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Short communication

High-performance liquid chromatographic determination of iobitridol in plasma, urine and bile

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Abstract

Iobitridol is a new non-ionic, low-osmolality contrast medium for urography and angiography. We have developed a method for determining iobitridol in body fluids using high-performance liquid chromatography with ultraviolet detection. The method, which is specific and reproducible, does not require an internal standard. Determinations can be carried out in body fluids against a set of standards in ethanol. The method was validated for the quantification of iobitridol in biological samples obtained during pharmacokinetic studies.

1. Introduction

Iobitridol is a new monomeric, non-ionic low-osmolality contrast medium for urography and angiography. Like all other iodinated contrast agents currently on the market, iobitridol is a tracer of extracellular fluid. It does not bind to plasma proteins and is mainly eliminated in urine during the hours following intravenous administration in rats [1]. In addition, iobitridol can be dialysed [2].

Preclinical pharmacokinetic studies carried out during the development of iobitridol called for the adaptation of a determination method. One of the analytical methods frequently used to determine iodinated contrast agents in body fluids is X-ray fluorescence [3–5] but its lack of specificity and sensitivity is a major disadvantage. More sensitive and specific methods, using high-performance liquid chromatography, have also been described in the literature. Some of them [6,7] require a cumbersome liquid–liquid extraction step (multiple steps) before chromatographic analysis. Others are much simpler to apply and require only limited pretreatment of biological samples [8–11].

This is the case of the reversed-phase high-performance liquid chromatography method described here, which is simple to use for determinations of iobitridol in body fluids (bile, urine and plasma from rabbits and urine from dogs).

2. Experimental

2.1. Test substance

Iobitridol (5-[3-hydroxy-2-(hydroxymethyl)-propionamido] - N,N′ - dimethyl - N,N′ - *bis* - (2,3 - dihydroxypropyl) - 2,4,6 - triiodoisophthalamide)

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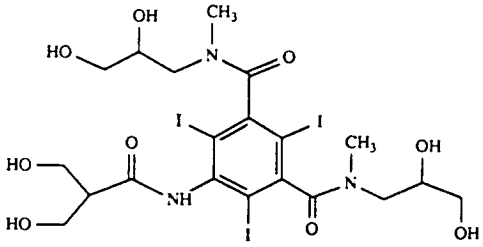

Fig. 1. Iobitridol (Xénétix).

(Fig. 1) was furnished by Guerbet—GCA (Aulnay sous Bois, France).

2.2. Materials

A Shimadzu HPLC system (Touzart et Matignon, Vitry sur Seine, France) with an isocratic pump (LC-6A), a flow controller (SCL-6A), an automatic injector (SIL-6A) and a UV detector (SPD-6AV) was used.

2.3. Sample preparation

The biological samples were diluted with ethanol (Prolabo, Paris, France) and vortex-mixed. Rabbit: urine was diluted 1:100; bile, 1:10; plasma, diluted 1:20; dog: urine diluted 1:100.

The tubes were then cooled for 15 min at −20°C, centrifuged for 10 min at 4000 rpm (2667 *g*) and 5 μl of the supernatant was injected into the HPLC system.

2.4. Chromatographic analysis

Iobitridol was chromatographed at room temperature on a Brownlee Spheri 5 RP18 column (220 × 4.6 mm, 5 μm). It was equipped with a 30 × 4.6 mm precolumn whose characteristics were identical to the main column. The eluent consisted of acetonitrile (10%) and 0.01 *M* $NaH_2PO_4 \cdot 2H_2O$ solution (90%) (Prolabo, Paris, France). The flow-rate was 1 ml/min. The detection wavelength was 239 nm (sensitivity 0.01 AUFS). Peak heights were integrated. The analysis time for each sample was 5 min. A set of standards at five concentrations of iobitridol in ethanol, i.e. 5.25, 10.5, 21, 70 and 105 μg iodine/ml, was analysed in parallel. The calibration curve was calculated by linear regression.

3. Results and discussion

Under the chromatographic conditions described above, the retention time of iobitridol was 2.7 min.

The linearity of the detector response was verified for the set of standards in ethanol solution over the concentration range of 5.25 to

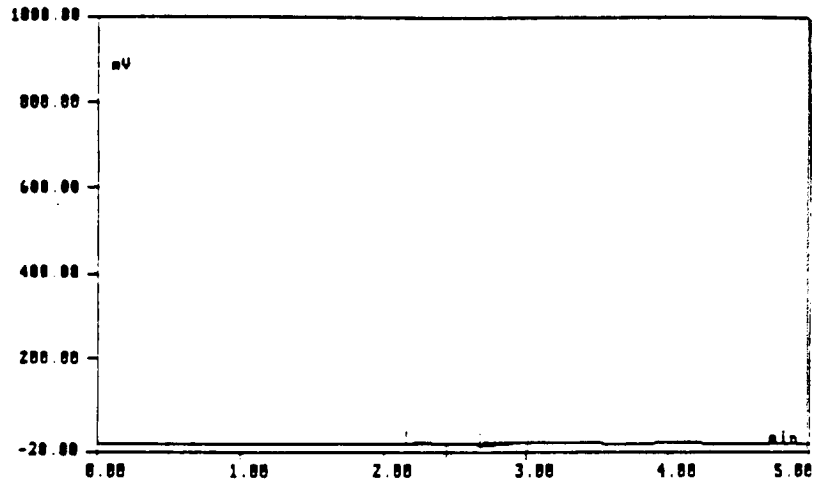

Fig. 2. Blank chromatogram of dog urine.

