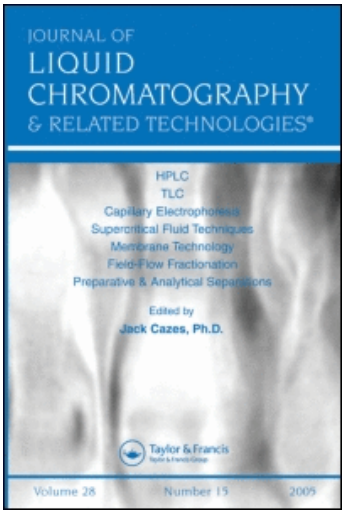

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Lim, C. K. and Rinsler, M. G.(1978) 'The Determination of Ioglycamic Acid in Bile and in Urine by High Performance Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 1: 5, 659 — 667
To link to this Article: DOI: 10.1080/01483917808060025
URL: http://dx.doi.org/10.1080/01483917808060025

JOURNAL OF LIQUID CHROMATOGRAPHY, 1(5), 659-667 (1978)

THE DETERMINATION OF IOGLYCAMIC ACID IN BILE AND IN URINE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A fast, simple and specific method for the determination of ioglycamic acid in bile and in urine is described. Reversed-phase ion-pair chromatography with methanol-water (67.5 : 32.5) in the presence of the counter ion tetrabutyl-ammonium phosphate as the mobile phase separates ioglycamic acid in 5 min at a flow rate of 1 ml/min. Under the same conditions urinary creatinine can be simultaneously determined and may be used as an endogenous internal standard. Bile and urine from a healthy subject were screened for possible metabolites but these are not detected.

INTRODUCTION

Ioglycamic acid (Figure 1) is a contrast medium used for the examination of the bile duct and the gall bladder. It is usually used as the di-N-methylglucamine salt (meglumine ioglycamate) or as the disodium salt (sodium ioglycamate) and is given intravenously by infusion or injection. In some patients it is believed (1) that ioglycamic acid is largely excreted by the kidneys and not by the liver. It is thus of interest to monitor the urinary concentration of ioglycamic acid and to compare the excretion rate by the liver and the kidneys during intravenous cholegraphy.

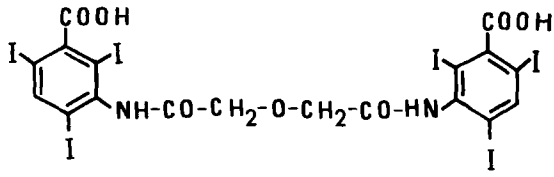


Figure 1. Ioglycamic acid.

The extent of the renal excretion of intravenous cholegraphic agents has been judged crudely by visualisation of the urinary tract on radiographs (2-4) but these give neither excretion rate nor the true urinary concentration of the radiological contrast agent (5). A spectrophotometric method has been reported (6) for the estimation of urinary meglumine ioglycamate based on the precipitation of ioglycamic acid with 2M sulphuric acid. The precipitate is redissolved in sodium hydroxide for spectrophotometric measurement.

We now report an improved, fast and specific method for the determination of ioglycamic acid in bile and in urine using high-performance liquid chromatography (HPLC).

As the mechanism of ioglycamic acid excretion by the liver is not clear we attempted, using HPLC and mass spectrometry, to detect any metabolites which might be present in the bile or in the urine.

EXPERIMENTAL

Material and reagents. Chloroform, ether and methanol were AnalaR grade (BDH, Poole, U.K.). Acetonitrile was HPLC grade (Rathburn Chemicals Ltd., Peebleshire, U.K.). Meglumine ioglycamate (Biligram) was from Schering AG, Berlin, G.F.R. PIC reagent A

(containing tetrabutylammonium phosphate and buffered at pH 7.5) was from Waters Associates, Milford, Mass., U.S.A. and was prepared by diluting one bottle of the reagent to 1 litre with water.

