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Improved assay for R(−)-apomorphine with application to clinical pharmacokinetic studies in Parkinson's disease

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Abstract

A high performance liquid chromatographic assay for the quantitative determination of apomorphine in human plasma is described. Sample clean-up and concentration was optimised using solid-phase extraction on C18 cartridges, enabling rapid and sensitive determination of apomorphine and potential metabolites. The limit of apomorphine quantification, using fluorescence detection, was 0.5 ng/mL. The assay was stability-indicating, and allowed the detection of analytes in the presence of commonly co-administered anti-Parkinsonian drugs. Apomorphine was stable in frozen plasma containing 0.14% (w/v) ascorbic acid for 98 days, and through four freeze-thaw cycles. The assay has been used in clinical pharmacokinetic studies of apomorphine in patients with Parkinson's disease, and in preliminary studies of novel apomorphine delivery devices in volunteers.

Keywords: Apomorphine; Parkinson's disease; HPLC; Solid phase extraction; Plasma; Pharmacokinetics

1. Introduction

R(−)-apomorphine (I, Fig. 1) is a potent D1 and D2 dopamine receptor agonist used in the management of Parkinson's disease. Apomorphine is used primarily as an adjunct to oral anti-Parkinsonian therapies and has been shown to reliably control the disabling motor fluctuations that affect patients with Parkinson's disease, despite the optimisation of other anti-Parkinsonian medication [1–4]. Due to extensive first-pass metabolism apomorphine is administered parenterally, usually as multiple intermittent subcutaneous injections or as a continuous subcutaneous infusion during waking hours. The main adverse effect of subcutaneous apomorphine treatment is the development of cutaneous nodules at the injection site. Recently the use of alternative administration routes such as sublingual, buccal and needle-free injection have been investigated, in an attempt to improve drug delivery.

Enantiomeric interconversion, methylation, sulphation and glucuronidation of R(−)-apomorphine (the licensed product) have each been proposed as minor metabolic pathways in vivo. A previous investigation using chiral chromatography found no evidence of racemic interconversion [5]. Auto-oxidation of apomorphine to quinone species (III, Fig. 1) is a potentially important factor in apomorphine metabolism, however the significance of such species in terms of an anti-Parkinsonian response remains to be established [5,6]. Several methods have been described for the determination of apomorphine in plasma [5,10–16]. These were associated with various limitations mainly related to analyte instability during liquid-liquid extraction procedures. Apomorphine readily undergoes oxidation, especially in solution under alkaline and acidic conditions (the latter commonly used for liquid-liquid extractions of apomorphine from plasma), and in the presence of oxygen and light. M^{2+}-catalysis of apomorphine auto-oxidation has also been reported [7–9]. We have developed a new apomorphine assay addressing these stability issues for use in pharmacokinetic studies of existing and novel apomorphine delivery systems. The assay is stability-indicating, and allows the low-level detection of apomorphine and potential metabolites (III and IV, Fig. 1)

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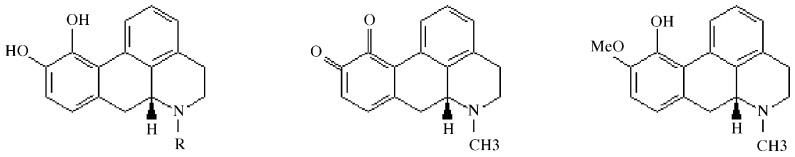

I apomorphine: R = CH_3 III apomorphine orthoquinone IV apocodeine

II NPA: R = $CH_2CH_2CH_3$

Fig. 1. Structure of apomorphine and selected related compounds.

in the presence of co-administered anti-Parkinsonian drugs. We have also addressed the stability and long-term storage of clinical samples.

2. Experimental

2.1. Reagents

R(−)-apocodeine HCl (purity 99% minimum), R(−)-apomorphine HCl (purity 99% minimum), ascorbic acid (ACS reagent), 2-mercaptoethanol (electrophoresis reagent), R-propylnorapomorphine HCl (NPA) (purity 99%) and Sigmacote (silanising agent) were purchased from Sigma–Aldrich Company Ltd. (Poole, UK).

Diaminoethanetetra-acetic acid sodium salt, methanol (HPLC Grade), orthophosphoric acid, sodium dihydrogen orthophosphate and sodium metabisulphite were obtained from Fisher Scientific (Loughborough, UK). Heptanesulphonic acid sodium salt "HiPerSolv for HPLC" was purchased from BDH Merck (Leicester, UK), and R(−)-apomorphine orthoquinone was supplied by SPA Contract Synthesis (Coventry, UK). All water used was 18 MΩ cm^{-1} water prepared using an Elga UHQ2 unit (Vivendi Water Systems, Wirksworth, UK).

