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Simultaneous determination of levodopa, its main metabolites and carbidopa in plasma by liquid chromatography

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Abstract: An ion-pair reversed-phase liquid chromatographic method for the simultaneous determination of levodopa, 3-*O*-methyldopa, 3,4-dihydroxyphenylacetic acid, homovanillic acid and carbidopa in plasma designed for clinical trials performed to study the effect of peripheral catechol-*O*-methyltransferase inhibitors on the metabolism of levodopa is described. The high sample throughput of over 50 samples per day of the method makes it ideal for the assay of the large number of samples encountered in clinical trials. After protein precipitation with perchloric acid the analytes are completely separated within 15 min and determined down to a plasma concentration of 20 ng ml^{-1} using amperometric detection at 800 mV relative to an Ag/AgCl reference electrode. For all analytes the within-day precision defined as a relative standard deviation ($n = 8$) is lower than 7 and 3% at plasma concentrations of 20 and 40 ng ml^{-1}, respectively. As the method is specific and highly reproducible, the most important factor affecting accuracy is the stability of the analytes during storage and analysis.

Keywords: *Reversed-phase ion-pair liquid chromatography; electrochemical detection; plasma; levodopa and metabolites; carbidopa.*

Introduction

Levodopa (L-dopa), the medication of choice for the treatment of Parkinson's disease, is principally metabolized by catechol-*O*-methyltransferase (COMT) into 3-*O*-methyldopa (3-OMD), and by an aromatic amino acid decarboxylase (dopadecarboxylase, DDC) to dopamine (DA). DA is rapidly deaminated by monoamine oxidase (MAO) into 3,4-dihydroxyphenylacetic acid (DOPAC), which subsequently is methylated to homovanillic acid (HVA) by COMT. When a peripherally acting DDC inhibitor such as carbidopa is used to improve the bioavailability of L-dopa, *O*-methylation becomes a quantitatively even more important metabolic pathway [1]. Inhibition of the extracerebral COMT activity might thus improve further the bioavailability of L-dopa [2]. A method for simultaneous determination of L-dopa, 3-OMD, DOPAC, HVA and carbidopa in human blood plasma was designed for human pharmacokinetic trials performed to assess the effect of new peripheral COMT inhibitors on the metabolism of L-dopa. Good separations of such chemically diverse catechol derivatives have been obtained using ion-pair reversed-phase high-performance liquid chromatography (HPLC) [3]. Electrochemical detection (ED) offers good selectivity and sensitivity for these catechols and methoxycatechols. The plasma concentrations of the analytes of interest span from tens to hundreds of nanograms per ml after a single dose of 100 mg L-dopa/25 mg carbidopa. With such concentrations it is not necessary to employ selective and enriching sample preparation methods such as alumina adsorption [4–8], liquid–liquid extraction of the diphenylborate complexes as ion-pairs [9, 10] for the free catechols or cation-exchange for the free and methylated catechol amines and catechol amino acids [11, 12], which are generally required for lower concentrations of such analytes in HPLC–ED assays.

Rapid and simultaneous HPLC–ED determination of the chemically diverse compounds of interest after a single-step sample preparation procedure is possible if a non-selective sample preparation method such as direct injection of deproteinized plasma is employed. Methods for simultaneous determination of relatively high concentrations of L-dopa and 3-OMD in serum or plasma directly after protein

precipitation with perchloric acid [13, 14] or after extraction of the deproteinated plasma using C-18 cartridges [15] have been described. Very low concentrations of L-dopa and 3-OMD in human plasma were determined after protein precipitation with trichloroacetic acid by a gradient HPLC run during which the potential of the ED was changed [16]. In two methods where L-dopa, 3-OMD, DOPAC and carbidopa were determined after protein precipitation the incomplete chromatographic resolution of 3-OMD and carbidopa was compensated for by using two detection potentials [17] or ED and fluorimetric detection in series [18]. This paper presents a precise and highly selective HPLC–ED method for simultaneous determination of L-dopa, its three main metabolites and carbidopa in plasma after rapid protein precipitation. The concentration range, limit of determination and high sample throughput of the method make it suitable for large clinical studies on the pharmacokinetics and metabolism of levodopa administered in combination with carbidopa and COMT inhibitors.