Preparation of dimethylioglycamate. Ioglycamic acid was precipitated from meglumine ioglycamate by adding 2M sulphuric acid. A solution of diazomethane in ether (20 ml) was stirred in an ice-bath and ioglycamic acid (500 mg) was added in small portions. The mixture was stirred for a further 30 min after all the ioglycamic acid had been added. It was then stirred at room temperature to remove excess diazomethane. The solvent was removed under reduced pressure, using a rotary evaporator, and the colourless residue, which is soluble in organic solvents (alcohols, chloroform) was identified as the dimethylester of ioglycamic acid by its mass spectrum (M^+ 1156). Tetramethyl-ioglycamic acid was also formed by further methylation of the -NH groups.

High-performance liquid chromatography. A Partisil-10 ODS column (25 cm x 4.6 mm I.D.) was used with a Waters Associates Model 6000A solvent delivery system with a U6K injector and a Cecil CE212 (Cecil Instruments, Cambridge, U.K.) variable wavelength ultraviolet detector set at 237 nm, the absorption maximum of ioglycamic acid. Preliminary purification was performed on a pre-column (5 cm x 4.6 mm I.D.) packed with C_{18} Corasil (Waters Associates). Bile (10 µl), collected by duodenal drainage from a tube introduced orally, and urine (10 µl of a ten-fold dilution) were injected untreated directly into the liquid chromatograph.

Ioglycamic acid was analysed using methanol-water with PIC reagent A (67.5 : 32.5) as the solvent system and elution was at 1 ml/min. For the analysis of bile the solvent system was adjusted to methanol-water with PIC reagent A (62.5 : 37.5) for improved resolution. Acetonitrile-water (80 : 20) was used as the mobile phase for the separation of dimethylioglycamate and elution was again at 1 ml/min.

RESULTS AND DISCUSSION

High-performance liquid chromatography. Ioglycamic acid is highly insoluble in organic solvents, its separation by adsorption chromatography is therefore not practicable. It is also quite acidic, easily ionisable in aqueous solution, thus no retention can be achieved on a reversed-phase column employing aqueous eluents. Ion suppression by using acidic solvent systems, on the other hand, tends to precipitate ioglycamic acid. Since it is completely ionised in aqueous solutions it can be separated by bonded phase anion exchange chromatography. This approach was not adopted, however, because we also wished to measure creatinine, which is a major component of urine and as creatinine is basic it will not be retained on the anion exchange column. The simultaneous measurement of creatinine during determination of compounds present in urine is advantageous as it was believed that urinary creatinine levels are relatively constant over several hours. Creatinine can therefore in theory be used as an endogenous internal standard.

Reversed-phase ion-pair chromatography is well established as an alternative to ion exchange chromatography and has the advantage of allowing the simultaneous assay of acids, bases and neutral species. The separation of standard creatinine and ioglycamic acid is shown in Figure 2. The solvent system consisted of methanol-water with PIC reagent A (67.5 : 32.5) and complete resolution was achieved in 5 min at a flow rate of 1 ml/min. The retention of ioglycamic acid was obviously due to the presence of the counter ion tetrabutylammonium phosphate, i.e.

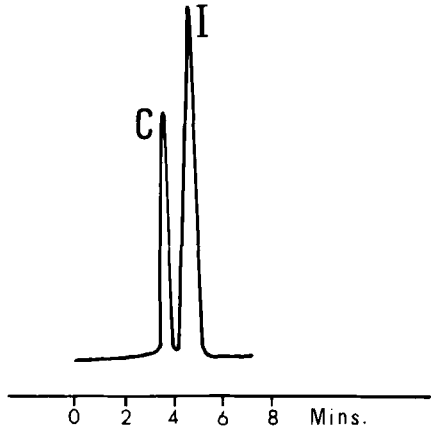
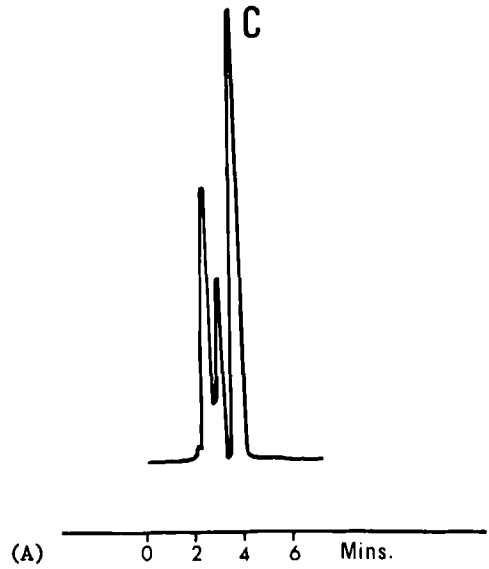