2.2. Chromatography system

The HPLC system consisted of a ConstaMetric 3200 isocratic solvent delivery system (ThermoElectron, Manchester, UK), an AS-950 autosampler and FP-920 spectrofluorometer (excitation and emission wavelengths of 270 nm 450 nm, respectively) (Jasco, Great Dunmow, UK), and a UV6000P photodiode array detector (wavelength range of 198–798 nm) from ThermoElectron (Manchester, UK). Gold grade glass (2 mL) autosampler vials were employed (Chromacol Ltd., Welwyn Garden City, UK). The chromatography method was adapted from Priston and Sewell [15]. Separation was achieved using a Columbus (octyldecylsilane 5 μm 110 Å) analytical column (150 mm × 4.6 mm I.D.) purchased from Phenomenex (Macclesfield, UK). The pre-column consisted of a Security Guard cartridge system (octyldecylsilane) (containing 2 cartridges of dimensions 4 mm × 3 mm I.D.) (Phenomenex, Macclesfield, UK). Analyte retention on the Columbus column was achieved primarily with hydrophobic interactions between the analytes and bonded phase. Polar interactions were minimised by extensive capping of residual silanol groups. The combination of this, combined with high carbon loading, resulted in the resolution of apomorphine degradation peaks that co-eluted with apomorphine when alternative stationary phases were used. The constituents of the mobile phase, expressed as final concentrations in the mobile phase, were: 0.25 M sodium dihydrogen orthophosphate and 0.25% (w/v) heptane sulphonic acid (adjusted to pH 3.30 with orthophosphoric acid), 0.003% (w/v) EDTA and 40% (v/v) methanol. The flow rate was 1 mL/min and the system was run at ambient temperature. Data acquisition was performed using Borwin Chromatography software (v. 1.22.3, JMBS Developpements, Grenoble, France) and Chromquest (ThermoElectron, Manchester, UK). Typical chromatograms of extracted plasma samples are given in Fig. 2.

2.3. Preparation of standard solutions

In order to prevent auto-oxidation, stock standard solutions of apomorphine, NPA (the internal standard), apomorphine orthoquinone and apocodeine (I–IV, respectively) were prepared in the following diluent: 0.10% (w/v) EDTA and 0.15% (w/v) ascorbic acid (referred to as diluent A). The concentration of

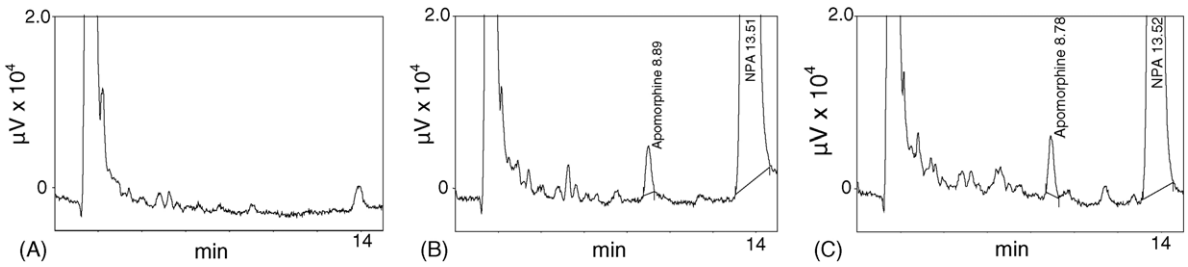

Fig. 2. Typical chromatograms (A) 10 min prior to apomorphine dose (1.2 mL of plasma extracted); (B) 181 min after subcutaneous administration of 2.0 mg bolus apomorphine (2.0 mL of plasma extracted, containing 0.6 ng/mL apomorphine); (C) pooled plasma from healthy volunteers spiked to 1.0 ng/mL apomorphine (1.0 mL of plasma extracted).

stock standard solutions was 1 mg/mL, except for NPA, which was 0.1 mg/mL. Silanised amber borosilicate glassware was used. Standards, and diluent A, were stored at 4–8 °C for up to 28 days. (Diluent A was also employed as the autosampler flushing solution.)

A working solution of 5 μg/mL NPA in diluent A was prepared and multiple aliquots of 1.2 mL were stored at 4–8 °C for up to 20 weeks.

Standards for assay validation and quality control (QC) were prepared in pooled plasma obtained from at least three healthy volunteers. QC standards were stored at −20 °C in multiple 1.2 mL aliquots for assay with each analytical run.

