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Short Communication

Simple isocratic high-performance liquid chromatographic method for measurement of iodixanol in human plasma

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ABSTRACT

A simple isocratic high-performance liquid chromatographic method was developed for the determination of iodixanol in human plasma. Samples containing an internal standard were prepared for analysis using a simple clean-up procedure based on Sep-Pak C_{18} solid-phase extraction and chromatographed using a size-exclusion column with purified water as a mobile phase. The iodixanol peak was completely separated from the peaks of an internal standard and endogenous substances on this column. Three geometric isomers (*exo–exo*, *endo–exo* and *endo–endo* forms) of iodixanol could be eluted as a single peak. The method was found to be applicable to pharmacokinetic studies of iodixanol in human plasma.

INTRODUCTION

Iodixanol, 5,5-[(2-hydroxy-1,3-propanediyl)bis(acetylimino)]bis[N,N′-bis-(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide], is a new non-ionic dimeric contrast medium [1,2]. This agent is a dimer of iohexol, which is well known as a non-ionic monomeric contrast medium. A method of determining iohexol in biological fluids has been reported by Edelson *et al.* [3]. Iodixanol is not metabolized, but excreted in an unchanged form via the kidney, as are others of this class of diagnostic agents. This agent has three geometric isomers: *exo–exo*, *exo–endo* and *endo–endo* forms. This study presents a simple high-performance liquid chromatographic (HPLC) method of eluting the isomers as a single peak for the quantitation of iodixanol in human plasma.

EXPERIMENTAL

Chemicals

Iodixanol (MW 1550) and the internal standard (I.S.) for the assay, 5-amino-N,N′-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenecarboxamide (MW

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705), were obtained from Nycomed (Oslo, Norway). The water used in the preparation of solutions or of the HPLC mobile phase was of Milli-Q quality (Millipore, Bedford, MA, USA). Other chemicals were obtained from commercial sources and were of reagent grade. Sep-Pak C_{18} cartridges (hold-up volume, 0.70 ml per filled cartridge; packing material, 360 mg per cartridge) were purchased from Waters Assoc. (Milford, MA, USA).

Chromatography

The liquid chromatograph used (880-PC, JASCO, Tokyo, Japan) was equipped with a variable-wavelength UV detector (UV-8000, Tosoh, Tokyo, Japan) at 254 nm. The automatic sample processor was a Tosoh AS-8000 equipped with a 20-μl sample loop. Chromatography was performed on a YMC-Pack S-5 60A DIOL size-exclusion column (300 mm × 8 mm I.D., 5 μm particle size, Yamamura Chemical Labs., Kyoto, Japan) at ambient temperature. The mobile phase was only water, which was pumped at a flow-rate of 1.0 ml/min.

Plasma preparation

Sep-Pak C_{18} cartridges were primed with 5 ml of methanol followed by 5 ml of water. To a tube containing 0.1 ml of plasma or diluted plasma, 0.1 ml of internal standard solution (43.2 μg/ml) and 0.8 ml of water were added. For standard samples, 0.1 ml of a standard solution of iodixanol and 0.7 ml of water were added instead of 0.8 ml of water. Samples were then loaded onto the Sep-Pak cartridges and drawn through under a vacuum. After all of the plasma had been drawn through, each cartridge was washed with 3 ml of water. Samples were then eluted with 2 ml of methanol. The eluent was evaporated with a centrifuging vacuum evaporator (EC-57C, Sakuma, Tokyo, Japan). After reconstitution in 400 μl of water, the samples were filtered with 0.45-μm membrane filters (Type HV, Japan Millipore Industry, Tokyo, Japan). A 20-μl aliquot was analyzed by HPLC as described above.

Recovery

Recoveries of iodixanol and I.S. from the Sep-Pak cartridges were determined using each compound as the external standard for the other as follows. (a) Plasma samples spiked with iodixanol and I.S. were loaded onto the Sep-Pak cartridges; (b) plasma samples spiked with iodixanol were loaded onto the Sep-Pak cartridges and I.S. was added to the effluent before injection for HPLC assay; and (c) plasma samples spiked with I.S. were loaded onto the Sep-Pak cartridges and iodixanol was added to the effluent before injection for HPLC assay. Recoveries were calculated by the comparison of peak-height ratios between the results of (a) and (b) and of (a) and (c).