Figure 2. Separation of creatinine and ioglycamic acid
Column : Partisil - 10 ODS
Solvent: MeOH-water (67.5 : 32.5) with PIC reagent A.
Flow rate : 1 ml/min
C = Creatinine
I = Ioglycamic acid

by the ion-pair mechanism. It was likely, however, that creatinine was separated by the ion suppression mechanism as it is a weak base.

In a preliminary study bile and urine specimens were collected from one subject for analyses both before and after intravenous infusion of meglumine ioglycamate. In urine, both creatinine and ioglycamic acid were present in high concentration and a ten-fold dilution was necessary before analysis by HPLC. Figure 3 shows the difference obtained between pre-infusion (A) and post-infusion urine (B). The post-infusion urine clearly showed a large peak occuring at a retention time corresponding to ioglycamic acid. This peak is absent in pre-infusion urine. The urinary creatinine levels were quite constant in both specimens.

To achieve complete resolution from impurities, the solvent system was modified to methanol-water with PIC reagent A



(62.5 : 37.5) when bile was analysed. Figure 4 shows the chromatograms obtained from bile before (A) and after (B) influsion with ioglycamic acid. This again indicated a high concentration of contrast agent present in the bile after infusion.

Background contamination was never a problem as ioglycamic acid was the major component in post-infusion bile and urine.

Quantitation. The quantitation of ioglycamic acid was carried out using standard chromatographic procedures of peak area measurement. The linearity and reproducibility of the chromatography were sufficiently good that an internal standard was not used. Creatinine,however, can be used as an endogenous internal standard when urinary ioglycamic acid is being determined. The detection wavelength (237 nm) was the absorption maximum of ioglycamic acid, and thus provides both specificity and sensitivity to the method.

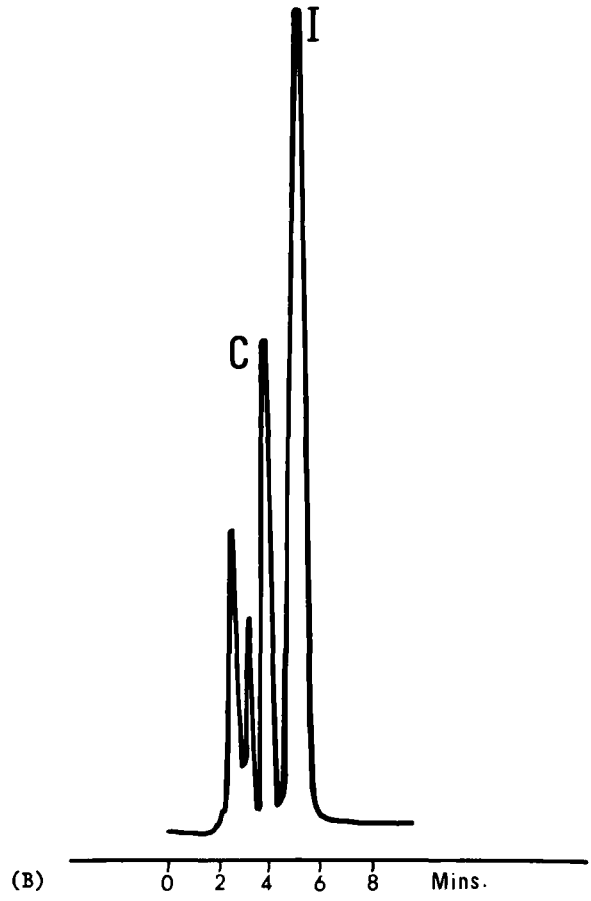


Figure 3. HPLC chromatograms of (A) pre- and (B) post-infusion urine. Conditions as for Fig. 2.