2.4. Blood sample pre-treatment

Venous blood was collected into a 7 mL EDTA k3 vacutainer tube (Southern Syringe Services, Bristol, UK). Blood was transferred into a polypropylene tube containing ascorbic acid (to give a final concentration of 5 mM ascorbic acid), gently mixed, then centrifuged (4 °C, 1250 × *g* × 5 min). The resulting plasma was removed and stored in a polypropylene tube at −20 °C until required for HPLC analysis. When required, plasma was thawed at 4–8 °C, and 1% (v/v) 2-mercaptoethanol in water added to give a final concentration of 0.01% (v/v). This was found to be the most effective anti-oxidant for preservation of apomorphine in plasma prior to extraction, but due to its strong odour was not suitable for use outside the laboratory. This mixture was then centrifuged (4 °C, 1250 × *g* × 5 min) in order to pellet precipitated protein and the resultant supernatant was transferred to a polypropylene tube [15]. Plasma was then spiked with internal standard (to give a final concentration of 100 ng/mL), mixed gently and left to equilibrate at 4–8 °C for 5 min prior to solid-phase extraction.

2.5. Extraction procedure

The extraction method was adapted from Priston and Sewell [15]. Apomorphine was extracted under vacuum using Bond-Elut octyldecylsilane 1 mL 100 mg solid phase extraction columns (Varian Sample Preparation Ltd., Surrey, UK). The hydrophobic characteristics of this bonded phase gave excellent retention of the analytes, enabling thorough washing to remove interferences. The vacuum manifold used was a Techelut unit (HPLC Technology, Macclesfield, UK). The solid-phase extraction column was conditioned with 2 mL methanol followed by 2 mL water. The plasma sample (between 0.25 and 2.5 mL in volume) was then applied to the solid phase extraction column and washed with 2 mL water followed sequentially by 1 mL 10% (v/v) methanol in water, 1 mL 20% (v/v) methanol in water, 1 mL 50% (v/v) methanol in water, and finally, 200 μL of 2% (w/v) sodium metabisulphite in diluent A (an additional step, necessary to prevent apomorphine degradation during elution). The column matrix was not allowed to dry out at any of the conditioning and washing stages. The column was transferred to a primed "elution" manifold port (see below) and analytes were eluted to dryness with 800 μL of eluting solution, i.e. 40:60 (v/v) methanol: 0.25 M sodium dihydrogen orthophosphate (to pH 3.30 with orthophosphoric acid), into an autosampler vial containing 200 μL 2% (w/v) sodium metabisulphite in diluent A. This optimised eluting solvent with the addition of additional anti-oxidants resulted in the preservation of apomorphine during the extraction procedure. The vial was sealed and placed at 4 °C prior to assay by HPLC.

In order to avoid contamination of subsequent extractions with residual analyte, the "elution port" was primed by washing with 5 mL of 0.01 M HCl in 50% (v/v) methanol:distilled water, followed by 10 mL of distilled water and then air-dried using suction generated by the vacuum pump.

2.6. Validation

2.6.1. Assay selectivity

Plasma samples collected from six healthy volunteers were subjected to the assay procedure and the chromatograms inspected for the presence of interference by endogenous compounds. In addition, the following potentially co-administered drugs were tested for chromatographic interference: bromocriptine, cabergoline, co-beneldopa, co-careldopa, domperidone, entacapone, lisuride, paracetamol, pergoline, pramipexole, selegiline, co-danthrusate and co-danthramer as were the proposed apomorphine metabolites R(−)-apocodeine HCl and R(−)-apomorphine orthoquinone (Fig. 3).

Peak purity values for apomorphine and NPA (1 μg/mL), as non-extracted standards in diluent A and also following extraction from plasma, were obtained. Peak purities were calculated using a scan threshold of 5 mAU and a peak coverage requirement of 95%, and were expressed as a spectral similarity index (S.S.I.), where S.S.I. = 1 represents a pure peak. In the comparison of spectra for identification purposes, a S.S.I. of greater than or equal to 0.980 was indicative that a match has been obtained [17].

