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Determination of dopexamine hydrochloride in human blood by high-performance liquid chromatography with electrochemical detection

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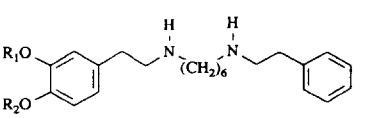
Abstract

A method is described for the determination of dopexamine hydrochloride at concentrations of 5 to 1000 ng/ml in human blood using electrochemical detection. The method uses a Hypersil ODS column and a mobile phase containing heptane sulphonate, orthophosphoric acid, diisopropylamine and disodium EDTA. Blood samples are stabilised immediately after collection by the use of dipotassium EDTA and a high concentration of sodium metabisulphite. The sample preparation procedure consists of a simple de-proteinisation with perchloric acid. The method is accurate, with inter-assay accuracies ranging from 100 to 104%, and is free of interference by blood from different individuals. Known and potential metabolites of dopexamine hydrochloride and a wide range of drugs do not interfere with the method. The method is precise with inter-assay coefficients of variation of 10.6% at 5 ng/ml and of less than 4.2% at higher concentrations. Stabilised blood samples may be stored for over six months at −25°C prior to analysis.

1. Introduction

Dopexamine hydrochloride (FPL 60278AR; Dopacard; I, Fig. 1) is a structural analogue of dopamine synthesised at Fisons plc, Pharmaceutical Division. Dopexamine hydrochloride displays many of the qualities required of a short-term treatment for low cardiac output states, and possesses the additional benefit of preserving or



Derivative	R_1	R_2
I	H	H
II	H	CH_3
III	CH_3	H
IV	SO_3H	CH_3
V	CH_3	SO_3H
VI	SO_3H	H
VII	H	SO_3H

Fig. 1. Structures of dopexamine hydrochloride (I) and derivatives, which are known or potential metabolites of the compound.

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enhancing vital organ perfusion. Its effects are achieved through its principal actions as a combined dopaminergic and β_2-adrenergic agonist. Cardiac output is increased mainly by afterload reduction, while blood pressure is maintained by a degree of positive inotropy. The drug has novel profiles of pharmacological [1] and cardiovascular [2] activity when compared with dopamine. Dopexamine hydrochloride is sold by Fisons in various, mainly European, countries for the treatment of cardiac failure by intravenous infusion and is licensed to Porton Products for this and other applications worldwide. To support clinical studies on the drug and to provide pharmacokinetic information, methods for the determination of dopexamine hydrochloride in biological fluids were required. Since sensitive and specific methods for dopamine and other catecholamines have been developed using HPLC in conjunction with electrochemical detection this approach was utilised for the determination of dopexamine hydrochloride.

Due to the chemical instability of catecholamines and metabolic transformation in biological matrices such as plasma [3], methods for their determination employ stabilisation procedures to prevent degradation of the compounds prior to analysis. The facile oxidation of catecholamines by atmospheric oxygen is often prevented by the addition of an anti-oxidant together with a metal-chelating agent to complex any metal ions which could catalyse the oxidation. We stabilised dopexamine hydrochloride by adding blood samples as soon as possible after collection to tubes containing dipotassium EDTA and a large amount of solid sodium metabisulphite (50 mg/ml), which was optimal for stability. This stabilisation procedure prevents both oxidative and metabolic degradation of dopexamine hydrochloride. However, substantial haemolysis of the blood occurs under these conditions preventing the separation of plasma by centrifugation. Dopexamine hydrochloride was therefore determined in whole blood rather than in plasma. The resulting blood analysis method is described in this paper.

2. Experimental

2.1. Materials

Dopexamine hydrochloride (4-{2-[6-(2-phenylethylamino)hexylamino] - ethyl} - 1,2 - benzenediol hydrochloride; I, Fig. 1) is a product of Fisons plc, Pharmaceutical Division (Loughborough, UK). The derivatives of dopexamine hydrochloride (II–VII, Fig. 1) were synthesised in these laboratories. Other drugs were purchased from Sigma (Poole, UK), when available, or were purchased as formulations. Diisopropylamine (99%) was obtained from Aldrich (Gillingham, UK). Sodium heptane-1-sulphonate (HPLC grade) and all other chemicals (all AR grade) were purchased from FSA Laboratory Supplies (Loughborough, UK). Plastic blood sample tubes (2, 5 and 10 ml) containing dipotassium EDTA as anticoagulant were obtained from Sarstedt (Leicester, UK). Plastic universal containers (30 ml) were obtained from Medfor Products (Fleet, UK).