The 3-OMD to L-dopa concentration ratio in plasma may be important for the clinical response to L-dopa in Parkinsonian patients [19]. This method should also be useful in monitoring drug and metabolite plasma concentrations of such patients.

Experimental

Chemicals

L-Dopa, carbidopa and α-methyldopa were obtained from Orion Pharmaceutica (Espoo, Finland). 4-*O*-Methyldopa (4-OMD), 3,4-dihydroxyphenylpyruvic acid (DHPPA), nitecapone, OR-611 and OR-1323 were synthesized by Orion Pharmaceutica. DOPAC, HVA, 3-OMD, DA, 3,4-dihydroxyphenyl glycol (DHPG), 3,4-dihydroxymandelic acid (DOMA), vanillylmandelic acid (VMA), noradrenaline (NA), adrenaline (A), 3-methoxy-4-hydroxyphenyl glycol (MHPG), 3-methoxy-4-hydroxyphenylethanol (MHPE), 3-methoxytyramine (3-MT), 5-hydroxyindole-3-acetic acid (5-HIAA) and 5-hydroxytryptamine (5-HT) were purchased either as the free acid, free base or salt from Sigma (St. Louis, MO, USA). 1-Octanesulphonic acid sodium salt (HPLC grade) was obtained from Eastman Kodak (Rochester, NY, USA). Ethylene glycol, 2-butanol, citric acid, sodium metabisulphite, disodium salt of ethylenediaminetetraacetic acid (disodium EDTA), perchloric acid (all of analytical grade) and triethylamine (synthetic grade) were purchased from Merck (Darmstadt, FRG). Acetonitrile and tetrahydrofuran (HPLC-grade) were obtained from Rathburn (Walkerburn, UK). Orthophosphoric acid (85%, AnalaR grade) was purchased from BDH Limited (Poole, UK). Sodium hydroxide (10 M) and hydrochloric acid (1 M) were obtained from Orion Pharmaceutica. Ultrapure reagent-grade water was obtained by means of a Milli-Q system (Millipore, Milford, MA, USA).

High-performance liquid chromatography

A Hewlett–Packard Model 1090 chromatograph (Waldbronn, FRG) was used to deliver the mobile phase in isocratic mode at a flow rate of 1.10 ml min^{-1}. The temperature was set at 28°C. The injection volume of the autosampler of the 1090 chromatograph was 30 μl. The Spherisorb ODS2 (150 × 4.6 mm i.d., 5 μm particle size, Phase Separations, Deeside, UK) analytical column was protected by a μBondapak C-18 guard column (10 μm particle size, Guard-PAC, Waters, Milford, MA, USA). The glassy carbon working electrodes of the LC4B amperometric detector (Bioanalytical Systems Inc., West Lafayette, IN, USA) were configured in the dual-parallel mode and used to oxidize the compounds of interest at 800 mV relative to an Ag/AgCl reference electrode. The sensitivity was set at 10 nA. The output from the detector was transmitted to a 3392A recording integrator (Hewlett–Packard, Avondale, PA, USA). The mobile phase consisted of buffer–acetonitrile–tetrahydrofuran (918:74:8, v/v/v) (pH* 2.0–2.15). One litre of the aqueous buffer contained 2.0 g of citric acid, 600 mg of sodium *n*-octanesulphonate, 50 mg of disodium EDTA, 13.2 ml of phosphoric acid, 250 μl of triethylamine and 1.0 ml of 2-butanol. The apparent pH (pH*) of the mixed eluent was adjusted to 2.0–2.15 using 10 M sodium hydroxide. The eluent was filtered through a 0.45 μm cellulose acetate membrane filter (Sartorius, Göttingen, FRG) and degassed with a stream of helium before use.

Preparation of standard solutions

A stock solution of the analytes was prepared monthly at a concentration of 80 μg ml^{-1} in 0.01 M hydrochloric acid containing

0.1% of sodium metabisulphite and 0.01% of disodium EDTA. The stock solution was protected from light and stored at 4°C. For preparation of calibration samples, nine working standard solutions were freshly prepared from the stock solution by dilution with 0.01 M hydrochloric acid.