2.6.2. Stability-indication study

A stability-indication study was performed to establish whether apomorphine and NPA could be distinguished from degradation products, particularly those produced during the

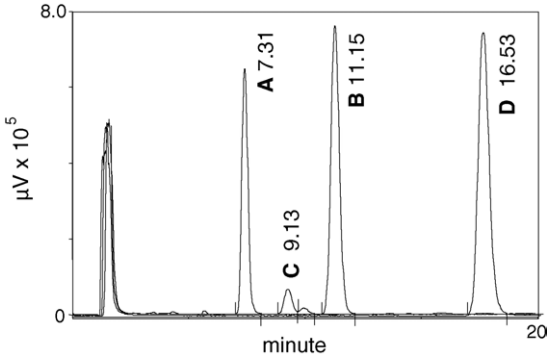

Fig. 3. Assay selectivity with respect to proposed apomorphine metabolites. (A) R(−)-apomorphine HCl in diluent A (50 ng/mL); (B) R(−)-NPA HCl in diluent A (100 ng/mL); (C) R(−)-apocodeine in diluent A (50 ng/mL); (D) R(−)-apomorphine orthoquinone in diluent A (140 ng/mL). Retention times given as peak labels.

"clean-up" procedure. Thus apomorphine (1 mL of 0.5 μg/mL in distilled water) and NPA (1 mL of 1.0 μg/mL in distilled water) were incubated separately with each of the following: (i) 1 mL of 0.1 M hydrochloric acid; (ii) 1 mL of 0.1 M sodium hydroxide; (iii) 1 mL of 6 vol hydrogen peroxide; (iv) 1 mL distilled water; (v) 1 mL distilled water at 4–8 °C (as a control for the effects of heating). The acidic and alkaline solutions were neutralised after the incubation period by addition of 1 mL of 0.1 M sodium hydroxide and 0.1 M hydrochloric acid, respectively. Finally, each test mixture and control solution was made up to volume with distilled water to give a final concentration of 50 ng/mL for apomorphine, and 100 ng/mL for NPA. Solutions of apomorphine (50 ng/mL) and NPA (100 ng/mL) in diluent A stored at 4–8 °C acted as controls for the use of distilled water as a diluent.

The stability-indication study was repeated with apomorphine and NPA standards prepared in diluent A in the first instance, instead of distilled water.

2.6.3. Calibration

Calibration standards were prepared in control-pooled plasma (range: 0.50–70 ng/mL, $n = 9$). The volume of stock R(−)-apomorphine HCl solution used for spiking was between 0.30 and 2.50% of the total volume of the calibration standards [18]. The linearity of the standard curve was evaluated using least-squares linear regression.

The limit of detection (LOD) was defined as the lowest concentration of apomorphine that could be distinguished from the noise level at a signal:noise ratio of 3:1. The limit of quantitation (LOQ) was defined as the lowest concentration of apomorphine that could be measured with a relative error of ±20% from the nominal concentration, and with a coefficient of variation (C.V.) of <20% [19].

Absolute recovery of analyte in plasma were calculated for all 1 mL extractions of comparing the peak area of the standard in plasma to that of a standard in diluent A which had not been subjected to solid phase extraction (range: 1–70 ng/mL ng/mL, $n = 8$).

The linearity of response over a volume range of 0.25–2.5 mL of plasma was also examined. Volumes of 0.25, 0.5, 1.0, 2.0 and 2.5 mL of plasma, at apomorphine concentrations of 3 and 20 ng/mL, were assayed (incorporating the internal standard). This equates to an analyte mass range of 25–300 ng.

2.6.4. Assay precision

The intra-batch precision of apomorphine and NPA as solid-phase extraction eluate was determined at each QC concentration. Thus eluate resulting from two separate 1 mL extractions of plasma was pooled (to give a sufficient volume for multiple injections on to the analytical column) and five injections were made.

Intra- and inter-day coefficients of variation for the assay were determined by the analysis of seven QC samples (seven times in one day, or once a day for seven days, respectively, analysed in duplicate). Absolute recoveries at each QC concentration were determined by comparing the peak area of the standard in plasma to that of a standard in diluent A.

The criteria used for acceptable assay precision were that the C.V. should not exceed 15%, and the mean value should be within ±15% of the nominal value for accuracy [18].

2.7. Stability studies

The stability of apomorphine (1 ng/mL and 20 ng/mL) in pooled control plasma was tested under the following conditions: (i) storage at −20 °C for 161 days (using sampling intervals of day = 0, 1, 2, 4, 7, 10, 14, and weekly thereafter); (ii) freeze-thaw cycles (over four consecutive days) in which a single aliquot of spiked plasma was assayed, placed at −20 °C overnight, then thawed to 4–8 °C, re-assayed and re-frozen. QC standards (stored at −20 °C) were assayed each day as controls, not having been subjected to multiple freeze-thaw cycles.

The stability of apomorphine and internal standard in solid-phase extraction eluate stored at 4–8 °C was tested over 31 days (using sampling intervals of day = 0, 1, 4, 7, 10, 14, 21, 28 and 31). The apomorphine:NPA peak area ratio, rather than the absolute potency of the analytes, was used as a marker of stability of the extract.

