydrochloride (Sterling-Winthrop) and N-1-propylnorapomorphine hydrochloride (Sterling-Winthrop).

### *Apparatus*

The HPLC–ED system consisted of a Waters M6000 pump at a flow-rate of 1.0 ml/min and a Bioanalytical Systems LC-4 amperometric detector set at 0.85 V and 20 nA/V. This was equipped with a TL-5 thin-layer glassy carbon working electrode *versus* a Ag/AgCl reference electrode. A Micromeritics 725 autoinjector with a 10 μl injection loop was used.

The HPLC–UV detection system consisted of a Varian 5000 pump and autosampler (20-μl injection loop) at a flow-rate of 1.2 ml/min. A Waters 441 UV detector with a cadmium lamp at 229 nm was used at a sensitivity of 0.05 a.u.f.s. Fisher Recordall Series 5000 recorders were used in both systems. Columns used in both systems were stainless steel Whatman Partisil PXS 10/25 ODS-3.

*Mobile phases*

For HPLC–ED: (I) water–methanol (550:450) which was 0.01 *M* overall in potassium dihydrogen phosphate and 0.0028 *M* overall in 1-octanesulfonic acid sodium salt (ionic strength 0.0128 *M*, apparent pH 5.59); (II) water–methanol–acetonitrile–phosphoric acid (600:200:200:1) which was 0.0028 *M* overall in 1-octanesulfonic acid sodium salt, and 0.0001 *M* overall in disodium EDTA (ionic strength 0.092 *M*, apparent pH 2.39). For HPLC–UV detection: (III) water–methanol–phosphoric acid (550:450:1) which was 0.017 *M* overall in sodium chloride and 0.0031 *M* overall in 1-octanesulfonic acid sodium salt (ionic strength 0.1089 *M*, apparent pH 2.68).

*Naloxone standard linearity*

Naloxone hydrochloride standard solutions were prepared at *ca.* 0.1 mg/ml in each of the following solvents: (1) water for HPLC–ED with mobile phase I, (2) methanol–water (1:1) for HPLC–ED with mobile phase II and (3) water–methanol–phosphoric acid (550:450:1) for HPLC–UV detection with mobile phase III. Four serial 1–10 dilutions were made for each of the three standard solutions in their respective solvents.

*Selectivity*

Solutions of the opiates listed above were prepared in water and further diluted to *ca.* 0.02 mg/ml and chromatographed using mobile phase I for HPLC–ED.

*Linearity of recovery from simulated samples*

*HPLC–ED.* Naloxone hydrochloride solutions were prepared in placebo which corresponded to Sterling-Winthrop naloxone hydrochloride injection at 0.4 and 0.02 mg/ml. Solutions were also prepared in placebo containing 0%, 80% and 120% of each of these levels. Solutions were diluted in water to a level giving 0.002 mg/ml for the 100% samples.

*HPLC–UV.* The same initial concentrations of simulated samples as above were diluted with water–methanol–phosphoric acid (550:450:1) to give 0.01 mg/ml for the 100% samples.

All sample solutions were chromatographed, injecting samples alternately with standards. These were prepared at 0.002 mg/ml in water and 0.01 mg/ml in water–methanol–phosphoric acid (550:450:1) for HPLC–ED and HPLC–UV detection, respectively.

## RESULTS AND DISCUSSION

Routine operation of an HPLC detector for dosage form analysis and quality control must give accurate and reproducible results, linearity of detection, stability indicating properties and low maintainence costs. The solid state electronics and relatively simple construction of commercial amperometric detectors would appear to afford these properties and make them useful alternatives to the standard UV detector for compounds which contain oxidizable or derivatizable functionalities. The high sensitivity of these detectors so often quoted is of little consequence for quality control work although it is available if needed.

The thin-layer glassy carbon working electrode used with the BAS LC-4 amperometric detector gave little problem in daily operation. A brief 15-min warmup was all that was required for stabilization provided the mobile phase was pumped overnight through the cell at a low flow-rate. Use of helium purging or other extensive pretreatment of the mobile phase other than the usual deaerating was found to be unnecessary here. Also no special precaution with the length of and submersion of the outlet tubing to prevent drop pulses had to be taken. When the system was continuously run for 28 h no deterioration in baseline was noted with peak responses of a standard varying no more than ± 5.8% of the initial value over this time. While this assumes no change in the chromatographic system and standard stability, one possible cause of decreased peak response is buildup of oxidation products on the glassy carbon electrode. If this occurs a simple resurfacing technique can be carried out. This involves polishing the electrode transducer cube with a sequence of alumina slurries from 5.0 to 0.3 $\mu$m in distilled water on a polishing cloth. Following each polishing adherent particles are removed by sonication in distilled water.