Preparation of samples

Human plasma samples, to which 50 μl of a 10% sodium metabisulphite solution per ml had been added before freezing, were stored at −70°C until required for analysis. The plasma samples were thawed at room temperature. Immediately after thawing, 0.5-ml aliquots of plasma, 50 μl of 0.01 M hydrochloric acid and 50 μl of ethylene glycol–triethylamine (95:5, v/v) were pipetted into a 1.5 ml polypropene microcentrifuge tube and vortex mixed for 3 s. The protein was precipitated by adding 500 μl of 0.6 M perchloric acid, containing 0.5% sodium metabisulphite and 0.05% disodium EDTA. The precipitation was performed by adding the perchloric acid solution slowly under continuous vortex mixing and by letting the samples stand in an ice-water bath for 10 min. The samples were then vortex mixed for 5 s and centrifuged at 3500*g* for 10 min at 4°C. The supernatants were transferred to 2 ml plastic syringes and filtered through a small dead-volume 0.45 μm membrane filter (Millipore, Milford, MA, USA) into the injector vials.

Calibration samples were prepared daily in 1.5 ml polypropene microcentrifuge tubes by spiking 0.5 ml human plasma, to which 50 μl of a 10% sodium metabisulphite solution per ml had been added, with 50-μl aliquots of working standard solutions containing 10, 20, 40, 100, 200, 400, 800 or 1000 ng of each analyte. After spiking, the samples were immediately vortex mixed. The equilibration time before protein precipitation was about 5 min.

Quantitative analysis

Two calibration curves were constructed daily for each of the five analytes using the peak heights, obtained after chromatographing the calibration samples and subtracting the peak heights due to endogenous analytes in the plasma used for calibration. The equations for the curves were calculated by linear regression analysis using at least four calibration points per curve. One curve covered the concentration range from 20 to 200 ng ml^{-1}, the second from 200 to 2000 ng ml^{-1}. For the test samples, the concentration of the analytes was calculated from the peak heights using the equations of the appropriate calibration lines.

The recovery of the sample preparation method was determined at three concentration levels. Reference solutions, containing the same amounts of the analytes and reagents as the plasma samples, were made by replacing the plasma with 0.5 ml of 0.01 M hydrochloric acid. The recoveries were calculated as the ratios of the peak heights of the analytes of the spiked plasma samples to the peak heights of the analytes for the corresponding reference solutions.

Results and Discussion

Specificity

For the simultaneous determination of the five chemically diverse analytes, non-selective protein precipitation was used as the sample preparation method. Good separation and selective detection of the substances of interest thus were mandatory for the specificity of the method.

Chromatograms of a standard solution, unspiked plasma, spiked plasma and a typical sample obtained from a pharmacokinetic study are shown in Fig. 1. The asymmetry factors of the peaks, given in Fig. 1, were determined by chromatographing a solution containing 83 ng ml^{-1} of each analyte, the concentrations corresponding to a plasma concentration of 200 ng ml^{-1} of each analyte. As seen from the figure, all analytes were completely separated in 15 min and could thus be determined using a single detector potential. As reported by previous workers [16, 20, 21] the potential required for simultaneous and reliable detection of the free and methylated catechols was found to be 800 mV. Higher potentials gave slightly higher peaks for the *O*-methylated catechols, but simultaneously the selectivity of detection for plasma samples decreased.

Endogenous DOPAC, L-dopa, HVA and 3-OMD were detected in most unspiked plasma samples. The mean ratio of the peak heights of 10 different unspiked plasma samples to the corresponding peak heights of the calibration samples spiked with 20 ng ml^{-1} of each analyte were (SD, n = 10) 0.27 (0.09), 0.18 (0.14), 0.36 (0.09) and 0.46 (0.12) for DOPAC, L-dopa, HVA and 3-OMD, respectively. Neither