The stability at 4–8 °C of 5 μg/mL NPA in diluent A was investigated over a 24-week period. A fortnightly sampling interval was employed. Injections of test solutions (100 ng/mL) were bracketed injections of freshly prepared NPA in diluent A.

The acceptable limits for analyte stability were obtained from the precision experiments given in Section 2.6.4, i.e. limits for the long-term storage of frozen QC samples were ±2 × standard deviation obtained for the intra-day precision of QC samples ($n = 7$).

3. Results

3.1. Assay validation

3.1.1. Assay selectivity

Although co-danthrusate and co-danthramer are not licenced for use in Parkinson's Disease, these laxatives are occasionally used in practice. Co-elution and interference from these drugs was eliminated by increasing the 50% (v/v) methanol:water washes from 1 × 1 mL to 3 × 1 mL. This had the effect of removing the interfering compounds from the solid phase extraction column (to waste), without detriment to the isolation and subsequent elution of apomorphine.

Assay of selegilene solution resulted in the detection of a peak at the retention time window of apomorphine. However, following solid phase extraction the interference was entirely removed from the extract, with approximately 93% (in terms of peak area compared to the non-extracted solution) of the interfering compound eluted in the 50% methanol wash and approximately 6% in the 2% (w/v) sodium metabisulphite wash.

Peak spectra analysis revealed high peak purity (S.S.I > 0.990) for apomorphine and NPA (1 μg/mL) as non-extracted standards in diluent A, and also following extraction from plasma. Furthermore, the spectra of non-extracted and extracted analyte exhibited high similarity (S.S.I. > 0.992).

Table 1
Fraction of initial analyte peak area remaining (%) following forced degradation

Reaction conditions		Apomorphine	NPA
Diluent A (control)	4–8 °C	100% after 30 min	100% after 30 min
Distilled water	4–8 °C	81% after 30 min	98% after 30 min
	60 °C	65% after 30 min	25% after 30 min
0.1 M hydrochloric acid	60 °C	4% after 10 min	10% after 10 min
	25 °C	4% after 3 s	–
0.1 M sodium hydroxide	60 °C	2% after 10 min	4% after 10 min
	25 °C	8% after 3 s	-
6 vol hydrogen peroxide	60 °C	79% after 30 min	76% after 30 min

3.1.2. Stability-indication study

Apomorphine and NPA degraded under each the four reaction conditions, and degradation products were observed in each case (Table 1 and Fig. 4). Both apomorphine and the internal standard NPA were clearly resolved from all hydrolytic and oxidative degradation products. The stability-indication study was repeated, using the same conditions as described, except that apomorphine and NPA standards were prepared in diluent A in the first instance instead of distilled water. Degradation of each analyte was markedly reduced compared to standards in distilled water.

3.1.3. Calibration

The standard plot was linear for a concentration range of 0.50–70 ng/mL ($y = 0.0158x + 0.0034$, $R^2 = 0.9951$, $n = 9$). The mean (S.D.) relative error of the observed versus calculated data points was −4.2 (9.4)%. The mean absolute value of the relative error of the observed versus calculated data points was 8.1%. The LOD was 0.03 ng/mL (extracted from 2.5 mL of plasma). The LOQ was 0.5 ng/mL (extracted from 2 mL of plasma).

The mean absolute recovery of apomorphine was 72% (S.D. 5%, C.V. 7%) ($n = 8$). The mean absolute recovery of NPA was 67% (S.D. 2%, C.V. 3%) ($n = 8$). The recovery of each analyte, whilst being less than maximal, was considered to be acceptable given the consistency in recovery throughout the calibration standard range [20].

The mean concentrations of apomorphine for the extractions of 3 and 20 ng/mL from 0.25 to 2.5 mL plasma were calculated to be 3.0 ng/mL (S.D. 0.2 ng/mL, C.V. 5.3%) and 19.0 ng/mL (S.D. 0.5 ng/mL, C.V. = 2.4%), respectively ($n = 6$). There was no indication that the capacity of the solid phase extraction bonded phase had been reached. This was evidenced by the linearity of the response with increasing mass of analyte ($y = 1.3 \times 10^5 x - 1.9 \times 10^5$, $R^2 = 0.9936$, $p < 0.001$).

3.1.4. Assay precision

Precision for the apomorphine:NPA peak area ratio following multiple injection of pooled eluate compared well with the intra-batch precision of peak area ratios for non-extracted standards in diluent A (for the latter, C.V. was 0.36% and 0.52% for 1 ng/mL and 20 ng/mL, respectively $n = 5$) (Table 2).