An experiment performed to determine linearity of scaling in the detector gave results shown in Fig. 1. Injection of the same naloxone hydrochloride standard solution at detector settings from 50 to 1 nA/V at 0.85 V gave the hyperbolic response curve which nearly coincides with the theoretical curve.

Linearity of detector response and minimum quantifiable level for naloxone are shown in Fig. 2. The electrochemical detector gave a linear response over 4–5 decades of dilution (mobile phases I and II) from $1 \cdot 10^{-1}$ to $1 \cdot 10^{-5}$ mg/ml corresponding to 1000 to 0.1 ng injected. Also included is the linearity curve over 4 decades of standard dilution by UV detection at 229 nm (curve D). This falls at *ca.* 1–1.5 log units of peak response below that for ED under optimized conditions. Curve C is an ED curve under deteriorated electrode surface conditions using mobile

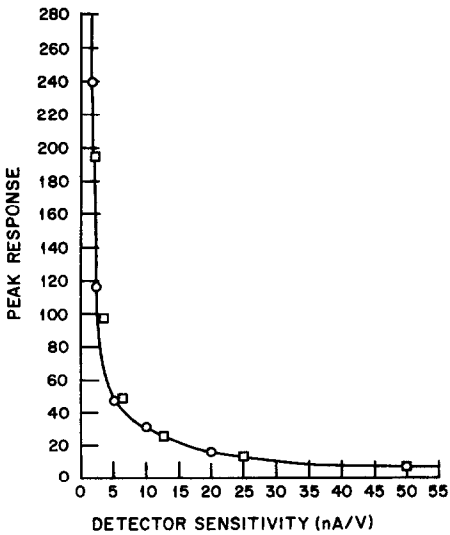



Fig. 1. Relationship between peak response and amperometric detector sensitivity setting for naloxone showing linearity of scaling in detector. Mobile phase II with detector at 0.85 V were used with other chromatographic conditions as in text. O, Experimental; □, theoretical.

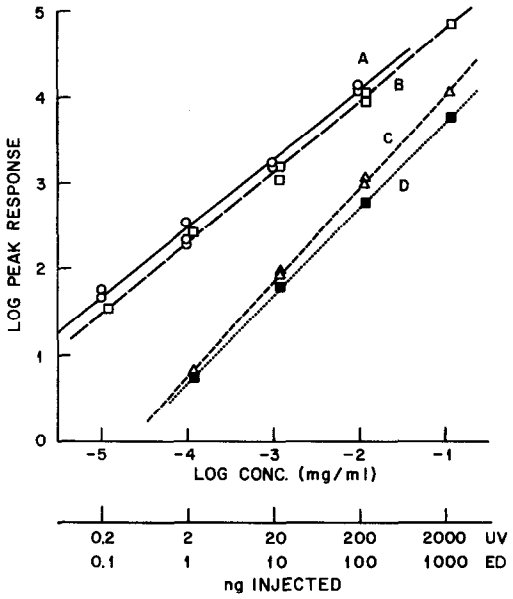



Fig. 2. Relationship between log normalized peak response and log concentration for naloxone giving minimum quantifiable levels. Peak responses were normalized to 0.5, 0.1 and 0.1 nA/V for ED curves A, B, and C, respectively, and to 0.005 a.u.f.s. for UV curve D.

phase II. The curve obtained under the same conditions after resurfacing was B. A 10–20 fold increase in peak response commonly followed resurfacing of the electrode.

Signal-to-noise ratios measured at the minimum detectable level for each of the four curves are given in Table I. The UV chromatogram is obviously a much cleaner picture than the deteriorated ED curve at 2 *versus* 1 ng, respectively, while an optimized ED curve (A or B) is much better yet.

Hydrodynamic voltammograms for naloxone standards in mobile phase I are shown in Fig. 3. At 0.1 mg/ml the linear curve was obtained while at 0.001 mg/ml the more familiar sigmoidal curve representing a polarographic wave was obtained. The inflection point estimated from the latter curve is 0.75 V. The 0.85 V used for oxidative detection of naloxone in this study is therefore appropriate in view of background noise amplification at higher voltages.

