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Short communication

Determination of naloxone in human plasma by high-performance liquid chromatography with coulometric detection

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Abstract

Naloxone, the analyte and the internal standard, sumatriptan, are extracted from plasma using solid-phase extraction columns. Chromatography and detection are performed using isocratic reversed-phase high-performance liquid chromatography (HPLC) with coulometric end-point detection. The standard curve was linear over the range 0–50 ng/ml of naloxone in plasma. The reproducibility, the coefficient of variation (C.V.) of the method over the range of the standard curve was 6.2–11.2%. The recovery averaged 90.4±8.9%. A plasma profile following i.v. administration of naloxone in one normal healthy volunteer is presented.

Keywords: Naloxone

1. Introduction

Naloxone is an opiate antagonist which is used in the treatment of overdose. It has also been used to identify control of various systems by endogenous opiates. Assay techniques for the measurement of naloxone in plasma have been described, these include both chromatographic and radioimmunoassay (RIA) methods. One method described an HPLC procedure for the measurement of morphine antagonists (including naloxone) in plasma, using electrochemical end-point detection [1]. The authors suggested an assay limit of detection for naloxone of 2–5 ng/ml. A further assay described the measurement of naloxone using HPLC with electrochemical detection, however in contrast, solid-phase extraction (SPE) rather than liquid–liquid extraction was used, an ion-pairing agent was employed to enhance the selectivity of the extraction [2]. An assay sensitivity of 1 ng/ml was described.

In contrast, RIAs have been described for the determination of naloxone in human plasma [3,4]. The reported limits of detection for these assays was about 0.025 ng.

Recently naloxone has been used as a specific ligand in PET (positron emission tomography) scanning studies. This usually involves naloxone being administered intravenously to healthy volunteer subjects or patients. The hope being that a constant or near constant plasma drug level might be sustained. It is necessary to monitor the plasma drug profile over the period of the PET scanning study so that

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changes in blood flow in localised areas of the brain may be correlated with this. Hence the need for us to establish a sensitive and specific assay of our own, for the measurement of naloxone in plasma. The early chromatographic methods described for measuring naloxone in plasma were rather lengthy procedurally and the RIAs, although highly sensitive, the materials used in them are difficult to purchase.

Described here is a simple, cheap to run, sensitive and accurate HPLC assay which uses an internal standard, SPE and coulometric end-point detection. The chemical structures for naloxone and the internal standard sumatriptan are given in Fig. 1.

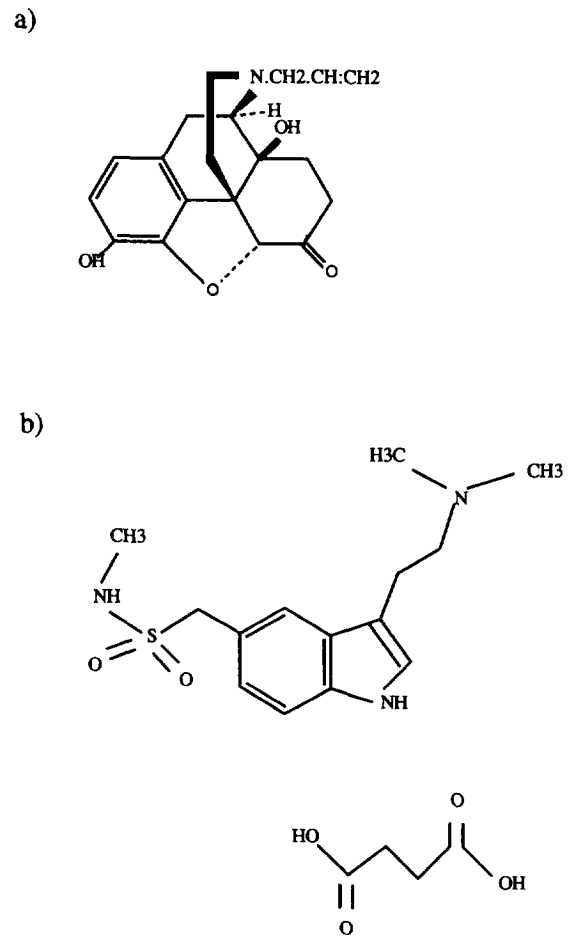

Fig. 1. Structures of (a) naloxone and (b) the internal standard, sumatriptan succinate.

2. Experimental

2.1. Materials

All reagents used in this procedure were of the highest grade available. Drug-free plasma for the preparation of standards and the quality control pools were obtained from normal healthy volunteer subjects. Naloxone and the internal standard were kindly donated by Roche Products (Welwyn Garden City, UK). The SPE columns (Isolute, 50 mg CBA) were purchased from Jones Chromatography (Hengoed, UK).

2.2. Apparatus

The HPLC apparatus consisted of a Jasco Model 980 intelligent pump, a Rheodyne 7125 injection valve fitted with a 50-μl loop, an ESA Coulochem 2 detector with a Model 5020 guard cell and a Model 5011 analytical cell, a 100×4 mm, 5 μm cyanopropyl column cartridge (Capitol HPLC, Edinburgh, UK) protected by a Brownlee Newguard C_2 precolumn (Anachem, Luton, UK) and a Varian Model 4400 integrator (Varian, Walton-on-Thames, Surrey, UK).