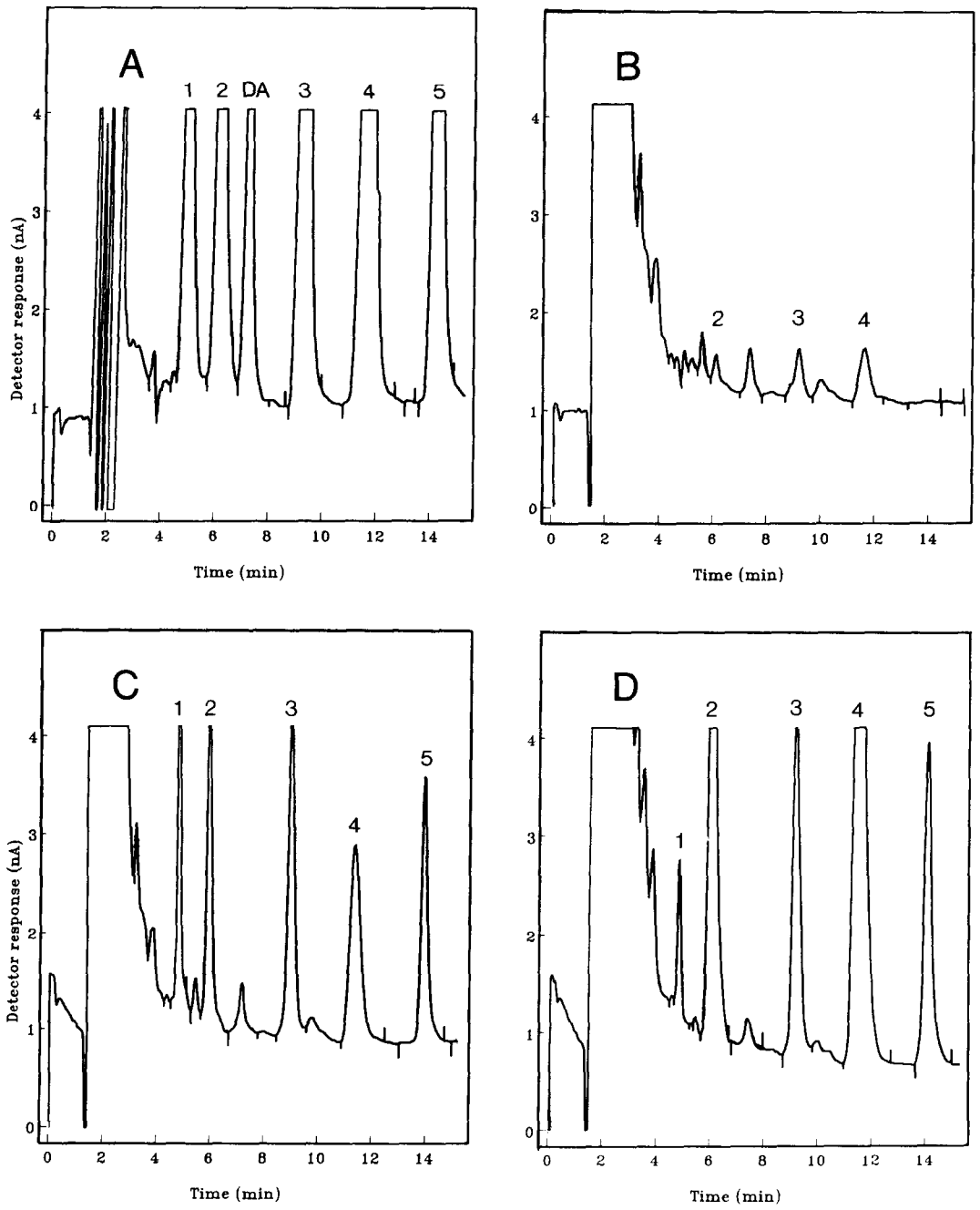

Figure 1
Chromatograms of (A) a solution containing 364 ng ml^{-1} of each analyte; (B) an unspiked plasma sample; (C) a calibration sample spiked with 80 ng ml^{-1} of each analyte; and (D) a plasma sample obtained from a volunteer 4 h after oral administration of 100 mg L-dopa/25 mg carbidopa. Peaks (asymmetry factors at 10% of peak height): 1 = DOPAC (0.83), 2 = L-dopa (1.1), 3 = HVA (1.0), 4 = 3-OMD (1.2), 5 = carbidopa (0.92), DA = dopamine.

quantitable concentrations of dopamine nor peaks close to the retention time of carbidopa were detected in the chromatograms of the unspiked plasma samples.

