

Short Communication

Determination of cabergoline in plasma and urine by high-performance liquid chromatography with electrochemical detection

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ABSTRACT

A sensitive and selective high-performance liquid chromatographic method for the determination of cabergoline in plasma and urine has been developed. After buffering plasma and urine samples, cabergoline was extracted with a methylene chloride-isooctane mixture, back-extracted into 0.1 M phosphoric acid, then analysed by reversed-phase high-performance liquid chromatography. Quantitation was achieved by electrochemical detection of the eluate. The linearity, precision and accuracy of the method were evaluated. No interference from the biological matrices (human plasma and urine) was observed. The assay was still inadequate in terms of sensitivity for the quantitation of cabergoline plasma concentrations after a single oral dose of 1 mg of the drug to humans, but was successfully used in the determination of the urinary excretion of the drug.

INTRODUCTION

Cabergoline (Cab), N-[3-(dimethylamino)propyl]-N-[(ethylamino)carbonyl]-6-(2-propenyl)-ergoline-8 β -carboxamide (Fig. 1), is an ergoline derivative selected for its specific, potent and long-lasting dopaminergic activity. This effect has been observed in animals [1-4], in healthy volunteers [5,6] and in hyperprolactinaemic patients [7,8] and has made the drug very interesting for the treatment of the inhibition and suppression of lactation, of several hyperprolactinaemic disorders and in Parkinson's disease. Cab had also shown good tolerance in preliminary studies in

humans [6,9] and has been submitted to extensive clinical trials in several countries.

The need to correlate the pharmacological activity of the drug with suitable pharmacokinetic

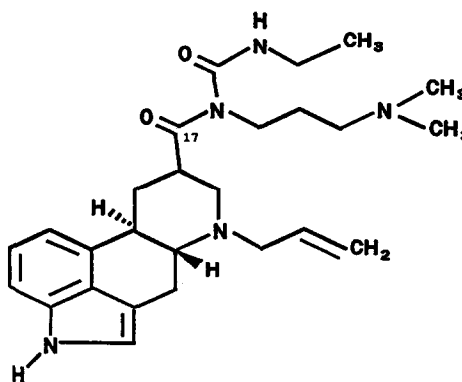


Fig. 1. Structure of cabergoline.

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data required the development of an analytical method for the determination of Cab in biological fluids, but the high potency of the drug and consequently the low doses administered made this difficult. In addition, the evidence that the drug is extensively metabolized [10] required an analytical method with high specificity.

Cab shows poor UV absorption and a lack of fluorescence, as well as low volatility and thermal stability; therefore high-performance liquid chromatography (HPLC) with UV or fluorescence detection and gas chromatographic techniques, even when coupled with mass spectrometry, were inappropriate or unable to achieve the sensitivity needed for pharmacokinetic studies.

HPLC coupled with electrochemical detection gives a good performance in terms of sensitivity and specificity and is convenient for compounds with low thermal stability. An assay was therefore developed using the selective extraction of Cab from plasma and urine followed by reversed-phase HPLC with electrochemical detection of the eluate. This method is a useful application of HPLC to the determination of ergoline compounds in biological fluids for which, previously, only less specific radioimmunoassay or radioreceptor assay methods were used in kinetic studies after therapeutic doses in human subjects [11].

EXPERIMENTAL

Materials and solutions

Cab was supplied by the Chemical Development Department of Farmitalia Carlo Erba and was at least 98% pure. All other chemicals and solvents were of analytical-reagent grade from Farmitalia Carlo Erba (Milan, Italy). Borate buffer (0.5 M, pH 9) was prepared from 3.09 g of boric acid adjusted to pH 9 with 1 M NaOH and diluted to 100 ml in a calibrated flask with HPLC-grade water. Phosphate buffer (75 mM, pH 3) was prepared by dissolving 10.2 g of KH_2PO_4 in 1 l of HPLC-grade water and adjusting to pH 3 with 1 M H_3PO_4 . A Cab stock solution was prepared by dissolving a weighed amount of the compound in 0.1 M H_3PO_4 . From this solution (stable for at least one week if stored at 4°C in the dark), working solutions were prepared daily by suitable dilution with 50 mM

H_3PO_4 . All glassware was silanized before use by treatment with a dimethyldichlorosilane-toluene solution (7%, v/v), followed by double rinsing with absolute ethanol and chloroform.

Apparatus

The HPLC system consisted of a Constametric 3000 pump (Milton Roy, LDC, Riviera Beach, FL, USA), a Rheodyne 7125 injection valve fitted with a 50- μl loop, a Model 5100 Coulochem electrochemical detector equipped with a Model 5020 guard cell and a Model 5011 analytical cell (5 μl volume) (ESA, Bedford, MA, USA). Peak integration was performed by a Spectra Physics Model 4270 recorder-integrator (Spectra Physics, Santa Clara, CA, USA).

