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Note

Rapid determination of renal contrast media in biological fluids by means of high-performance liquid chromatography

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Several iodinated organic acids are very frequently used for intravenous urography. The reason for their usefulness is the fact that they are rapidly excreted by the kidneys. Some of them are excreted solely by glomerular filtration but most of them are also secreted via a carrier-mediated mechanism in the proximal tubules.

In the course of a fundamental study on the kinetics of urinary excretion of a number of agents, secreted by the renal tubules of the mammalian kidney, we needed a rapid, sensitive and quantitative method for the detection of some contrast media in plasma and urine. Several existing methods [1, 2] are laborious, lack the required specificity or need the use of radioactive materials which imposes special handling and safety measures.

The procedure described in this paper enables the quantitative detection of renal contrast media in a simple specific way, by means of high-performance liquid chromatography (HPLC). Although developed for iodopyracet (I) and iodamide (II) (Fig. 1), the procedure is applicable to some other contrast media as well, e.g. metrizoic acid, diatrizoic acid, iothalamic acid and acetrizoic acid.

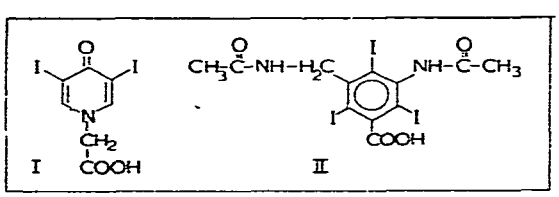

Fig. 1. Structural formulae of iodopyracet (I) and iodamide (II).

EXPERIMENTAL

Materials

Iodopyracet and iodamide were obtained from Dagra (Diemen, The Netherlands). LiChrosorb RP-8 (5 μm), tetrabutylammonium hydrogen sulphate and Tris [tris(hydroxymethyl)aminomethane] were obtained from Merck (Darmstadt, G.F.R.). All other reagents used were of analytical grade.

Apparatus

The equipment used was a Waters Assoc. (Milford, MA, U.S.A.) high-pressure liquid chromatograph consisting of a M 6000A pump, a 46K universal injector and a M440 absorbance detector. I was detected at 280 nm, whilst II was detected at 254 nm.

HPLC conditions

A stainless-steel column (10 × 0.46 cm I.D.) was packed with LiChrosorb RP-8 (5 μm). Chromatography was performed using a mixture of 15% water and 85% methanol containing 0.01 *M* tetrabutylammonium hydrogen sulphate and 0.01 *M* Tris. The elution rate was 1.5 ml/min. A typical chromatogram obtained from a plasma sample is shown in Fig. 2.

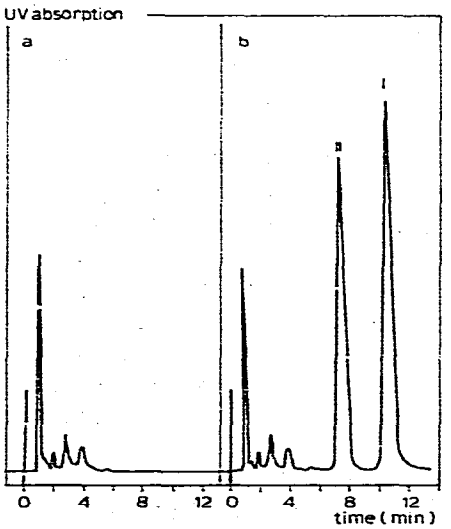

Fig. 2. Chromatograms of (a) blank plasma and (b) plasma containing iodopyracet (I) and iodamide (II).