TABLE I

NALOXONE STANDARD SIGNAL-TO-NOISE RATIOS AT THE MINIMUM QUANTIFIABLE LEVELS BY ED AND UV DETECTION

| *Curve (Fig. 2)* | *Mobile phase* | *Detection* | *Minimum quantifiable level (ng)* | *Signal-to-noise ratio* |
|---|---|---|---|---|
| A | I | ED | 0.1 | 10.4 |
| B | II | ED | 0.1 | 6.8 |
| C | II | ED | 1.0 | 0.5 |
| D | III | UV | 2.0 | 5.6 |

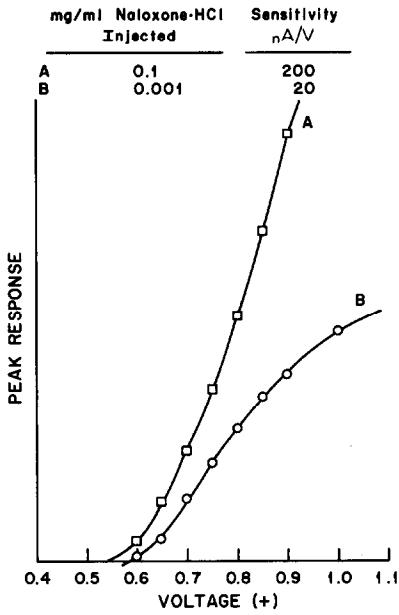
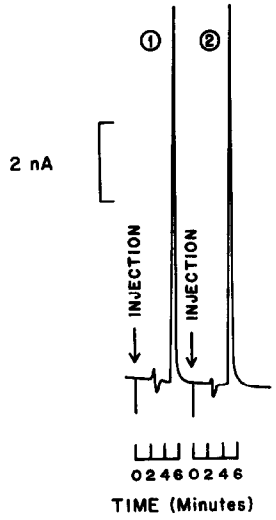



Fig. 3. Hydrodynamic voltammograms of naloxone at 0.1 and 0.001 mg/ml using mobile phase I.



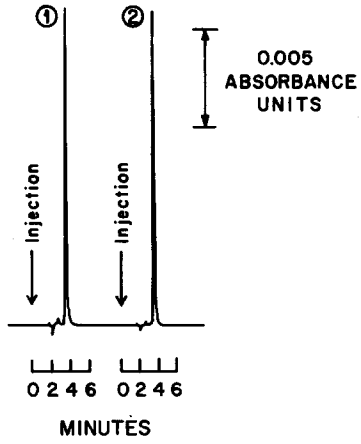




Fig. 4. Chromatograms of naloxone standard and 100% simulated sample using HPLC–ED with mobile phase I at 0.85 V and 20 nA/V. 1 = Naloxone simulated sample, 0.002 mg/ml; 2 = naloxone standard, 0.002 mg/ml.

Fig. 5. Chromatograms of naloxone standard and 100% simulated sample using HPLC–UV detection with mobile phase III at 229 nm and 0.05 a.u.f.s. 1 = Naloxone standard, 0.01 ng/ml; 2 = naloxone simulated sample, 0.01 mg/ml.

TABLE II

NALOXONE RECOVERIES FROM SIMULATED SAMPLES USING ED

| *0.02 mg/ml* | | | *0.4 mg/ml* | | |
|---|---|---|---|---|---|
| *Added (mg)* | *Found (mg)* | *Recovery (%)* | *Added (mg)* | *Found (mg)* | *Recovery (%)* |
| 0.0 | 0.008 | – | 0.0 | 0.0 | – |
| 0.0 | 0.013 | – | 0.0 | 0.0 | – |
| 1.608 | 1.585 | 98.57 | 32.5 | 32.08 | 98.71 |
| 1.608 | 1.635 | 101.68 | 32.5 | 32.56 | 100.18 |
| 2.01 | 1.978 | 98.41 | 40.2 | 39.71 | 98.78 |
| 2.01 | 2.045 | 101.74 | 40.2 | 40.06 | 99.65 |
| 2.412 | 2.390 | 99.09 | 47.9 | 47.88 | 99.96 |
| 2.412 | 2.349 | 97.39 | 47.9 | 46.75 | 97.60 |
| Average recovery (%) | | 99.48 | | | 99.15 |
| R.S.D. recovery (%) | | 1.82 | | | 0.98 |
| Slope | | 0.984 | | | 0.989 |
| Intercept | | $1.68 \cdot 10^{-2}$ | | | $4.66 \cdot 10^{-2}$ |
| Correlation coefficient | | 0.9995 | | | 0.9998 |