Chromatographic conditions

The separation was performed on a Nucleosil C_{18} column (250 mm \times 4 mm I.D., particle size 5 μm) (Macherey-Nagel, Düren, Germany). The mobile phase was a mixture (20:80, v/v) of acetonitrile-75 mM phosphate buffer (pH 3).

To minimize the background noise, the buffer was filtered through a 0.22- μm filter and the mobile phase was degassed under vacuum prior to use. The flow-rate was 0.6 ml/min and the column was maintained at room temperature ($22 \pm 1^\circ\text{C}$).

Detection conditions

To optimize the response of the electrochemical detector, a voltammogram was obtained. It was obtained by replicate injections of a standard solution of Cab while the applied potential of the test electrode was changed linearly. Peak heights were measured and plotted against the applied potential. The operating potential (+0.65 V) was chosen to be near the top of the sigmoid curve obtained, approximately 0.05 V after the beginning of the upper plateau. A lower voltage (+0.35 V) was applied to detector 1 (conditioning detector) to minimize the interferences and to reduce the background current with an improvement in the baseline stability and hence in the signal-to-noise ratio.

Sample preparation

Plasma or urine (1.0 ml) was placed in a 10-ml

conical glass centrifuge tube and mixed with 0.5 ml (plasma) or 1 ml (urine) of 0.5 M borate buffer (pH 9). After the addition of 2.5 ml of a methylene chloride–isooctane mixture (2:3, v/v) the tubes were capped, shaken on a rotary mixer for 5 min and centrifuged at 1200 g for 15 min to separate the two phases. This extraction step was repeated, then the collected organic phase was extracted with 0.1 ml of 0.1 M phosphoric acid by vortex-mixing for 1 min. After centrifugation at 1200 g for 10 min, the organic phase was discarded and the aqueous phase was washed with 1 ml of *n*-hexane by vortex-mixing. Finally, after the removal of the *n*-hexane, an aliquot (50 μ l) of the aqueous solution was injected onto the column.

Determination of unknown and quality control samples

Analyses of blank plasma or urine spiked with known amounts of Cab were carried out to determine the response factor of the compound. Standard curves were obtained in the range 1.7–0.22 ng/ml (plasma) and 2.5–0.30 ng/ml (urine).

The precision and accuracy of the method were determined by repeated analyses of plasma and urine samples spiked with Cab over the whole concentration range of the standard curves. Cab was determined by peak-height measurements from the standard curve prepared daily.

Chromatographic system performance test

On each day of analysis, the performance of the HPLC system was assessed by monitoring three parameters: the reproducibility of the response, the column efficiency and the peak tailing, evaluated according to the United States Pharmacopoeia [12]. The limit values accepted were $\pm 5\%$ for reproducibility [as coefficient of variation (C.V.) of five replicate injections of 0.6 ng of Cab], $N > 6400$ for column efficiency and $T < 1.3$ for peak tailing.

RESULTS AND DISCUSSION

Under the described chromatographic conditions, Cab gave a sharp peak with a retention time of 14 min (Fig. 2). A 50-pg aliquot of a standard solution gave a signal-to-noise ratio better

than 5:1, illustrating the efficiency of the conditions for both the chromatographic separation and electrochemical detection. Strict conditions were defined to assess the suitability of the chromatographic system for the analysis of the biological samples.

The use of a guard cell set at +0.67 V and the overnight recycling of the mobile phase significantly reduced the background noise in the detection system and also increased the life-span of the detector cell (up to four to five months of continuous use). This allowed the optimum performance of the system to be obtained. Blank plasma or urine samples analysed in this way showed no significant peak at the retention time of the compound of interest (Fig. 2).

The specificity of the assay was checked with some Cab-related compounds that had been shown to be possible biotransformation products of the drug in animals and humans [10]. None of the compounds with a partial or total splitting of the chain linked on C₁₇ interfered in the determination of Cab. As Cab can be associated with L-DOPA in the treatment of Parkinson's disease, blank plasma samples spiked with microgram amounts of L-DOPA and its main metabolites, 3-O-methyldopa, dopamine and homovanillic acid, were assayed as described. Again, none of these compounds interfered with the determination of the compound of interest, further proof of the excellent specificity of the analytical method.

The linearity of this HPLC assay was evaluated from three separated calibration graphs obtained on different days in the range 1.7–0.22 ng/ml (plasma) and 2.5–0.30 ng/ml (urine) using least-squares linear regression analysis of the detector response *versus* the amount of Cab added to the sample. The mean parameters calculated were: slope = $56.0 \cdot 10^3$ (C.V. = 6.6%), intercept = $2.74 \cdot 10^3$ (C.V. = 48.2%), $r > 0.991$ and slope = $65.3 \cdot 10^3$ (C.V. = 6.7%), intercept = $-3.50 \cdot 10^3$ (C.V. = 53.0%), $r > 0.996$ for plasma and urine, respectively. When analysed by Student's *t*-test, the intercept values were not significantly different from zero ($p > 0.05$).