The retention time of some minor L-dopa-related catechol metabolites and some other potentially interfering endogenous compounds and drugs are listed in Table 1. The separation was free of interference from most of the compounds tested. Adrenaline was not completely resolved from DOPAC. Even under physical stress and COMT inhibition the endogenous concentration of adrenaline is, however, <1 ng ml^{-1} [22]. Thus only an exception-

Table 1
Typical retention times of the analytes and some potentially interfering compounds

Compound	Retention time (min)
3,4-Dihydroxyphenylpyruvic acid (DHPPA)	<3
3,4-Dihydroxyphenyl glycol (DHPG)	<3
3,4-Dihydroxymandelic acid (DOMA)	<3
Vanillylmandelic acid (VMA)	3.6
Noradrenaline (NA)	4.2
3-Methoxy-4-hydroxyphenyl glycol (MHPG)	4.5
Adrenaline (A)	4.8
3,4-Dihydroxyphenylacetic acid (DOPAC)	4.9 (0.3)*
Levodopa (L-dopa)	6.2 (0.4)
Dopamine (DA)	7.0
3-Methoxy-4-hydroxyphenylethanol (MHPE)	7.2
5-Hydroxyindole-3-acetic acid (5-HIAA)	7.4
Homovanillic acid (HVA)	9.1 (0.5)
Benserazide	10.5
α-Methyldopa	11.1
3-*O*-Methyldopa (3-OMD)	11.3 (0.6)
3-Methoxytyramine (3-MT)	12.7
4-*O*-Methyldopa (4-OMD)	14.4
Carbidopa	14.5 (0.6)
5-Hydroxytryptamine (5-HT)	15.6

* Baseline peak widths (min) of the analytes measured from the chromatogram of a 200 ng ml^{-1} sample.

ally high concentration of adrenaline would contribute substantially to the peak height of DOPAC at the limit of determination of the method. α-Methyldopa, a drug used for treatment of hypertension, eluted about 0.2 min before 3-OMD. As the peak of 3-OMD is 0.6 min broad, methyldopa interferes with the determination of 3-OMD if administered simultaneously.

4-*O*-Methyldopa (4-OMD) also is a potential metabolite of L-dopa. As this compound eluted almost simultaneously with carbidopa, the possible existence of a considerable amount of this potential metabolite in the plasma samples obtained from the pharmacokinetic studies was investigated. The ratio of the peak height of 4-OMD detected at 800 and 650 mV was 170. Plasma samples from six volunteers, collected 4 h after administration of a 100 mg L-dopa/25 mg carbidopa preparation, were analysed using an applied potential of 800 and 650 mV. The average concentration of carbidopa was 0.75 ng ml^{-1} higher when the voltage of the detector was 800 mV. This corresponds to 1.3% of the mean carbidopa concentration measured (59.3 ng ml^{-1}). Taking the precision of the method and the weak detector response of 4-OMD at 650 mV into consideration, it is obvious that no significant amount of 4-OMD coeluted with carbidopa. Plasma from patients on L-dopa therapy has, however, been shown to contain 4-OMD in concentrations of about 1% of that of 3-OMD [23].

The pH* of the mobile phase was found to be very important for the chromatographic resolution of the various analytes. As the properties of the column changed with time, it was necessary to adjust the pH* within the range 2.0–2.15 to maintain good selectivity. As the pH* of the mobile phase was increased, the retention time of the acidic metabolites (DOPAC and HVA) increased whilst the retention times of the amphoteric analytes decreased. The concentration of triethylamine in the mobile phase had very little effect on the acids whilst the retention times of the amphoteric analytes decreased as the concentration of triethylamine was increased. Triethylamine is ionized at the pH of the eluent and is a competing ion for these amphoteric compounds. When the ratio of triethylamine concentration in the samples and the eluent was changed, disturbing system peaks appeared in front of the DOPAC peak. When changing the triethylamine concentration of the eluent it is thus necessary to alter the triethylamine concentration of the samples simultaneously. Alterations in the mobile phase composition identically affected the retention times of the compounds of interest in the standards and in the biological samples. The identity of the compounds was also checked by testing the

detector response at various potentials (HVA and 3-OMD gave slight response at potentials below 650 mV) and by extraction experiments (only DOPAC and HVA were extractable with ethyl acetate from the acidified samples).

When solutions of the COMT inhibitors nitecapone, OR-611 and OR-1323 were injected, no peak was observed. Thus, the determination of L-dopa, its main metabolites and carbidopa was not influenced by the presence of these drugs in the plasma samples from studies where the effect of these COMT inhibitors on the pharmacokinetics of L-dopa was investigated.

