Journal of Chromatography, 570 (1991) 419–424
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6015

Short Communication

Selective and quantitative isolation and determination of apomorphine in human plasma

A. W. G. ESSINK*, C. P. G. G. LOHUIS, J. T. KLEIN ELHORST and W. J. RUTTEN

Department of Clinical Pharmacy, Medisch Spectrum Twente, Ariënsplein 1, 7511 JX Enschede (Netherlands)

(First received January 23rd, 1991; revised manuscript received May 6th, 1991)

ABSTRACT

A simple extraction system for the selective and quantitative isolation of apomorphine from human plasma is described. Apomorphine and N-*n*-propylnorapomorphine were isolated by complex formation between a borate group and the diol group of the apomorphines in an alkaline medium, this in combination with ion-pair formation. The reproducibility and linearity of this extraction method combined with high-performance liquid chromatography with electrochemical detection is excellent. The absolute mean recovery of apomorphine was 100%, the recovery of N-*n*-propylnorapomorphine was 98%. The detection limit of apomorphine in human plasma in the described system is approximately 0.5 ng/ml.

INTRODUCTION

Apomorphine is well known for its dopaminergic effects. Recently it has been introduced for the treatment of Parkinson disease. It has been shown to benefit most patients who are disabled by severe on–off fluctuations [1–4].

Owing to its extensive first-pass metabolism, apomorphine must be administered via non-oral routes, mostly subcutaneously by continuous infusion or by repeated injections. A large inter-patient variability in response makes it clear that there is a need to monitor plasma drug concentration in acute studies in which the actions of the drug are compared in subgroups of patients [5,6]. The same holds for pharmacokinetic studies in humans and pharmaceutical studies to find appropriate drug delivery systems. Therefore a selective, simple and fast extraction system as well as a sensitive method for the determination of apomorphine in human plasma is required.

For the determination of apomorphine several analytical methods have been developed, such as fluorimetry [7], gas chromatography [8] and particularly high-performance liquid chromatography (HPLC) combined with ultraviolet [9], fluorimetric [10] or electrochemical detection (ED) [11], of which HPLC–ED is the

0378-4347/91/$03.50

most sensitive. Various non-specific separation methods have been used as a clean-up and concentration step [5,10–12]. However, in all these methods the extracts still contain considerable amounts of impurities which interfere with the HPLC method.

This paper describes the selective and quantitative isolation of apomorphine from human plasma, combined with a sensitive HPLC method to assay apomorphine. This method has been used to follow the response of patients to apomorphine administration in clinical conditions.

EXPERIMENTAL

Reagents and materials

Apomorphine · HCl · $1/2H_2O$ (APO) was supplied by Bufa Chemicals (Castricum, Netherlands). N-*n*-Propylnorapomorphine (NPA) was a generous gift from the Sterling-Winthrop Research Institute (Rensselaer, NY, USA). Standard spectrometric analysis and analysis by HPLC of the apomorphines gave no indication of impurities. All other solvents and chemicals were analytical-reagent grade. Water was deionized and distilled before use. All glass was of Pyrex quality.

Sample collection

Blood samples were collected in tubes containing 5 mg of sodium metabisulfite and 15 mg of Na_2EDTA and stored immediately on ice. Plasma was obtained by centrifugation at 4°C.

Plasma samples which were not immediately assayed were stored at −70°C. Under these conditions apomorphine in plasma is stable for at least six months.

High-performance liquid chromatography

The HPLC system consisted of a solvent delivery system (Perkin-Elmer, Series 10), an electrochemical detector (Kipp-Analytica, Model 9205) which was maintained at an applied potential of 0.7 V (range, 20 mA; filter, 1 s), a stainless-steel column packed with Nova-Pak C_{18} (Waters, 150 mm × 3.9 mm I.D., No. 86344) and an integrator (Hewlett-Packard 3390A).

The flow-rate was set at 0.5 ml/min. The mobile phase was acetonitrile (24%) in 0.1 *M* sodium dihydrogenphosphate, containing 0.3 g/l sodium chloride and 0.76 g/l sodium 1-octanesulphonic acid adjusted to pH 3 with phosphoric acid. The mobile phase was filtered through a Millipore filter (0.2 mm) and degassed by ultrasonication.

Extraction procedures

To 2 ml of plasma in a glass tube 100 μl of internal standard, 0.5 μg/ml NPA, were added.

To extract the apomorphines 1 ml of ammonium chloride ammonium hydroxide buffer (pH 8.45) containing 0.2% diphenylboratecthyleneamine

(DPBEA) and 0.5% EDTA was added. After the addition of 5 ml of a mixture of *n*-heptane, 10% octanol and 0.25% tetraoctyl ammonium bromide (TOABr), the mixture was shaken by hand for 2 min and centrifuged (5 min, 1500 *g*, 4°C). The organic layer (4 ml) was transferred into another tube. Thereafter 4 ml of *n*-octanol and 1 ml of 0.01 *M* phosphoric acid were added. After shaking by hand for 2 min and centrifugation (5 min, 1500 *g*, 4°C), the organic layer was discarded and 100 μl of the aqueous phase were injected into the HPLC system.

