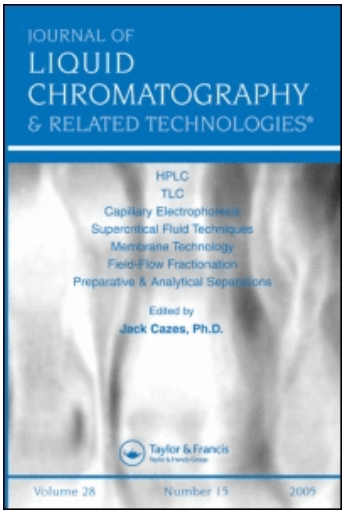

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IOHEXOL DETERMINATION BY DIRECT INJECTION OF SERUM ON THE HPLC COLUMN

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ABSTRACT

Iohexol, a non-ionic compound used as a contrast media for angiography and as a mesure to determine glomerular filtration rate, was measured in serum by injecting serum directly on the column after a 200 fold dilution. The analysis was accomplished in less than 5 min.

INTRODUCTION

Iohexol (N,N -Bis(2,3-dihydroxypropyl)-5-[N- (2,3-dihydroxy propyl)-acetamido]-2,4,6-triiodoisophthalamide), known also as Omnipaque, is a tri-iodinated, non-ionic radiographic contrast medium. It is used frequently for angiography. Because it is a non-metabolizable compound, does not bind to serum proteins, and is cleared by the kidney, iohexol has been advocated recently as a

good substance for measuring glomerular filtration rate (GFR) (1-3). Although creatinine clearance is commonly used for GFR determination, it is well recognized that it is not very accurate. While, inulin clearance is considered the gold standard for GFR measurement (4), it is not suitable for routine testing because it requires pump infusion and collection of multiple urine and blood samples. Several organic compounds labelled with radioisotopes have been used to replace the inulin test such as the ^{125}I-iothalmate, and ^{51}Cr- labeled ethylene- diaminetetraacetic acid (EDTA). These latter tests involve the administration of radioisotopes to the patient. Iohexol eliminates the latter problem and avoids the need for multiple sample collection while giving results for GFR similar to that obtained by the previous compounds (1-3) including inulin (4).

In order to be used routinely for GFR measurement a simple, rapid and sensitive assay method for this compound is needed. Serum iohexol has been analyzed previously by high-performance liquid chromatography (HPLC) after precipitation with perchloric acid (5,6) and measuring the released iodine by the ceric reaction (7). A commercial instrument, based on x-ray fluorescence, dedicated to the measurement of this compound, has been described (4,8) indicating further the importance of this test for GFR measurement. Here we describe a simple and rapid method for iohexol assay based on injecting diluted serum directly on the HPLC column without any pre-treatment. This technique is very simple and can be fully automated. Previously we have shown that

direct serum injection on the column speeds the assay and simplifies the automation (9).

MATERIALS AND METHODS

Equipment: The instrument consisted of a model 110 A pump (Beckman Instruments, Fullerton, CA), a model 440 ultraviolet detector set at 254 nm, 10 mA full scale (Waters Associates, Milford, MA) and a 10 uL loop injector (Rheodyne, Berkely, CA)

Column and Mobile Phases: Two cartridge columns were tested: (1) a 3um RP-18 Velosep column, 40 X 3.2 mm (I.D.), (Applied Biosytems, San Jose, CA) eluted with 8 mM phosphoric acid, pH 2.2 at a flow rate of 0.6 ml / min and (2) a 5um RP 18, 12.5 cm, Lichosphere 100(Merck, Cherry Hill, NJ) eluted with 10% methanol in phosphate buffer, 8 mmol/L, pH 2.2 at a flow rate of 1.3 ml/min.

Procedure: Serum (standard or controls) was diluted 200 fold with the elution solvent. An aliquot was injected directly on the column.

For comparison, a few samples were analyzed with acetonitrile deproteinization too. Equal volumes of serum, and acetonitrile (100 uL of each) were vortex-mixed for 15 s and centrifuged for 30 s at 15000 rpm. The supernatant was further diluted 100 times with the pump solvent and an aliquot was injected on the column.

Standards: Iohexol (Winthrop Pharmaceuticals, New York, NY) was diluted to prepare the different concentrations.

RESULTS AND DISCUSSION

The choice of an appropriate column in HPLC for direct serum injection is very important (9). Here we chose an inexpensive cartridge column to perform the assay. The use of a short length column packed with a small size particles offers a rapid, effecient and sensitive analysis. For iohexol elution, these columns do not require the use of high concentrations of organic solvents which tend to denature and precipitate proteins leading to an increase in pressure. Thus it is feasible to inject a reasonable amounts of diluted serum on these columns. Figure 1 illustrates that the chromatograms, by this method, are clean with no interferences. The retention time is about 4 min. None of the common drugs listed in Table 1 interfered with the assay. Acetaminophen, however, eluted very close to iohexol. Periodically we checked the resolution of the column by injecting a mixture of acetaminophen and iohexol. It is important to keep the dead volume of the connections to a minimum to obtain a good separation between acetaminophen and iohexol with the 3 um column. However, this was not a problem with the 5 um column. Of course acetaminophen can be detected by the same method; however, a 50 rather than 200 fold dilution of the sample is necessary to obtain adequate sensitivity.