Assay procedure

For the determination of I, II was used as an internal standard, whilst I was used as an internal standard for the determination of II. Standard solutions of the appropriate salts were prepared in water. Samples of plasma (1 ml) were transferred into a test tube and spiked with 100 μl of internal standard solution

(500 μg/ml). Next, 5 ml of methanol were added to precipitate the plasma proteins. After centrifugation at 1500 *g* for 20 min the supernatant was transferred into another tube and evaporated to dryness under a stream of dry filtered air in a water bath at 65°C. The residue was dissolved in 300 μl of water—methanol (10:90, v/v). After standing for 10 min, 10 μl of the clear supernatant were injected directly into the liquid chromatograph.

Urine samples were treated in a different way. After diluting 100 μl of the urine 10 times with mobile phase and spiking it with 100 μl of internal standard solution (500 μg/ml), 10 μl of the resulting solution were injected into the liquid chromatograph.

Calibration procedure

Samples of blank plasma (1 ml) or urine (0.1 ml) were spiked with various known amounts of the compound to be determined. After treating the calibration samples as outlined above and injecting them into the liquid chromatograph peak height ratios of compound to internal standard were plotted against the amount of compound added.

RESULTS AND DISCUSSION

Under the conditions employed no interfering substances from blank plasma appear in the chromatogram (Fig. 2). This holds true for urine as well.

The procedure described can also be used for the assay of other contrast media, such as metrizoic acid, diatrizoic acid, iothalamic acid and acetrizoic acid. For this last agent it is necessary to change the mobile phase to contain more methanol and less water.

The method involves no extraction, but the deproteinization of plasma should be performed carefully in order to obtain clear solutions for injection. The residue after the evaporation of methanol is not completely free of protein. Therefore it is taken up in the water—methanol (10:90) mixture in which the rest of the proteins settles in about 10 min. During protein precipitation, evaporation and redissolution no significant dissipation of analysed substances occurred. Pure methanol should not be used for this redissolution, since injection of the pure methanolic solution causes asymmetric peaks of the contrast media. Also a mixture with a higher water content is not useful since the protein remains emulsified.

Calibration graphs are linear up to concentrations of at least 100 μg/ml. For iodopyracet the linearity was checked further and appeared to prevail up to 1000 μg/ml. The limit of detection of the various compounds is about 0.2 μg/ml with the procedure described. By small modifications (less dilution) this limit may still be decreased. Over the whole range studied the standard deviations of the plasma and urine determinations were 2—4%.

The method reported here was successfully applied to pharmacokinetic studies aimed at describing kinetic drug interactions. As an example of a pilot study for these investigations Fig. 3 shows a plasma and an urinary excretion curve obtained after application of an intravenous dose of iodopyracet to a male beagle dog. The plasma curve shows three distinct phases, indicating the occurrence of a three-compartmental model. Iodopyracet is recovered almost

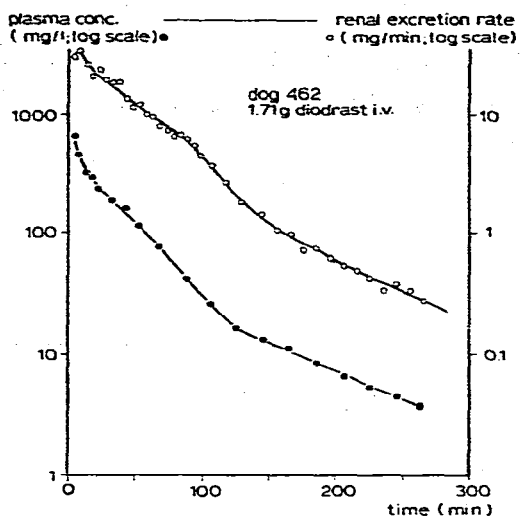

Fig. 3. Plasma and urinary excretion curves obtained after intravenous administration of iodopyracet in the form of its diethanolamine salt to a male beagle dog. Urinary excretion rates are shown at the midpoints of urine collecting periods.

completely unchanged from urine. By careful analysis of plasma and urine kinetics it is possible to obtain insight into the mechanisms of renal excretion and into the nature of possible drug interactions at the level of tubular secretion.

REFERENCES

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