2.2. Preparation of standard solutions

Standard solutions of dopexamine hydrochloride were prepared in dilute perchloric acid (10 mmol/l), containing disodium EDTA (1 mmol/l) and sodium metabisulphite (5 mmol/l). The stock standard solution at a concentration of 10 μg/ml was prepared in an acid-washed 100-ml glass volumetric flask. Since dopexamine hydrochloride in dilute perchloric acid adsorbs to glass the stock standard solution once prepared was rapidly dispensed in portions (approximately 2 ml) to 5-ml plastic blood sample tubes. These portions were stored at $-25° \pm 5°C$. The stored stock standard solution and a dilution (2.5 μg/ml) were used for the preparation of quality control samples. Working standard solutions (0.05 and 1.0 μg/ml) for the preparation of standard samples were prepared from the stored stock standard solution for each analysis. All the dilutions of the stored stock solution were performed in 10-ml plastic blood sample tubes.

2.3. Blood sample stabilisation procedure

Within one minute of collection, blood samples (5 ml) containing the drug were placed in 5-ml dipotassium EDTA-anticoagulated plastic blood sample tubes containing solid sodium metabisulphite (250 mg). The tubes were immediately shaken to dissolve the sodium metabisulphite. As soon as possible after this stabilisation the samples were rapidly frozen in a mixture of solid carbon dioxide and methanol, and were then stored at −25° ± 5°C.

Pooled blank blood used for the preparation of quality control and standard samples was similarly stabilised by the addition of sodium metabisulphite (50 mg/ml) and then stored at −25° ± 5°C. Dipotassium EDTA-anticoagulated blood collected from ten or more individuals, and then stored at −25° ± 5°C, was used for the preparation of the pooled material.

2.4. Preparation of quality control samples

Quality control samples containing dopexamine hydrochloride (5, 100 and 1000 ng/ml) were prepared in plastic universal containers by dilution of the diluted 2.5 μg/ml standard solution (0.05 and 1.0 ml) and dilution of the 10 μg/ml stock standard solution (2.5 ml) to 25.0 ml with stabilised pooled blank blood. Portions (1.5 ml) of these samples were dispensed into 2-ml plastic blood sample tubes which were rapidly frozen in a mixture of solid carbon dioxide and methanol and then stored at −25° ± 5°C until required.

2.5. Sample preparation procedure

Dilute perchloric acid (10 mmol/l), containing disodium EDTA (1 mmol/l) and sodium metabisulphite (5 mmol/l), was added to duplicate portions (0.5 ml) of the test and quality control samples in 5-ml plastic blood sample tubes. Standard samples at concentrations of 0, 5, 10, 25, 50, 100, 250, 500 and 1000 ng/ml were prepared by the addition of appropriate volumes of the working standard solutions of dopexamine hydrochloride (0.05 and 1.0 μg/ml) to stabilised pooled blank blood (0.5 ml) in 5-ml plastic blood sample tubes. Dilute perchloric acid containing disodium EDTA and sodium metabisulphite, as above, was added in appropriate volumes to the standard samples so that all the standard samples had received a total volume of 0.5 ml of aqueous solution.

Perchloric acid (0.4 mol/l, 1.0 ml) was added to the test, quality control and standard samples. After vortex-mixing the samples were shaken on a horizontal shaker (300 oscillations/min, 10 min) and then centrifuged (1800 *g*, 10 min). The supernatant (approximately 1.6 ml) was transferred to glass autosampler vials from which 200 μl samples were analysed.

2.6. Chromatographic equipment and conditions

A Hewlett-Packard 1084B liquid chromatograph equipped with an autosampler (Hewlett-Packard Limited, UK) and a Metrohm 656 electrochemical detector cell with a Metrohm 641 electrometer (Roth Scientific, Farnborough, UK) were used. The electrometer was set to a range of 50 nA. The glassy carbon working electrode of the detector was set at a potential of +0.55V versus a Ag/AgCl reference electrode. The detector, with the supplied water jacket removed, was placed in the column oven of the chromatograph. The output of the detector was integrated using a Spectra-Physics 4270 integrator (Severn Analytical, Gloucester, UK) set at an attenuation of 128 mV. A pre-column (250 × 4.6 mm I.D.) containing silica (25–40 μm particle size) was connected before the autosampler to condition the mobile phase. The Hypersil ODS (5 μm particle size) analytical column (250 × 4.9 mm I.D.) was purchased prepacked from Hichrom (Reading, UK). Both columns were placed in the column oven. The mobile phase consisted of a mixture of methanol (49%, by volume) and an aqueous solution of sodium heptane-1-sulphonate (4.04 g/l), disodium EDTA (1 mmol/l), orthophosphoric acid (10 ml/l) and diisopropylamine (22.5 ml/l). The mobile phase was filtered through a cellulose