The intra-day C.V. of the QC standards was less than 8.2%, with a mean absolute relative error less than 8.8%. The inter-day C.V. was less than 5.8%, with a mean absolute relative error less than 1.7%.

In summary, the assay operated within predefined limits for precision and accuracy (Table 2).

3.2. Stability studies

Each stability study chromatogram was scrutinised for the presence of peaks with equivalent retention times to those resulting from the forced degradation of apomorphine; no such peaks were found.

3.2.1. Stability of Apomorphine in Plasma at −20 °C Containing Ascorbic Acid

The limits for analyte stability were defined as ±2S.D. from the analyte concentration at time = 0, whereby the S.D. used was that demonstrated for the intra-day precision of apomorphine in plasma.

Based on these criteria, apomorphine was stable for 98 days (Table 3).

3.2.2. Freeze-thaw cycles: apomorphine in plasma

The limits for analyte stability were defined as ±2S.D. from the analyte concentration at time = 0, whereby the S.D. used was that obtained for the precision of the experimental controls. During the course of four freeze-thaw cycles, apomorphine concentration did not exceed the defined acceptable limits (Table 3). At a nominal apomorphine concentration of 1 ng/mL the mean (S.D.) observed concentration was 0.96 (0.07) ng/mL for the test solutions and 1.0 (0.02) ng/mL for the controls. At a nominal apomorphine concentration of 20 ng/mL the mean (S.D.) concentration was 17.52 (0.62) ng/mL for the test solutions and 18.04 (0.43) ng/mL for the controls.

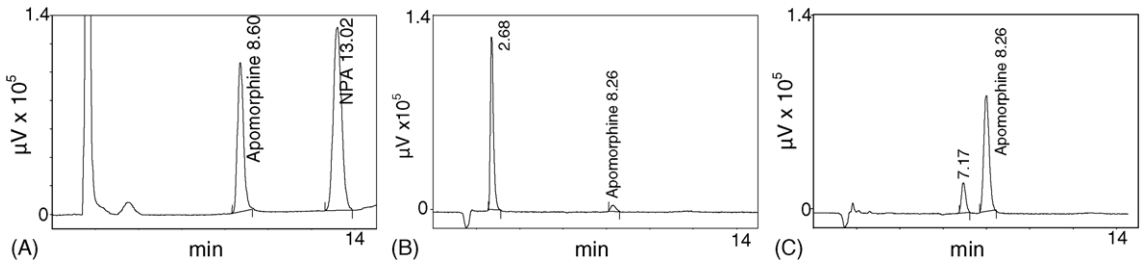

Fig. 4. Forced degradation of apomorphine: (A) R(−)-apomorphine HCl (50 ng/mL) and R(−)-NPA HCl (100 ng/mL) in diluent A (60 mins at 4–8 °C, control); (B) Reaction of apomorphine with HCl (3 s at 25 °C); (C) Reaction of apomorphine with H_2O_2 (60 mins at 60 °C). Retention times are given as peak labels.

Table 2
Assay precision for R(−)-apomorphine HCl extracted from pooled plasma from healthy volunteers

Nominal concentration (ng/mL)	Observed concentration (mean ± S.D.) (ng/mL)	C.V. (%)	Mean relative error (%)	Mean recovery (%)	
				Apomorphine	NPA
Pooled eluate (n = 5 injection)					
1		0.4			
2		0.8			
Individual extacts (n = 7)					
Intra-day					
0.50	0.54 ± 0.09	8.1	7.1	73.4	58.6
20.00	18.26 ± 0.76	2.1	−8.7	74.4	70.9
50.00	48.23 ± 0.79	1.6	−3.5	67.6	55.9
Inter-day					
0.50	0.49 ± 0.03	1.5	−1.1	75.6	65.9
1.00	0.87 ± 0.03	3.9	−1.1	78.3	55.9
20.00	19.69 ± 1.10	5.7	−1.6	66.1	60.9
50.00	50.46 ± 2.10	4.1	0.9	73.1	65.0

Table 3
Summary of stability studies of apomorphine and NPA

Experimental conditions	Analyte concentration (ng/mL)	Limits: 100% remaining ± x%	Period of stability	%Remaining at end of period of stability	%Remaining CV (%)
Apomorphine in plasma at −20 °C	1	7.88%	98 days	95.0	5.1 (n = 27)
	20	11.32%	98 days	93.4	6.0 (n = 27)
Apomorphine in plasma at −20 °C, freeze-thaw cycle	1	4.38%	4 cycles	98.0	1.9 (n = 5)
	20	4.72%	4 cycles	96.8	3.5 (n = 5)
NPA in diluent A at 4–8 °C	100	1.44%	20 weeks	101.0	1.1 (n = 13)
Plasma extract at 4–8 °C.	1	2.98%	31 days	97.4	3.8 (n = 9)
	20	1.66%	31 days	98.3	0.7 (n = 9)

x% = ±2S.D. from the analyte concentration at time 0, calculated using the C.V. of the precision of the experimental control.