Limit of determination and range of calibration

The practically usable lower limit of determination was estimated on basis of the sensitivity and selectivity of detection and the precision of the analytical method. The absolute detection limits were determined by chromatographing solutions of the pure analytes. At the signal-to-noise ratio of 3 the smallest detectable amounts of DOPAC, L-dopa, HVA, 3-OMD and carbidopa were 3, 3, 6, 9 and 14 pg, respectively. As 30 μl of the solution was injected the concentrations needed for detection were 0.1–0.5 ng ml^{-1}. The necessity to correct the calibration by the peak heights due to endogenous DOPAC, L-dopa, HVA and 3-OMD in the plasma used for calibration in combination with variation of the chemical noise caused by different plasma samples, resulted in some inaccuracy in calibrating the method at the low nanogram range. A practical lower limit of determination, giving a good precision and an acceptable accuracy, was 20 ng ml^{-1} for all five analytes.

Linear regression equations, calculated from the peak height/concentration data obtained by analysing spiked plasma, were used for calibration. The concentration range required for analysis of plasma samples in the pharmacokinetic studies was two orders of magnitude. To cover this range, calibrations from 20 to 200 ng ml^{-1} and from 200 to 2000 ng ml^{-1} were used. For all analytes the peak height/concentration relationship was highly linear over the entire concentration range and the correlation coefficients of the calibration curves were typically 0.999.

Recovery and precision

Conventional perchloric acid precipitation gave variable recoveries at low analyte concentrations. Triethylamine and ethylene glycol, assumed to change the protein binding equilibrium by competing for binding sites with the protonated amine groups and catechol-moieties of the analytes, were therefore added to the samples before protein precipitation. The recoveries given in Table 2 were high and reproducible. The recoveries exceeding 100% observed for L-dopa, HVA and 3-OMD are due to the endogenous analytes present in the plasma.

The within-day precision of the entire analytical procedure was studied by preparing and chromatographing spiked plasma samples at concentration levels of 20, 40, 80 and 800 ng

Table 2
Recovery of the analytes added to human plasma

Concentration (ng ml^{-1})	Recovery, (RSD)* (%) DOPAC	L-Dopa	HVA	3-OMD	Carbidopa
20	94.9 (1.3)	127 (2.0)	154 (3.3)	146 (3.0)	92.5 (6.3)
200	90.8 (1.4)	104 (2.4)	93.1 (0.6)	110 (1.3)	98.4 (1.8)
2000	90.5 (1.1)	100 (1.6)	88.7 (1.4)	98.0 (1.7)	98.6 (1.8)

*RSD = relative standard deviation, $n = 8$.

Table 3
Within-day precision of the method

Concentration (ng ml^{-1})	n	RSD* (%) DOPAC	L-Dopa	HVA	3-OMD	Carbidopa
20	8	1.3	2.0	3.3	3.0	6.3
40	8	2.2	2.0	2.0	1.8	2.4
80	6	0.7	1.6	0.6	1.6	0.7
800	6	1.2	1.6	1.3	1.2	1.4

*RSD = relative standard deviation of peak heights.

ml^{-1}. The relative standard deviations (RSD) of the peak heights are given in Table 3. The addition of ethylene glycol and triethylamine prior to protein precipitation and the slow addition of perchloric acid solution under continuous vortex mixing are essential for obtaining good precision especially at low analyte concentrations.

The day-to-day reproducibility of the method at two concentration levels was estimated from the concentrations determined for samples of a spiked plasma pool in 13 independently calibrated assays. Table 4 lists means, standard deviations and RSDs of these concentrations.

Stability

Because of their catechol structure the analytes are susceptible to oxidation. Sodium metabisulphite and EDTA therefore were added as antioxidants to the stock solutions and plasma samples. As reported for various catechol derivatives [24, 25], the acidic stock solutions of the analytes were found to be stable by comparing solutions stored for 1 month with freshly prepared solutions.

The stability of the analytes in plasma samples at 0, −20 and −70°C was followed for 4 h, 38 days and 61 days, respectively. The initial concentrations were 200 ng ml^{-1} at 0°C and 700–800 ng ml^{-1} at −20 and −70°C. The results are shown in Fig. 2. The concentration of all analytes decreased rather rapidly when the plasma samples were held in an ice-water bath. During 4 h some 30% of the initial amount of DOPAC and carbidopa decomposed at 0°C. At −20°C a slow decrease in the concentrations was observed for the non-methylated catechols, whereas the *O*-methylated derivatives were more stable. As free catechols are more susceptible to oxidation than the methylated derivatives these data suggest that the observed degradation is due to oxidation. When stored at −70°C the analytes were stable for over 1 month.