Calibration curve

The appropriate iodixanol stock solutions were used to prepare calibration

curves in the concentration range 0.679–130.4 μg/ml. The calibration graph was established by plotting the peak-height ratios of iodixanol to the internal standard *versus* the concentration of the substance in the standard samples. The graph was evaluated by the least-squares linear regression method.

Accuracy and precision

To determine accuracy, known amounts of iodixanol were added to drug-free plasma and concentrations of iodixanol were calculated using the standard curve. To determine the effect of dilution of plasma, known amounts of iodixanol were added to diluted plasma and concentrations of iodixanol were calculated using the standard curve prepared with intact plasma. The difference between the mean concentration estimated and the theoretical concentration was taken as the accuracy of the assay. Precision was estimated by determination of the intra- and inter-day coefficients of variation (C.V.).

Clinical application

Six healthy male volunteers participated in the pharmacokinetic study of iodixanol. Multiple blood samples were taken for 24 h after intravenous injection of iodixanol at a dose of 0.3 g equivalent to iodine per kg of body weight (0.3 gI/kg). Blood samples were immediately centrifuged and the plasma samples were separated. These samples were stored at −20°C until the time of analysis. Before

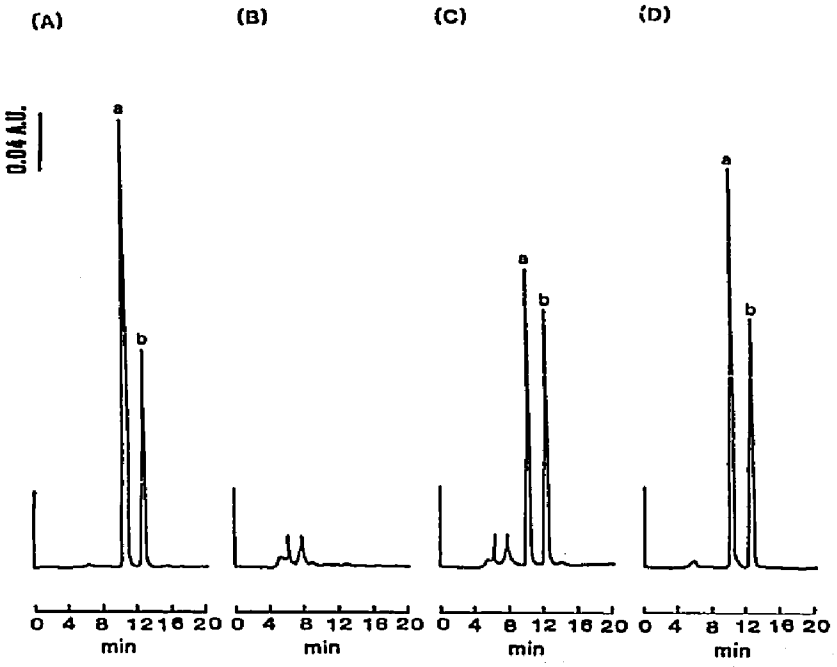

Fig. 1. Chromatograms of (A) standards of iodixanol (a) and internal standard (b), (B) drug-free human plasma, (C) drug-free plasma spiked with iodixanol (32.6 μg/ml) and I.S. and (D) plasma obtained 10 min after injection of 0.3 gI/kg iodixanol to a healthy volunteer (45.39 μg/ml of plasma).

TABLE I

WITHIN-DAY ACCURACY AND PRECISION FOR IODIXANOL

Concentration (μg/ml)	n	Concentration found (%)	C.V. (%)
0.679	5	97.7	6.7
1.019	5	100.3	3.5
4.075	5	97.8	2.6
16.30	5	101.6	1.0
65.20	5	100.9	1.1
130.4	5	98.9	0.9

assay, plasma samples were diluted with water as needed to keep the concentration in the range of calibration.

RESULTS AND DISCUSSION

Fig. 1 shows chromatograms of standards without plasma, of drug-free plasma, of drug-free plasma spiked with 32.6 μg/ml iodixanol and of plasma obtained 10 min after injection of 0.3 gI/kg iodixanol to a healthy volunteer. The iodixanol peak was completely separated from the peaks of I.S. and endogenous substances on a size-exclusion column, YMC-Pack DIOL, by elution with water. Substances in the range of molecular weights from 22 000 to 120 can be separated on this type of column, which has a pore diameter of 60 Å. The retention times of iodixanol and I.S. were 10.2 and 12.4 min, respectively, and the composition of the mobile phase did not affect the retention of these substances.