3.2.3. NPA in diluent A at 4–8 °C.

The limits for analyte stability were defined as ±2S.D. from the analyte concentration at time = 0, whereby the S.D. used was that demonstrated for the intra-day precision of NPA (100 ng/mL) in diluent A. It was demonstrated that for a period of 20 weeks, NPA concentration did not exceed the pre-defined acceptable limits (Table 3).

3.2.4. Plasma extract at 4–8 °C

The acceptable limits for analyte stability were defined as ±2S.D. from the apomorphine:NPA peak area ratio at time = 0, whereby the S.D. used was that obtained for the intra-batch precision of the apomorphine:NPA peak area ratio of the extract. The solid-phase extraction eluate remained stable up to and including day 31 (Table 3).

3.3. Application of the assay

Eleven patients with Parkinson's disease and eighteen healthy volunteers participated in our studies of apomorphine pharmacokinetics. Co-administered drugs included: amitriptyline, benzhexol, co-beneldopa, co-caroldopa, diazepam, domperidone, entacapone, paracetamol, pergolide, ropinerole, selegiline. Fig. 5 shows the plasma concentration-time profile following subcutaneous bolus administration of 2.0 mg (i.e. 35 μg/kg) apomorphine (Britaject®, Britannia Pharmaceuticals Ltd.) to a patient with Parkinson's disease. Other medications administered to this patient were domperidone, co-beneldopa and ropinerole. Concentration-time data were modelled using

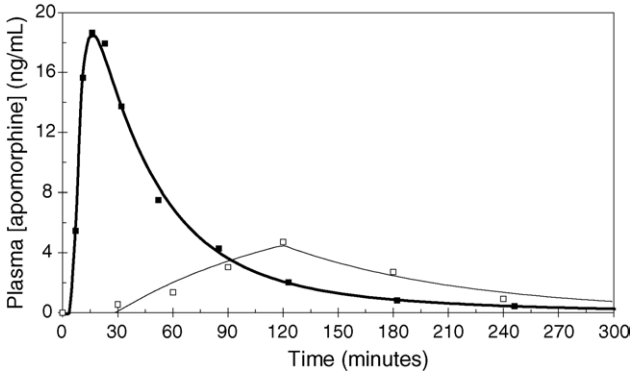

Fig. 5. Apomorphine kinetics following 2.0 mg subcutaneous injection at t = 0 to a patient with Parkinson's disease (filled squares), and 7.4 mg apomorphine administered via the buccal mucosa over 0–120 min to a healthy volunteer (open squares).

a two-compartment model with first-order input, first-order output, and a lag time. Also shown is the profile following a dose of 7.4 mg apomorphine administered via the buccal mucosa over 0–120 min to a volunteer. These concentration-time data were modelled using a one compartment model with first-order input, first-order output, and a lag-time.

4. Discussion

Evaluation of previously published LC methods for the quantification of apomorphine in plasma revealed significant limitations. The main difficulty was apomorphine degradation during liquid-liquid extraction, giving rise to LC peaks additional to the analyte and internal standard. This degradation was observed with several methods [12,14] and was probably attributable to M^{2+} catalysed oxidation under the acidic extraction conditions. The recovery of apomorphine was a particular concern with other methods [11,21] where recovery from pooled plasma at 20 ng/mL concentration was 47% and 4%, respectively. This level of recovery was not deemed acceptable for PK studies on prolonged infusion of apomorphine where plasma concentrations were expected to be low, particularly post-infusion.

The assay was selective for apomorphine, with the exception of dantron. Dantron was identified as a source of assay interference, however the interference could be eliminated from the solid-phase extraction eluate by employing additional 50% (v/v) methanol:water washes during solid-phase extraction.