105 μg I/ml. The mean accuracy was 102 ± 2% and the coefficient of variation among runs was less than 7%. The lower limit of quantitation was 5.25 μg I/ml.

The specificity of the method was verified in body fluids by comparing the chromatograms of reference body fluids not containing the test substance with chromatograms of the same fluids spiked with the test substance. The method showed high specificity, as shown by the chromatograms in Figs. 2 and 3.

Using reference fluids spiked with known quantities of iobitridol, recovery was verified at several concentrations and each concentration was tested 5 times. The results given in Table 1 show that the mean recovery for each fluid was high (between 91 and 114%) and reproducible, which makes it unnecessary to use an internal standard.

The high recoveries observed indicate the absence of matrix effects from any of the biological fluids tested. Thus, no errors are introduced when determinations are carried out in body fluids against a set of iobitridol standards in ethanol.

The lower limits of quantitation, taking into account the dilutions of the fluids, were as follows. Rabbits: 1.03 mg I/ml of urine; 0.068 mg I/ml of bile; 0.221 mg I/ml of plasma; dogs: 0.621 mg I/ml of urine.

Table 1
Percentage of recovery of iobitridol

Fluid	Concentration (mg I/ml)	Recovery (mean ± S.D.) (%)
Rabbit urine	1.03	114 ± 15
	7.06	103 ± 0
Rabbit bile	0.068	91 ± 9
	0.100	103 ± 3
	0.140	102 ± 0
	0.540	106 ± 5
Rabbit plasma	0.221	94 ± 15
	0.275	91 ± 1
	0.540	104 ± 8
	0.620	109 ± 0
	0.800	104 ± 1
Dog urine	0.62	107 ± 4
	1.03	102 ± 4
	6.86	105 ± 3

The range of linearity for determinations in body fluids is identical to that observed in ethanol.

In sum, a specific and reproducible method for determining iobitridol in body fluids by high-performance liquid chromatography was developed and validated. It does not involve a cumbersome step for pretreatment of biological samples, and because of the short analysis time (5 min), it is possible to inject a large number of samples in one day. This analytical method has been used for determinations in samples ob-

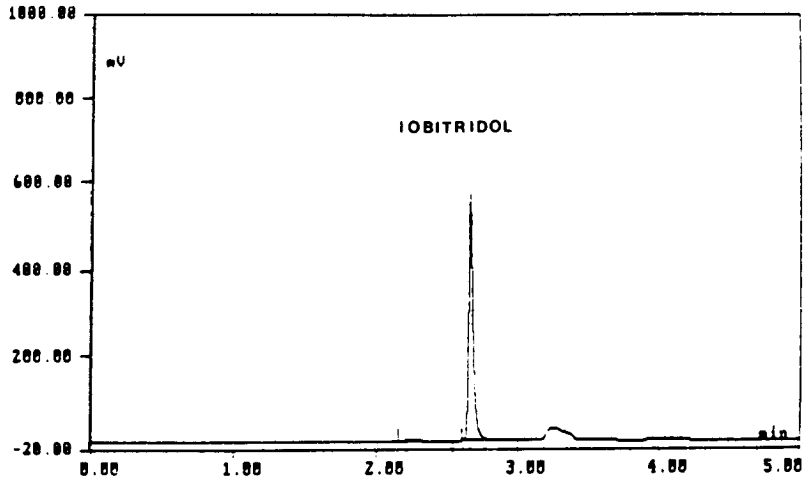

Fig. 3. Spiked chromatogram of dog urine.

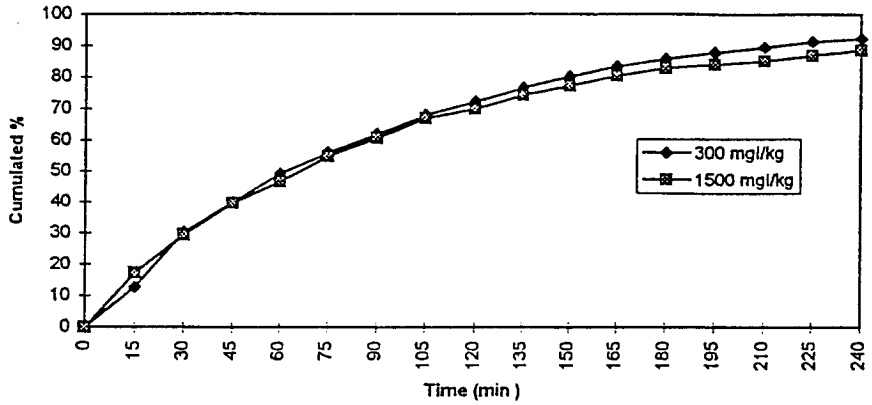

Fig. 4. Urinary excretion of iobitridol in anesthetized rabbit.

tained during preclinical pharmacokinetic studies of iobitridol (for example in urinary excretion studies in rabbit; Fig. 4) and can be validated for other fluids from other species.

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