The intra-day precision expressed as the C.V. of replicated analyses ranged from 2.93 to 8.81% and from 5.34 to 10.14% in plasma and urine, respectively. The inter-day precision ranged from

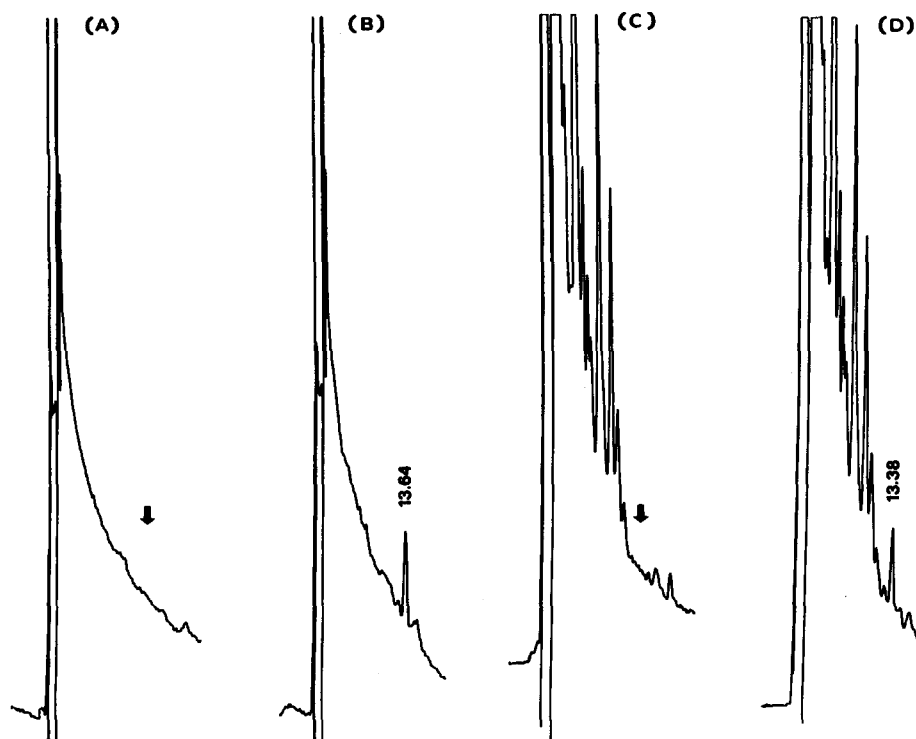


Fig. 2. Representative chromatograms obtained from extracts of (A) blank human plasma, (B) blank human plasma spiked with 0.4 ng/ml Cab, (C) blank human urine and (D) blank urine spiked with 0.4 ng/ml Cab.

5.74 to 11.94% in plasma and from 5.21 to 11.09% in urine.

The accuracy evaluated on three different days and expressed as the percentage ratio of the amount found to the amount added to plasma (or urine) was $99.33 \pm 6.78\%$ and $99.25 \pm 4.94\%$ (mean \pm S.D.) for plasma and urine, respectively.

The extraction recovery was evaluated at four different concentrations in plasma and urine by comparing the peak height obtained from extracted samples and from the direct injection of unextracted Cab standard solutions.

The mean values obtained on four different days were $69.5 \pm 7.25\%$ and $67.9 \pm 5.15\%$ in plasma and urine, respectively ($n = 4$).

The lowest limit of quantitation was assumed to be 0.25 and 0.30 ng/ml in plasma and urine, respectively. At these concentrations the assay still shows good linearity, precision and accuracy.

The described method was applied to the determination of the urinary excretion of Cab in hyperprolactinaemic patients after the adminis-

tration of single oral doses of 0.5, 0.75 and 1 mg of the drug [13]. In this study the urinary excretion of Cab could be determined in several subjects up to 168 h after the dose. The urinary half-life of Cab evaluated from urinary excretion rate data ranged from 62 to 91 h, whereas the mean percentage of unchanged drug excreted in 0–168 h urine was $0.7 \pm 0.1\%$ (standard error of the mean), less than one twentieth of the total radioactivity excreted in the same time period in volunteers receiving the radiolabelled drug [10].

In a further study carried out in healthy volunteers who received a single oral dose of 1 mg of Cab, it was shown by the less specific radioimmunoassay analysis that plasma concentrations of the unchanged drug were less than 100 pg/ml at all times tested [14]. These results confirm that the sensitivity of the HPLC assay developed, although successful for the determination of the urinary excretion, would be insufficient to determine Cab concentrations in plasma after a single oral dose of the drug. However, the selectivity of the HPLC method could make the assay applica-

ble to the determination of steady-state plasma concentrations in patients with Parkinson's disease where, in most cases, repeated doses of more than 1 mg are given [15] and consequently plasma concentrations higher than the lowest limit of quantitation of the HPLC assay are expected.

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