nitrate filter (0.45-μm pore size) before use. A flow-rate of 1.2 ml/min was used. The temperature of the column oven was adjusted within the range 37°C to 43°C to obtain retention times for dopexamine hydrochloride between 7.0 and 7.3 min.

2.7. Standardisation

The linear equations of standard curves were calculated by regression analysis following logarithmic transformation of both response and concentration.

3. Results and discussion

3.1. Chromatography

Typical chromatograms of standard and blank samples and chromatograms of pre- and post-dose samples from a patient are shown in Figs. 2 and 3. The large broad peak which tails until the retention time of dopexamine hydrochloride results mainly from sodium metabisulphite added to the samples.

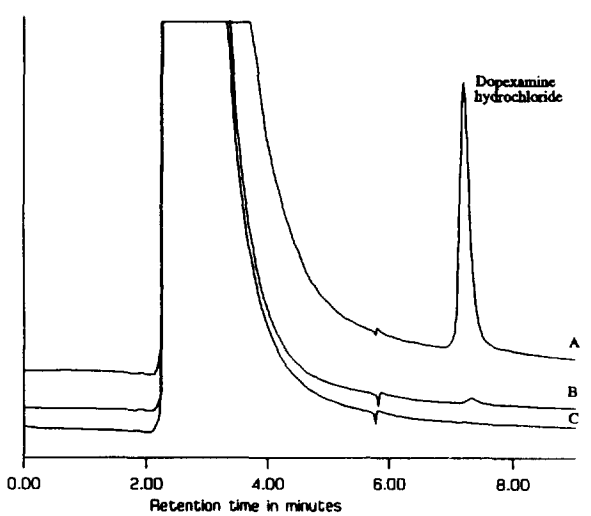

Fig. 2. Chromatograms of standard blood samples containing dopexamine hydrochloride at 250 ng/ml (A) and at 5 ng/ml (B) and a dopexamine hydrochloride free blank blood sample (C).

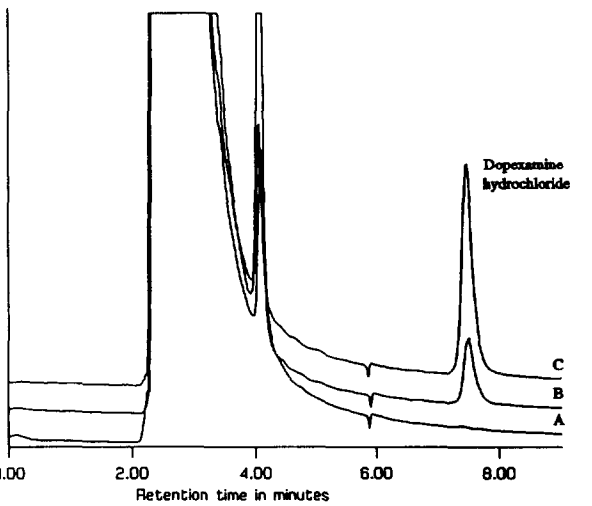

Fig. 3. Chromatograms of a pre-dose (A) and post-dose blood samples containing dopexamine hydrochloride at 64 ng/ml (B) and at 204 ng/ml (C) from a patient.

3.2. Statistical validation

The correlation coefficients of the linear equations for the logarithmically-transformed standard curves were 0.9994, or better, confirming their linearity. The intra-assay and inter-assay accuracy and precision of the method (Table 1) was determined by the repetitive analysis of quality control samples (5, 100 and 1000 ng/ml) and also of similarly prepared samples containing dopexamine hydrochloride at concentrations of 10, 20 and 250 ng/ml. The inter-assay precision, expressed as the coefficient of variation, varied from 2.6 to 4.2% over the concentration range 10–1000 ng/ml and was 10.6% at 5 ng/ml. The inter-assay accuracy varied from 100 to 104% over the concentration range 5–1000 ng/ml.