TABLE III

NALOXONE RECOVERIES FROM SIMULATED SAMPLES USING UV DETECTION

| *0.02 mg/ml* | | | *0.4 mg/ml* | | |
|---|---|---|---|---|---|
| *Added (mg)* | *Found (mg)* | *Recovery (%)* | *Added (mg)* | *Found (mg)* | *Recovery (%)* |
| 0.0 | 0.0 | – | 0.0 | 0.0 | – |
| 0.0 | 0.0 | – | 0.0 | 0.0 | – |
| 1.608 | 1.58 | 98.63 | 31.8 | 31.7 | 99.68 |
| 1.608 | 1.62 | 100.75 | 31.8 | 31.9 | 100.31 |
| 2.01 | 2.00 | 99.50 | 40.2 | 40.3 | 100.25 |
| 2.01 | 1.98 | 98.51 | 40.2 | 39.9 | 99.25 |
| 2.412 | 2.43 | 100.75 | 48.3 | 48.0 | 99.38 |
| 2.412 | 2.44 | 101.16 | 48.3 | 48.4 | 100.21 |
| Average recovery (%) | | 99.82 | | | 99.85 |
| R.S.D. recovery (%) | | 1.25 | | | 0.47 |
| Slope | | 1.003 | | | 0.998 |
| Intercept | | $-5.72 \cdot 10^{-3}$ | | | $1.35 \cdot 10^{-2}$ |
| Correlation coefficient | | 0.9998 | | | 0.99996 |

TABLE IV

CAPACITY FACTORS OF COMPOUNDS CHROMATOGRAPHED USING ED AND MOBILE PHASE I

| *Compound* | *k'* |
|---|---|
| Oxycodone | 1.24 |
| Naloxone | 1.28 |
| Naltrexone | 1.43 |
| Hydromorphone | 1.62 |
| Cyclazocine | 3.28 |
| Levorphanol | 3.76 |
| N-1-Propylnorapomorphine | 4.14 |
| 8-Acetylpentazocine | 4.90 |

Initial use of mobile phase II for HPLC–ED of naloxone injection resulted in non-linear recoveries from simulated samples with dilutions made in methanol–water (1:1). Peak splitting behavior was observed which depended on the amount of sodium chloride present in the injection solvent and in the mobile phase. Use of mobile phase I modified from Lake *et al.*[14] with the addition of the octanesulfonate pairing ion alleviated these problems and gave acceptable linearity and recovery data.

Figs. 4 and 5 are comparable chromatograms of naloxone standards and simulated samples by HPLC–ED and HPLC–UV detection run at 0.002 and 0.01 mg/ml, respectively. The increased sensitivity of ED was used here with the additional five-fold dilution to give the results shown in Table II. Average recoveries of 99.48

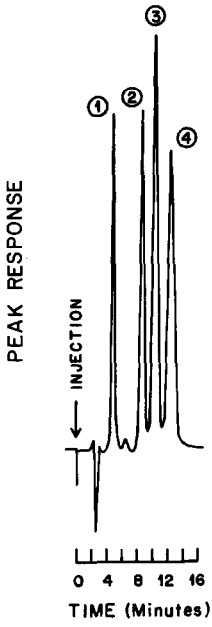



Fig. 6. Selectivity of chromatographic amperometric detection method for naloxone (1), cyclazocine (2), levorphanol (3) and 8-acetylpentazocine (4) with mobile phase I at 0.85 V and 5.0 nA/V.

and 99.15% were obtained for linearity curves of 0.02 and 0.4 mg/ml simulated samples. The 80–120% injections correspond to 16–24 ng injected. A minor carryover from standards was seen at the 0.02 mg/ml level blank solutions but not the 0.4 mg/ml blanks and did not detract from the linearity.

Table III gives the recovery and linearity data for UV detection of naloxone injection at 0.02 and 0.4 mg/ml. Here again excellent recoveries are seen at 99.82 and 99.85%. The 80 to 120% samples corresponded to 160–240 ng injected.

Selectivity of the paired-ion reversed-phase HPLC–ED method is shown by capacity factor ($k'$) results for various opiates given in Table IV. Fig. 6 is a chromatogram of naloxone and three other opiates with ED run in combination at 0.001–0.02 mg/ml using 0.85 V and 5 nA/V settings. Stability indicating properties of this assay are further suggested by the structures of known degradation products. These include noroxymorphone and 6-hydroxynaloxone (EN 2265) as well as potential oxidation products noroxymorphone-N-oxide and dimerized "pseudonaloxone" which maintain their phenolic hydroxides intact making their amperometric detection feasible[16].

The present results indicate that ED for HPLC is applicable to quantitation of drugs in dosage forms and fulfills statistical requirements on the level of UV detection. Little additional effort needs to be expended in the operation of an electrochemical detector over a UV detector except periodic resurfacing of the electrode which can be accomplished in 10 min. This method requires analytes with oxidizable functional groups for use with a glassy carbon electrode although a gold–mercury amalgam and mercury electrodes are available for reductive detection which reactions will require further exploration.

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