The average recovery of spiked serum samples was 97 and 101 % for 400 and 200 mg/L (triplicate assays). The test is linear between 25-1000 mg/L , Fig 2. The coefficient of variation is 3.1%

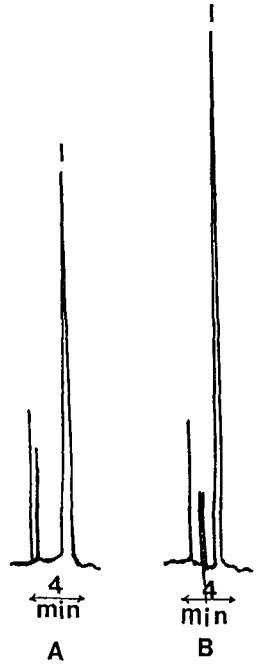

Fig 1 Chromatogram of serum iohexol (1, 800 mg/L)) from a patient receiving iohexol: A) on the 3 um column, and B) on the 5 um column.

Table 1

List of The Drugs Which Did Not Interfere With Iohexol Analysis.

Drug	mg/L
Theophylline	40
Phenytoin	30
Phenobarbital	50
Carbamazepine	13
Lidocaine	6
Procainamide	12
N-acetylprocainamide	10
Quinidine	5
Salicylic acid	100

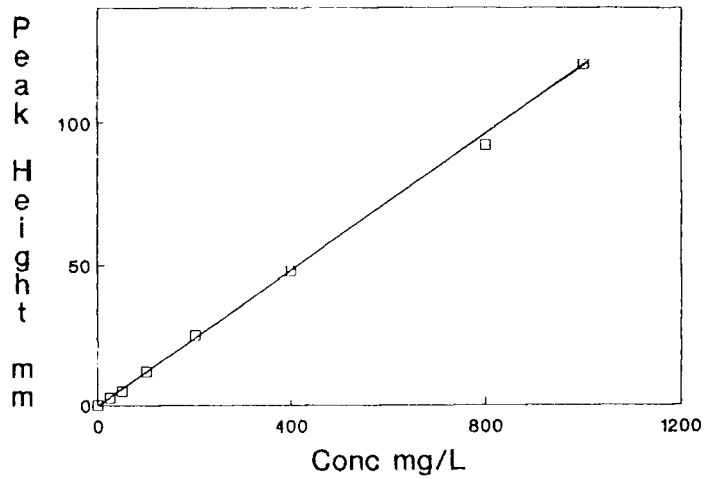

Fig 2. Linearity of iohexol analysis.

(N=15, mean 400 mg/L). The minimum detection limit (3 SD of baseline) was 25 mg/L. We used a 50 times dilution for samples below 40 mg/L thus being able to measure values about 10 mg/L. The capacity factor and the peak height did not change by changing the pH of the buffer between pH 2.2 to 7.5.

We compared this method to that of acetonitrile deproteinization for 14 samples , Fig 3. The regression analysis showed a good correlation between the two methods, with r very close to 1.0 . Figure 4 illustrates iohexol pharmacokinetics. The level of this drug varies greatly over time, decreasing from about 15,000 mg/L immediately after injection of the dye to a low value of about 20 mg/l after 24 hours. However for glomerular filtration rate the majority of the values will be between 20-400 mg/L depending on the dose, time, and renal clearance rate. Both

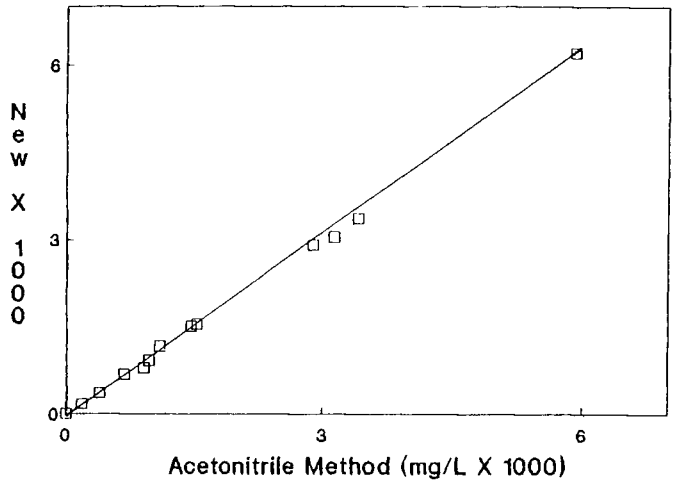

Fig 3. Comparison of iohexol analysis by direct serum injection and by acetonitrile deproteinization. New method= Acetonitrile X 1.03 -29.86, r=0.99, n=14).

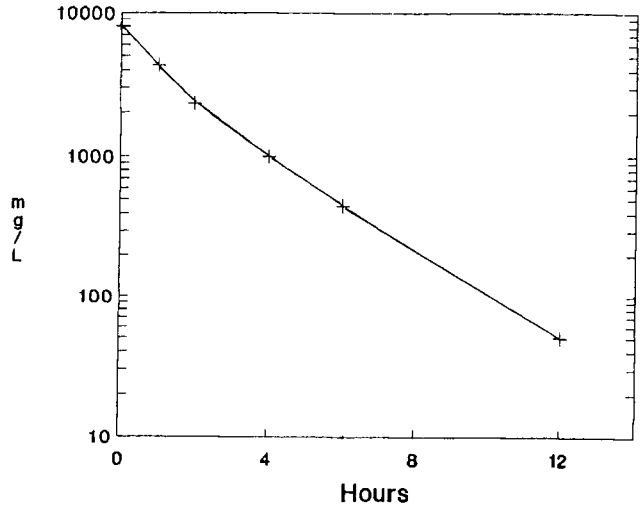

Fig 4. Pharmacokinetics of iohexol in a subject injected with a total dose of 90 g.

columns gave similar results. However, the separation, in general, and especially between iohexol and acetaminophen was better on the 5 um column. Every 30-50 injections the column was reversed and washed with 20 ml acetonitrile. Because of the low pressure drop and the high sample dilution(9) these cartridge columns can be used for at least 200 injections. Thus the cost per injection is less than $0.50. However, the savings in labor and time and suitability for automation are much greater than the cost of the column.

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