The electrode potentials for the detectors were selected following injection of fixed amounts of the analyte and the internal standard over the range 0.3–1.0 V (see Fig. 2). The selected potentials for the guard cell and detectors 1 and 2 were 0.9, 0.45 and 0.85 V, respectively. The detector response time was 2 s.

The HPLC mobile phase was 0.04 *M* potassium phosphate buffer (pH 5.3)–methanol (40:60, v/v). This was filtered and degassed prior to use. The flow-rate was 1.0 ml/min.

For naloxone analysis, peak heights were measured. The analyte concentration was assessed by using the slope of the standard curve for peak-height ratios.

2.3. Procedures

Whole blood was taken into lithium heparin (anticoagulant) tubes, centrifuged and the plasma

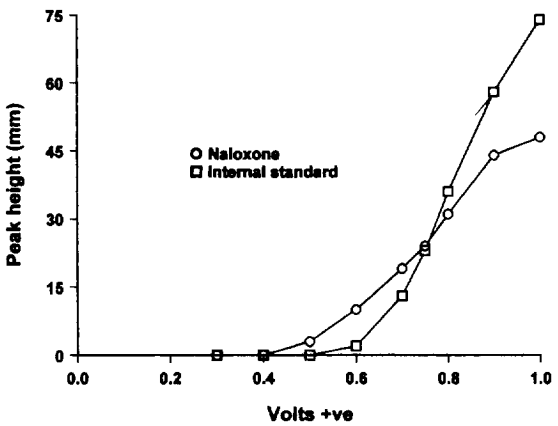

Fig. 2. Voltammogram of naloxone and the internal standard, sumatriptan for detector 2 (the analytical electrode) at different potentials. These were determined when the guard cell and detector 1 were at zero potential. The full scale deflection was 0.3 nA.

separated and then stored at −25°C until required for assay.

Stock solutions of naloxone and the internal standard were prepared at an initial concentration of 100 μg/ml in methanol, from these standards for assay were prepared freshly each day. Naloxone standard was made up in drug-free plasma in the range 0–50 ng/ml. Prior to SPE loading, 15 ng of the internal standard, contained in a small volume, was added to each 1 ml volume of standard or sample. The SPE (CBA type) columns were conditioned with full column volumes of methanol and water, in that order. The vacuum was diverted to stop columns from drying out and the standards and samples were then transferred to the columns. The vacuum was reapplied and the materials allowed to pass through. Each column was washed with two column volumes of water. Columns were then taken to dryness under vacuum. The vacuum was diverted and the vacuum manifold needles wiped dry with tissue. A rack containing 10×75 mm glass tubes was inserted into the manifold. Analytes were eluted with one column volume of 1% ammonia in methanol. The eluates were evaporated to dryness under vacuum at 50°C. Samples and standards were reconstituted into mobile phase (150 μl), vortex mixed and injected onto the HPLC system. Standards and samples were stable for a minimum of one week in the dry state and for at least 3 days in solution.

3. Results

Resolution of the analytes and sensitivity was determined by injection of an extracted plasma standard (see Fig. 3). The retention times for naloxone and the internal standard were 5.3 and 6.1 min, respectively. The linearity of the detector response was verified over the standard range. This was done by spiking drug-free plasma with known amounts of naloxone (in triplicate). A calibration curve was then

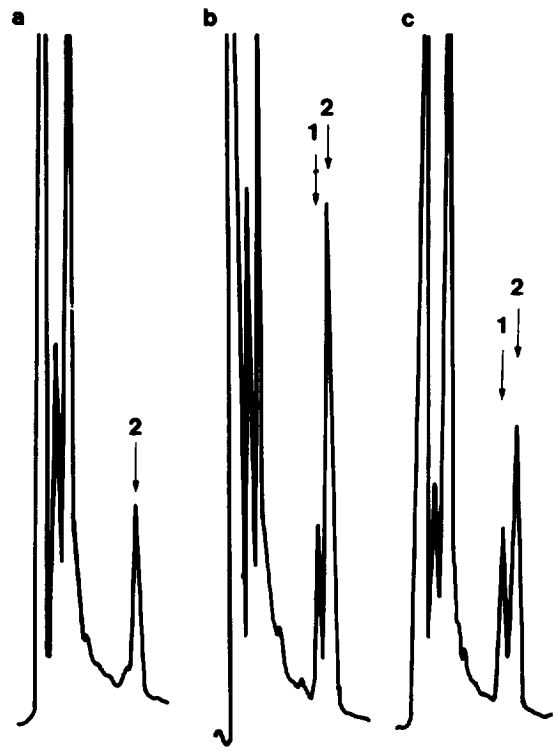

Fig. 3. Chromatograms of (a) blank drug-free plasma, (b) drug-free plasma spiked with 10 ng/ml of naloxone and (c) sample from normal volunteer following i.v. administration of 400 μg/kg naloxone (calculated to be 23.2 ng/ml). Peaks 1 and 2 represent naloxone and the internal standard, sumatriptan, respectively. The retention times for peaks 1 and 2 were 5.3 and 6.1 min, respectively.