The stability of the analytes during freeze–thawing cycles was studied by freezing spiked plasma samples containing the antioxidants at −70°C and thawing them. A small (2–3%) but significant (Student's *t*-test, $P < 0.05$) decrease in analyte concentrations for samples subjected to three freezing–thawing cycles was observed when compared to samples subjected to one such cycle. Degradation resulting from repeated freezing and thawing was observed only for carbidopa in a previous study [17].

The stability of the analytes at ambient temperature after protein precipitation was studied by rechromatographing 200 ng ml^{-1} calibration samples after 5 and 22 h. The ratios of the peak heights, measured for the calibration samples and freshly prepared reference solutions, remained almost unchanged during 22 h. Student's *t*-test revealed no statistically significant difference between the ratios at the three time points. Thus, after sample preparation, the analytes are sufficiently stable to enable automated runs of up to 80 samples without problems.

Accuracy

As the recoveries were high, and the method was linear and precise within the calibration range, it was not necessary to use an internal

Table 4
Between-day reproducibility of the assay

Compound	Mean concentration* (ng ml^{-1})	SD† (ng ml^{-1})	RSD‡ (%)
DOPAC	55	3.9	7.1
	665	20	3.0
L-Dopa	70	5.8	8.3
	734	32	4.4
HVA	74	4.3	5.8
	757	24	3.2
3-OMD	67	4.7	7.0
	718	27	3.8
Carbidopa	67	5.0	7.5
	713	29	4.1

*Mean concentration determined for homogenous samples.
†SD = standard deviation of the concentrations determined on 13 different days.
‡RSD = relative standard deviation of the determined concentrations.

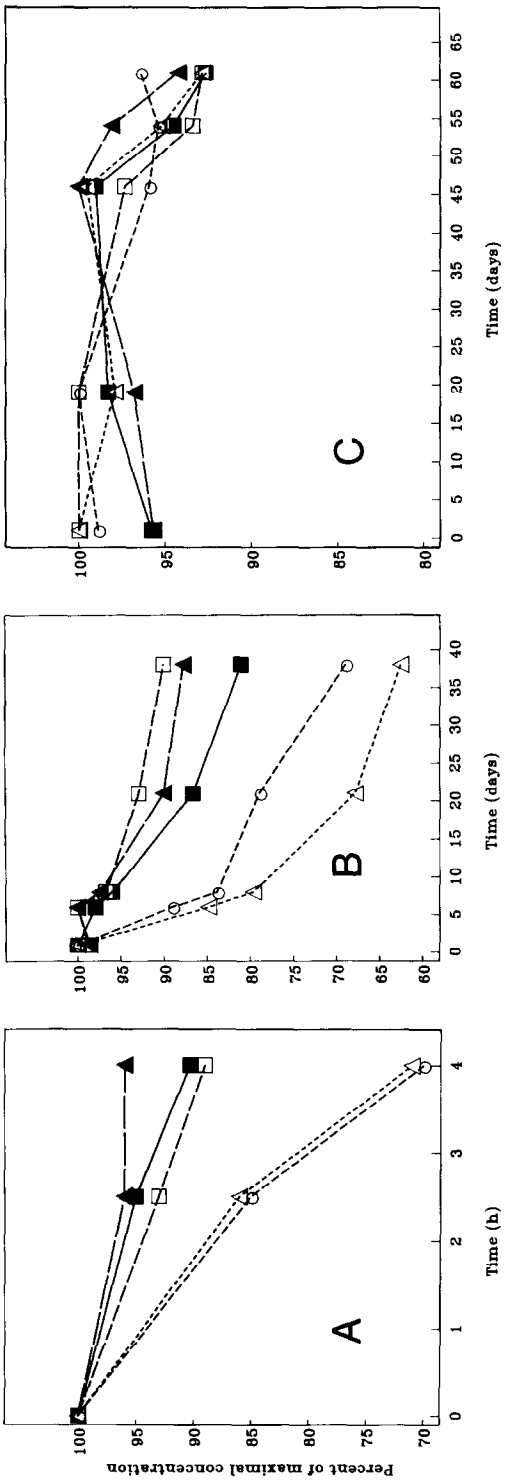

Figure 2
Stability of DOPAC (△), L-dopa (■), HVA (□), 3-OMD (▲) and carbidopa (○) in plasma containing sodium metabisulphite at (A) 0°C, (B) −20°C, and (C) −70°C. Each point represents the mean of two to three determinations.