3.3. Specificity

Matrix interference by blood from different individuals was investigated by the analysis of blank blood samples from ten donors. These samples were also analysed following the addition of dopexamine hydrochloride at three concentrations of 5, 20 and 250 ng/ml. None of the blank blood samples were found to contain

Table 1
Intra-assay and inter-assay accuracy and precision

Dopexamine hydrochloride concentration (ng/ml)	Intra-assay (n = 10)		Inter-assay (n = 6)	
	Accuracy (%)	Coefficient of variation (%)	Accuracy (%)	Coefficient of variation (%)
5	106	8.5	104	10.6
10	100	3.3	100	4.2
20	99	4.2	102	3.6
100	99	3.3	102	3.0
250	96	2.2	102	2.6
1000	96	2.9	101	4.1

constituents which were detected as dopexamine hydrochloride (Table 2). There was no evidence of a consistent bias in the determination of the three added concentrations in any of the blood samples (Table 2). Moreover, at each added concentration the mean accuracy and coefficient of variation of the determinations in the ten individual blood samples were comparable to those determined in pooled blank blood (Table 1). The method is therefore considered to be free of matrix interference.

The specificity of the method with respect to possible interferences by the derivatives II–VII (Fig. 1) was determined. Some of the derivatives are metabolites of dopexamine hydrochloride which are excreted in the urine or faeces by animals and by man [4] whilst the other derivatives are potential metabolites which could occur in the blood. Solutions (1000 ng/ml) of the derivatives in dilute perchloric acid (10 mmol/l), containing disodium EDTA (1 mmol/l) and sodium metabisulphite (5 mmol/l), were prepared and injected directly onto the analytical column to determine their content of dopexamine hydrochloride, if any. The derivatives were also added at concentrations of 1000 ng/ml

Table 2
Investigation of inter-individual specificity

Individual subject number	Determined concentration of dopexamine hydrochloride (ng/ml)			
	Added concentration (ng/ml)			
	0	5	20	250
1	< 2	4.5	21.0	258
2	< 2	4.4	19.1	261
3	< 2	6.7	19.7	268
4	< 2	4.9	19.2	258
5	< 2	5.1	20.1	254
6	< 2	5.4	19.3	258
7	< 2	5.1	19.9	263
8	< 2	5.1	21.1	265
9	< 2	4.8	21.3	262
10	< 2	5.3	21.0	269
Accuracy (%)	–	102	101	105
C.V. (%)	–	12.5	4.3	1.8

to stabilised pooled blank blood samples. These samples were processed through the method to determine if any of the derivatives were converted to dopexamine hydrochloride during the analysis procedure. The mixture of the sulphate derivatives (VI and VII, Fig. 1), synthesised by sulphation of dopexamine hydrochloride, was found to contain 7% by weight dopexamine hydrochloride whereas the other derivatives (II–V) contained none, or only very small amounts of 0.6%, or less. The concentrations of dopexamine hydrochloride determined in the blood samples containing the added derivatives were similar to the concentrations directly determined in the solutions of the derivatives. Even after four days at ambient temperature there was no increase in the amount of dopexamine hydrochloride in the extracts prepared from the blood samples containing the mixture of derivatives VI and VII, which were synthesised together and not separated. Thus, none of the derivatives tested therefore interfered with the quantitation of dopexamine hydrochloride, either directly or by conversion to dopexamine hydrochloride during the analytical procedure.

Possible interference with the method by the drugs listed in Table 3 was determined by directly injecting solutions of the drugs onto the column and monitoring with a UV absorbance detector and the electrochemical detector connected in series. None of the drugs were found to interfere with the method.

3.4. Stability

The stability of dopexamine hydrochloride in stabilised blood stored at $-25° \pm 5°C$ was determined by the analysis after storage of blank blood samples containing added concentrations of 5, 10, 20, 100, 250 and 1000 ng/ml. Concentrations of 4.8, 9.9, 19.6, 102, 255 and 1023 ng/ml were determined in samples stored for one week and concentrations of 4.7, 10.3, 20.4, 103, 257 and 1063 ng/ml were determined in samples stored for thirty-three weeks.