Table 1
Inter- and intra-assay precision and accuracy data for the determination of naloxone in human plasma

Nominal value (ng/ml)	Observed value (ng/ml)		C.V.[a] (%)	
	Intra-assay	Inter-assay[b]	Intra-assay	Inter-assay
5	5.2 ±0.58	5.6±0.6	11.2	11.2
12	11.82±0.61	12.8±0.8	5.2	6.8
40	41.03±3.2	40.8±2.5	7.8	6.2

[a]The precision (C.V.) of the method was calculated from results for pooled drug-free plasma spiked with known amounts of naloxone).
[b]The inter-assay data was taken from 3 assay runs on separate days ($n=5$ for inter- and intra-assay experiments).

calculated using the peak-height ratio of the analyte, naloxone to the internal standard versus the concentration of the analyte. The mean equation for the calibration curve was $y=31.1x-1.13$ ($r=0.998\pm0.004$, $n=6$).

The inter- and intra-assay precision and accuracy profiles (C.V.) are given in Table 1. The absolute recovery of naloxone from spiked drug-free plasma was 90.4±8.9% (20 ng/ml, $n=14$).

A plasma profile of naloxone, following intravenous (i.v.) administration of naloxone in one normal healthy male volunteer who took part in a PET scanning study, is shown in Fig. 4. Naloxone was administered in two 400 μg/kg doses to this subject, the first was given as a bolus dose delivered over a short time interval at a time 30 min into the study procedure and the second being given at a time 65 min into the study. This was delivered for a period of 1 h.

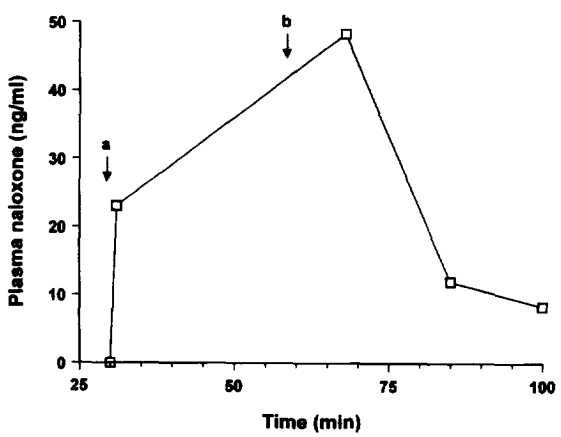

Fig. 4. Plasma concentrations of naloxone in one male volunteer following i.v. administration of 400 μg/kg (a) of naloxone in a single bolus at 30 min and (b) a top-up dose of 400 μg/kg delivered over 1 h from time 60 min.

4. Discussion

Described here is a simple and highly selective HPLC assay technique for the measurement of naloxone in human plasma. This utilizes coulometric end-point detection, SPE and an internal standard to monitor both extraction recovery and detector response variation.

The minimum amount of analyte detectable for the assay method (peak height equal to three times baseline noise) was 0.25 ng of naloxone on column. The detector response and the extraction procedure was linear over the range 0–50 ng/ml of naloxone in plasma.

It is not usual to utilize a mix of a C_2-guard column and a CN-analytical column, however for our use we found that a CN-analytical column alone gave more than adequate resolution of the analyte and therefore the use of a CN-guard column in addition would give unneeded extra resolution. Hence our use of a C_2-guard column which has only small retention properties for these compounds but nevertheless protects the analytical column.

The procedure shows a number of advantages and improvements over previous methods. For example this procedure utilizes SPE rather than previously described three-stage liquid–liquid extraction procedures [1] which is slow and would be expected to give much lower analyte recoveries. The assay described here also has advantages over a previously described assay [2]. The SPE technique used in our procedure is simpler and gains the same or greater

selectivity without the need of ion-pairing agents. When we tried the ion-pairing agent, octyl sodium sulphate, we found spurious peaks appearing in the chromatogram solvent front or shortly following the front, these we diagnosed as emminating from the ion-pairing agent.

Although this assay procedure is not as quick as an RIA method and does not have its sensitivity or capacity in terms of throughput, it is nevertheless a great deal more selective.

5. Conclusion

The method described here is fast, simple, cheap to run and can be set up easily in a routine clinical assay laboratory. The method has been used to successfully determine plasma naloxone in normal volunteers following i.v. administration.

References

[1] H. Derendorf, A. El-din, A. El-Koussi and E.R. Garrett, J. Pharm. Sci., 73 (1984) 621-624.
[2] R.W. Reid, A. Deakin and D.J. Leehey, J. Chromatogr., 614 (1993) 117-122.
[3] B.A. Berkiwitz, S.H. Ngai, J. Hemstead and S. Spector, J. Pharmacol. Exp. Ther., 195 (1975) 499-505.
[4] E.F. Hahn, R. Lahita, M.J. Kreek, C. Duma and C.E. Inturrisi, J. Pharm. Pharmacol., 35 (1983) 833-836.