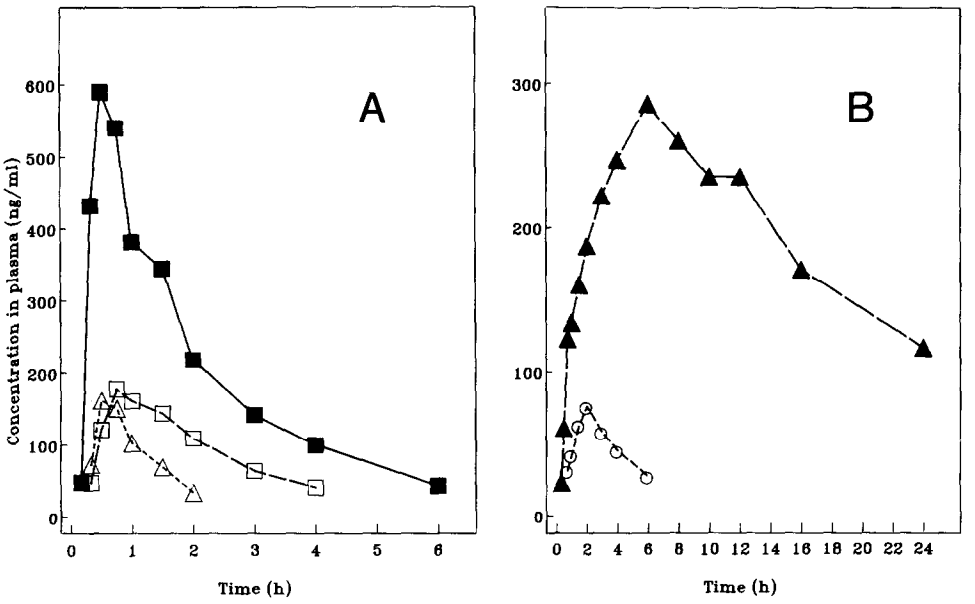

Figure 3
Plasma concentrations of (A) DOPAC (△), L-dopa (■) and HVA (□); and (B) 3-OMD (▲) and carbidopa (○) after an oral dose of 100 mg L-dopa/25 mg carbidopa.

standard to obtain accurate quantitation as in the case of some more complicated methods [16, 17, 24]. In the present method the most critical factors affecting accuracy are the stability of the analytes during storage and analysis and, at the low end of the calibration range, the specificity of the chromatographic method.

In plasma the non-methylated catechols are rather rapidly oxidized at 0°C or higher temperatures despite the addition of antioxidants, while they are stable in plasma at −70°C and at ambient temperature in the acidic solution after protein precipitation. It is therefore important to separate the plasma from blood samples and to freeze the samples at −70°C promptly and, after thawing, to precipitate the proteins without delay.

As seen from the chromatograms of blank plasma and from the high recoveries obtained for samples spiked with 20 ng ml^{-1}, the endogenous analytes have a considerable effect on the peak height at low concentration levels. To improve accuracy of quantitation it was therefore necessary to subtract the peak heights due to endogenous analytes in the blank plasma from the peak heights of the calibration samples.

Application

The method has been applied to the determination of levodopa, its three main metabolites and carbidopa in human plasma obtained from pharmacokinetic studies on the effect of COMT inhibitors on the metabolism of L-dopa. In such studies sample throughput is an important property of the method. As the sample preparation step is quick, chromatography becomes the throughput-limiting step of this method. Typically about 50–60 samples have been analysed per day.

An example of the plasma pharmacokinetic profiles obtained after oral administration of 100 mg L-dopa/25 mg carbidopa to one healthy volunteer is presented in Fig. 3. The plasma concentrations of DOPAC and HVA could be followed for about 2 and 4 h, respectively, whilst 3-OMD could be determined for over 24 h. L-Dopa and carbidopa concentrations typically exceeded the limit of determination for 6 h.

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