The assay was stability-indicating for degradation products produced under acidic, alkaline and oxidative conditions. Marked degradation of apomorphine and NPA occurred under the reaction conditions of the stability-indication test, most strikingly under acidic and alkaline conditions whilst in the absence of diluent A. Assay conditions were optimised with these findings in mind, in fact the inclusion of a anti-oxidant wash step (sodium metabisulphite in diluent A) was critical to the preservation of analyte levels in the final extract. We issue caution against the use of acidified solvents in the extraction of apomorphine from plasma, such as in the methods of Bolner [11], Priston and Sewell [15], and Sam et al. [14], and especially in the absence of anti-oxidants. We recommend that a stability indication is performed in order to recognise degradation products. The use of fluorescence detection, as opposed to electrochemical detection [14,21,22], allows the detection of apomorphine oxidisation products.

In conclusion, the method presented for the determination of R(−)-apomorphine in human plasma was shown to be specific, accurate and precise. In addition, the method was suitable for the quantitation of two apomorphine metabolites (apomorphine orthoquinone and apocodeine), although neither were detected in any clinical samples analysed in this study. This method has been used in studies of apomorphine pharmacokinetics in Parkinson's disease, and also in preliminary studies of novel delivery devices in healthy volunteers where the dose (i.e. 1.6–7.4 mg delivered over 120 mins) was lower than that encountered in the clinical Parkinson's disease studies (2–10 mg subcutaneous bolus).

References

[1] J.D. O'Sullivan, Lees.F A.J., Hosp. Med. 60 (1999) 816.
[2] R.B. Dewey Jr., J.T. Hutton, P.A. LeWitt, S.A. Factor, Arch. Neurol. 58 (2001) 1385.
[3] F. Stocchi, L. Vacca, M.F. De Pandis, L. Barbato, M. Valente, S. Ruggieri, Neurol. Sci. 22 (2001) 93.
[4] P. Kanovsky, D. Kubova, M. Bares, H. Hortova, H. Streitova, I. Rektor, V. Znojil, Movement. Disord. 17 (2002) 188.
[5] R. van der Geest, T. van Laar, P.P. Kruger, J.M. Gubbens-Stibbe, H.E. Bodde, R.A. Roos, M. Danhof, Clin. Neuropharmacol. 21 (1998) 159.
[6] F.C. Colpaert, W.F. Van Bever, J.E. Leysen, Int. Rev. Neurobiol. 19 (1976) 225.
[7] P. Lundgren, L. Landersjo, Acta Pharm. Suec. 7 (1970) 133.
[8] M.J. Priston, G.J. Sewell, Pharm. Sci. 1 (1995) 91.
[9] T. van Laar, Pharmacokinetics and Clinical Efficacy of Apomorphine in Patients with Parkinson's Disease, Leuven University Press, Leuven, Belguim, 1996.
[10] E. Ameyibor, J.T. Stewart, J. Chromatogr. B 686 (1996) 297.
[11] A. Bolner, L. Barbato, F. Tagliaro, A. Monge, F. Stocchi, G. Nordera, Forensic. Sci. Int. 89 (1997) 81.
[12] F. Durif, E. Beyssac, F. Coudore, M. Paire, A. Eschalier, M. Aiache, J. Lavarenne, Clin. Neuropharmacol. 17 (1994) 445.
[13] R.V. Smith, M.R. De Moreno, J. Chromatogr. 274 (1983) 376.
[14] E. Sam, S. Sarre, Y. Michotte, N. Verbeke, Eur. J. Pharmacol. 329 (1997) 9.
[15] M.J. Priston, G.J. Sewell, J. Chromatogr. B 681 (1996) 161.
[16] T. van Laar, R. van der Geest, M. Danhof, H.E. Bodde, P.H. Goossens, R.A. Roos, Clin. Neuropharmacol. 21 (1998) 152.
[17] Chromquest Users Guide, ThermoElectron, Manchester, UK, Revision B, 1998, p. 18.
[18] F. Bressolle, M. Bromet-Petit, M. Audran, J. Chromatogr. B 686 (1996) 3.
[19] T.C. Paino, A.D. Moore, Pharm. Technol. 9 (1999) 86.
[20] V.P. Shah, K.K. Midha, S. Dinhe, I.J. McGilvery, J.P. Skelly, A. Yacobi, T. Layloff, C.T. Viswanathan, C.E. Cook, R.D. McDowall, K.A. Pittman, S. Spector, Pharm. Res. 9 (1992) 388.
[21] R. van der Geest, P. Kruger, J.M. Gubbens-Stibbe, T. van Laar, H.E. Bodde, M. Danhof, J. Chromatogr. B 702 (1997) 131.
[22] E. Nicolle, P. Pollak, F. Serre-Debeauvais, P. Richard, C.L. Gervason, E. Broussolle, M. Gavend, Fundam. Clin. Pharmacol. 7 (1993) 245.