RESULTS AND DISCUSSION

Chromatography

Fig. 1 shows chromatograms of extracts of apomorphine and N-*n*-propylnorapomorphine from human plasma. As can be seen, the background is virtually free from interfering substances. Furthermore a satisfactory separation of apomorphine and NPA is achieved in the HPLC procedure (capacity factor (k'): apomorphine = 3.0, NPA = 6.2). Under the conditions of the assay plasma extracts can be injected every 15 min.

The devised method provides good accuracy for the determination of apomorphine in the concentration range 0.5–100 ng/ml. This range fits the level in human plasma in clinical practice; a level below 1 ng/ml is clinically irrelevant [4].

Table I shows peak concentration (C_{max}) of apomorphine in human plasma after subcutaneous injection of therapeutic quantities of apomorphine [13].

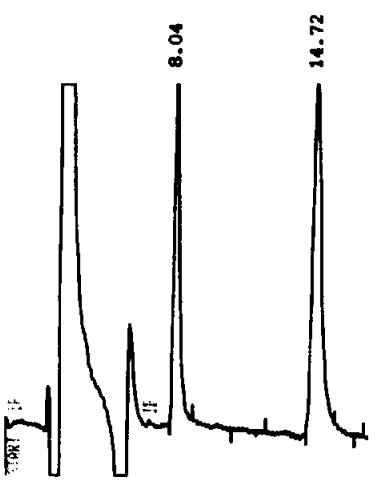

Fig. 1. Chromatograms of extracts from human plasma of a patient treated with apomorphine containing 25 ng/ml apomorphine (t_R = 8.04 min) and 25 ng/ml NPA (t_R = 14.72 min) added as an internal standard. The amounts of apomorphine and NPA chromatographed were 2.5 ng each.

TABLE I

PEAK PLASMA CONCENTRATION AFTER BOLUS INJECTIONS OF APOMORPHINE IN HUMAN PLASMA

Subject No.	Age (years)	Dose of apomorphine (mg)	Mean C_{max} (ng/ml)
1	53	1.0	8.6
2	29	1.5	19.0
3	64	1.5	20.5
4	57	3.0	43.4

Extraction procedure

The extraction method is a new application of the extraction principle for the isolation of catecholamines, described by Smedes *et al.* [14]. Apomorphine was isolated from plasma by complex formation between the borate group of DPBEA and the diol group of apomorphine in an alkaline medium. Because of its negative charge, this stable complex cannot migrate into the organic layer. Therefore an ion pair is formed by adding a TOABr as a cation to the organic phase. This ion pair is subsequently extracted into the organic phase, followed by back-extraction in an acid aqueous phase.

An additional advantage of this method is the stabilization of apomorphine, which is intrinsic unstable in solution. The complex formation protects apomorphine from decomposition during the extraction. This advantage has been stated for catecholamine extraction by Smedes *et al.* [14].

Reproducibility, linearity, recovery and sensitivity of the method

The linearity of the extraction method was tested with human spiked plasma with different concentrations of apomorphine. A linear relation between apo-

TABLE II

RECOVERY OF APOMORPHINE FROM SPIKED HUMAN PLASMA

Concentration (ng/ml)	Mean recovery (%)	*n*	R.S.D. (%)
Apomorphine			
5	98.3	5	2.4
25	98.9	5	2.5
50	100.7	5	2.2
N-n-Propylnorapomorphine			
50	98.8	5	2.2

morphine/NPA peak ratios in the concentration range 5–100 ng/ml apomorphine was obtained (slope = 0.041, y-intercept = 0.007, r = 0.9994).

The reproducibility (intra-assay variability) is excellent: the R.S.D. ranged between 1.2 and 4.7% (n = 5). The inter-assay variability was measured by taking a control sample (25 ng/ml) every day. The R.S.D. measured over several days was 8.7% (n = 6).

The recovery of the method was determined by spiking human plasma with known quantities of apomorphine and NPA covering the relevant "therapeutic" concentration level of apomorphine and extracting the samples as described in the *Extraction procedure* section, followed by analysis of the extracts by HPLC. The results of the measurements are given in Table II. The absolute mean recovery of apomorphine was 100%, the recovery of NPA 98%. This very high recovery of the apomorphines proves the stabilization of the apomorphines by complexation in the extraction procedure.

The reproducibility with the recovery studies was excellent (n = 5, R.S.D. = 2.2% for both).

The detection limit is approximately 0.5 ng/ml apomorphine (signal-to-noise ratio = approximately 7). As stated before, apomorphine levels which are relevant in clinical practice range from 1 to 100 ng/ml [4]. This range is easily achieved without any concentration step in the extraction procedure.

CONCLUSIONS

The method described for the extraction of apomorphine in human plasma, which is based on the complex formation between the diol group of apomorphine and the diphenylborate, is highly selective. It also gives a high recovery and an excellent reproducibility. In all these aspects this extraction method is superior to other extraction procedures for apomorphine described in literature [5,10,11]. The combination of this extraction method with HPLC and ED produces a powerful combination of selectivity and sensitivity which makes it very suitable for use in clinical pharmacokinetic studies.

ACKNOWLEDGEMENTS

Thanks are due to T. van Laar, M.D., and E. N. H. Jansen, M.D., Department of Neurology, Medisch Spectrum Twente, for their cooperation and support.

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