TABLE II

EFFECT OF DILUTION OF PLASMA ON DETERMINATION OF IODIXANOL

Concentration (μg/ml)	n	× 1[a]		× 20[b]		× 200[c]	
		Concentration found (%)	C.V. (%)	Concentration found (%)	C.V. (%)	Concentration found (%)	C.V. (%)
1.019	5	100.3	3.5	95.3	3.8	94.1	3.3
16.30	5	101.6	1.0	101.1	1.8	98.3	3.5
65.20	5	100.9	1.1	101.3	0.9	100.6	0.9

[a] Iodixanol was spiked in intact plasma.
[b] Iodixanol was spiked in plasma diluted twenty times with water.
[c] Iodixanol was spiked in plasma diluted 200 times with water.

TABLE III

INTER-DAY PRECISION FOR IODIXANOL

Concentration (μg/ml)	*n*	C.V. (%)		
		× 1[a]	× 20[b]	× 200[c]
16.30	5	0.8	1.2	2.3
65.20	5	2.6	2.1	3.0

[a] Iodixanol was spiked in intact plasma.
[b] Iodixanol was spiked in plasma diluted twenty times with water.
[c] Iodixanol was spiked in plasma diluted 200 times with water.

Iodixanol consists of three geometric isomers, complexes of *endo* and *exo* forms. To elute the isomers as a single peak, several types of column were examined, namely reversed-phase C_{18} and C_8 columns, cyano- and phenyl-type columns and size-exclusion columns. The YMC-Pack DIOL column was found to be suitable for this purpose, giving a sharp, single peak for the three isomers.

The recoveries of iodixanol and I.S. from Sep-Pak cartridges were 96.4 ± 1.1 and 93.2 ± 1.0%, respectively.

The precision in the concentration range 1.019–130.4 μg/ml was very good, C.V.s being 3.5% or less. For the 0.679 μg/ml samples, the C.V.s increased to 6.7% (Table I). The accuracy is given as the ratio of the concentration found to the theoretical value as a percentage (Table I), bias being 2.3% or less in the concentration range 0.679–130.4 μg/ml. It was found that the dilution of plasma samples did not affect the assay of iodixanol (Table II). The inter-day C.V.s were 2.6% or less (Table III).

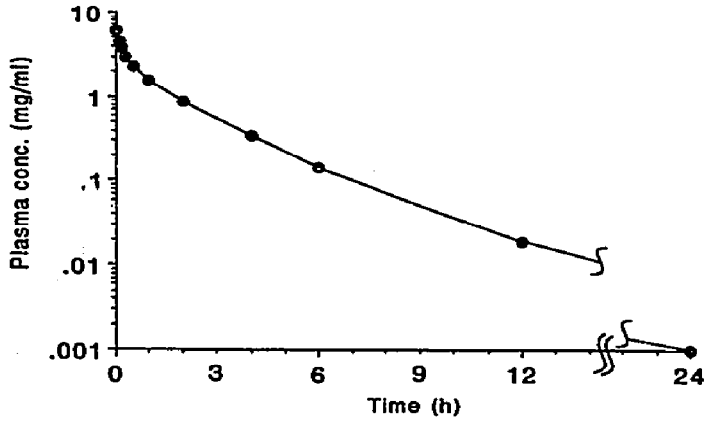

Fig. 2. Mean plasma concentration–time curve of iodixanol after intravenous injection of 0.3 gI/kg in six healthy volunteers.

The calibration curve was linear over the range 0.679–130.4 μg/ml, coefficients of regression being 0.99998. The minimum detectable quantity of iodixanol was about 0.5 μg/ml of plasma.

This method was successfully applied to the pharmacokinetic study in man. The concentration of iodixanol in plasma was determined after intravenous injection of iodixanol at a dose of 0.3 gI/kg (Fig. 2). Plasma half-lives in three phases were found to be 0.34 (α), 1.18 (β) and 3.24 h (γ).

In conclusion, the validation of HPLC showed that this method is acceptable for the quantitation of iodixanol in human plasma.

REFERENCES

1 T. Renaa and T. Jacobsen, *Acta Radiol.*, 370 (Suppl.) (1987) 9–11.
2 M. E. Oxaal, *Radiol. Diagn.*, 30 (1989) 311–314.
3 J. Edelson, G. Palace and G. Park, *J. Chromatogr.*, 274 (1983) 428–433.