Metabolites. Methylation of carboxyl groups by the liver is often a way of excreting acidic compounds, although conjugation with amino acids or sugars is also common.

Ion-pair chromatography using a wide variety of solvent compositions, failed to reveal the presence of ioglycamic acid conjugates. The dimethylester of ioglycamic acid was therefore

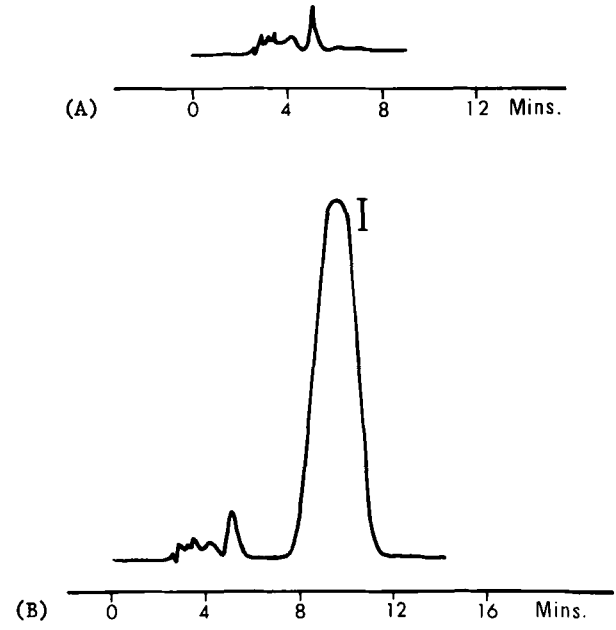


Figure 4. HPLC chromatograms of (A) pre- and (B) post-infusion bile.
Solvent : MeOH-water (62.5 : 37.5) with PIC reagent A.
Other conditions similar to Fig. 2.

investigated as a possible metabolite. This compound, prepared by treating ioglycamic acid with diazomethane, was completely soluble in chloroform and alcohols. It gave a sharp peak (Figure 5) when separated by reversed-phase chromatography using acetonitrile-water (80 : 20) as the mobile phase. Extraction of bile and urine with chloroform followed by HPLC analyses of the extracts showed no peak corresponding to dimethylioglycamate.

CONCLUSION

Reversed-phase ion-pair chromatography provides a fast and specific method for the determination of the biliary contrast medium ioglycamic acid in bile and in urine. The method described is also suitable for the measurement of urinary creatinine.

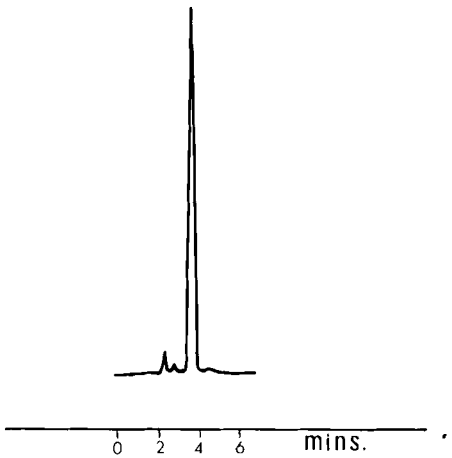


Figure 5. Separation of dimethylioglycamate
Column : Partisil - 10 ODS
Solvent : CH_3CN-water (80 : 20)
Flow rate : 1 ml/min

A preliminary study in one healthy subject suggested that ioglycamic acid was excreted unchanged by the liver and the kidneys, since no metabolites could be detected.

ACKNOWLEDGEMENT

We would like to thank Dr. J.D. Spencer for his assistance in obtaining the samples of bile and urine.

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