The stability of dopexamine hydrochloride at 100 ng/ml in blood containing a range of sodium metabisulphite concentrations (0.25 to 500 mg/ml) and stored at 4°C for three days was investigated. At a sodium metabisulphite concentration of 50 mg/ml there was a negligible loss of dopexamine hydrochloride of 2%. Large losses were found at smaller and higher concentrations of sodium metabisulphite, such as 29 and 65% at concentrations of 10 and 250 mg/ml, respectively. At concentrations of sodium metabisulphite of 100 mg/ml and above the blood had partially solidified and the losses may represent entrapment of the dopexamine hydrochloride. These results show that dopexamine hydrochloride was

Table 3
Drugs tested for interference

Dopamine hydrochloride	Lidocaine	Frusemide
Procainamide hydrochloride	Warfarin	Allopurinol
Quinidine hydrochloride	Colchicine	Digoxin
Dobutamine hydrochloride	Spironolactone	Atenolol
Isosorbide dinitrate	Paracetamol	Heparin
Cyclizine lactate	Dicloxacillin sodium	Diazepam
Ranitidine hydrochloride	Metoclopramide hydrochloride	Atropine
Cefuroxime sodium	Amiloride hydrochloride	Diclofenac sodium
Pancuronium bromide	Clindamycin hydrochloride	Nifedipine
Isoprenaline sulphate	Amiodarone hydrochloride	Pethidine
Netilmicin sulphate	Dipyridamole	Glibencyamide
Mexiletine hydrochloride	Amoxicillin	Salicylic acid
Hydrochlorothiazide	Metolazone	Gentisic acid
Acetylsalicylic acid	Captopril	Epinephrine

stable in blood (containing sodium EDTA and with sodium metabisulphite at 50 mg/ml) stored at −25°C for over thirty weeks and at 4°C for three days.

3.5. Recovery and limits of quantitation

The recovery of dopexamine hydrochloride from pooled blank blood was determined on six days by the injection of a solution (1000 ng/ml) of the compound onto the analytical column and comparison of the response obtained with the standard curve generated on that day. The recovery of dopexamine hydrochloride was 83.5 ± 3.1%. The limit of quantitation of the method was 5 ng/ml based on the accuracy and precision values listed in Tables 1 and 2.

3.6. Application

The applicability of the method is illustrated by a blood concentration–time profile (Fig. 4) in a volunteer subject administered dopexamine hydrochloride at an infusion rate of 4 μg/kg/min for 30 min. Immediately before this infusion the subject received dopexamine hydrochloride infusions at 1, 2 and 3 μg/kg/min for 10 min each (data not shown).

4. Conclusions

A method using HPLC with electrochemical detection for the determination of dopexamine hydrochloride in human blood at concentrations in the range 5–1000 ng/ml has been developed. The use of a high concentration of sodium metabisulphite (50 mg/ml) and dipotassium EDTA to prevent both metabolic and oxidative losses of dopexamine hydrochloride in collected blood samples is an essential part of the method. Sample preparation is simple and rapid, involving only de-proteinisation. The method is accurate and precise and free of interference by blood from different individuals. Known and potential metabolites of dopexamine hydrochloride, and a wide range of drugs do not interfere with the method.

Acknowledgement

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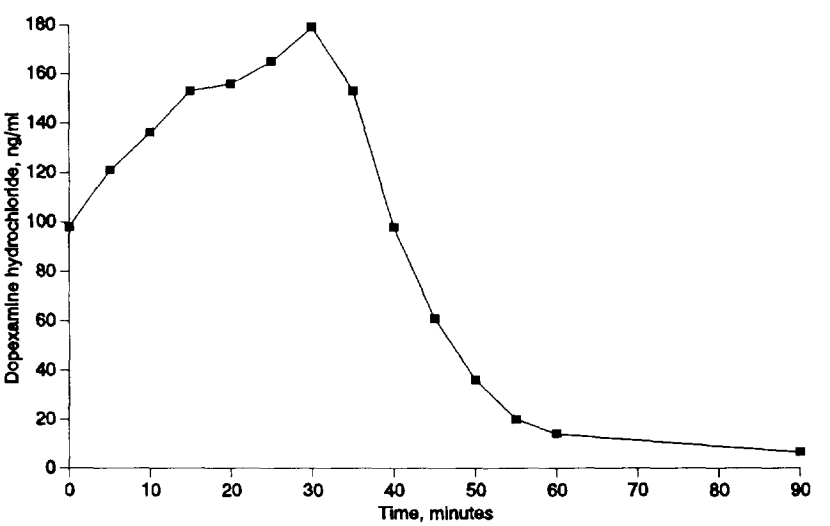

Fig. 4. A blood dopexamine hydrochloride concentration–time profile in a volunteer infused at 4 μg/kg/min for 30 min.

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