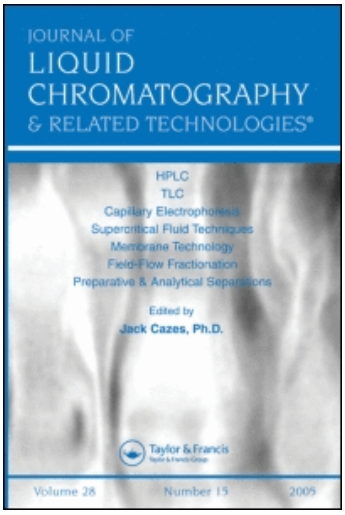

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

The Estimation of Serum Digoxin by Combined HPLC Separation and Radioimmunological Assay

J. C. K. Loo[a]; I. J. McGilveray[a]; N. Jordan[a]
[a] Drug Research Laboratories, Health Protection Branch, Ontario, Canada

To cite this Article Loo, J. C. K. , McGilveray, I. J. and Jordan, N.(1981) 'The Estimation of Serum Digoxin by Combined HPLC Separation and Radioimmunological Assay', Journal of Liquid Chromatography & Related Technologies, 4: 5, 879 — 886
To link to this Article: DOI: 10.1080/01483918108059982
URL: http://dx.doi.org/10.1080/01483918108059982

JOURNAL OF LIQUID CHROMATOGRAPHY, 4(5), 879-886 (1981)

THE ESTIMATION OF SERUM DIGOXIN BY COMBINED HPLC SEPARATION AND RADIOIMMUNOLOGICAL ASSAY

J.C.K. Loo, I.J. McGilveray and N. Jordan
Drug Research Laboratories,
Health Protection Branch,
Ottawa, Ontario, Canada, K1A 0L2

ABSTRACT

A specific method for the quantitation of digoxin in serum has been developed and applied to determination of drug concentrations in serum from digitalized patients. One ml of the supernatant from acetonitrile denatured serum, subsequently diluted with water, was chromatographed on a reversed phase HPLC column. The eluent corresponding to the retention time of digoxin was collected and analysed using a commercial radioimmunoassay kit. The recovery at 3ng/ml was 96.1 ± 3.0%. Endogenous substances, drugs and metabolites of digoxin do not interfere with the method. The mean value of the samples estimated by the present method was 84% of those determined by direct R.I.A.

INTRODUCTION

The studies of Gault et. al. (1) and those of the present authors (2) have demonstrated the possibility of degradation of orally administered digoxin by acid hydrolysis in the stomach prior to absorption. Digoxigenin, a major degradation product, possessing relatively little cardiac activity, was shown to be rapidly absorbed after oral administration. In addition, several radioimmunoassays (R.I.A.) for serum digoxin do not distinguish between digoxin and digoxigenin as antisera from these kits cross react significantly with digoxigenin (2).

Resolution of digoxin from its metabolites by HPLC followed by RIA determination (HPLC-RIA) demonstrated that the intact drug as well as its metabolites was present in serum obtained from digitalized patients with gastric hyperacidity (3). Recently, Gibson et. al. demonstrated by using a similar technique, that R.I.A. overestimates the amount of digoxin in plasma of renal failure patients (4).

The assays mentioned above (2-4), though accurate and specific, were tedious and not readily adaptable for routine monitoring of serum samples from digitalized patients.

The object of the present study was to develop a rapid method for the specific determination of digoxin in serum.

MATERIALS

Reagents

Digoxin 12α-^{3}H was obtained from New England Nuclear (Lachine, Québec, Canada). The Digoxin ^{125}I solid phase kits were purchased from Clinical Assays, a division of Travenol Labs Inc. (Cambridge, Mass., U.S.A.). Ethyl alcohol was obtained from Consolidated Alcohols Ltd (Toronto, Canada) and redistilled prior to use. HPLC grade acetonitrile was purchased from Fisher Scientific (Ottawa, Canada).

Apparatus

Samples were centrifuged in a Sorvall RC 2B centrifuge (Newtown, Conn., U.S.A.). A constant volume high performance liquid chromatograph from Waters (Milford, Mass., U.S.A.) containing a Model 6000A pump, a U 6K injector (fitted with a 2.6 ml loop) and a model 440 detector

set at 254 nm, was used. A reverse phase column, (ODS Spherisorb 5 µ, 3.2 x 250 mm; Altex, Berkeley, Cal., U.S.A.) was used.

Mobile Phase

The mobile phase, 3.3% ethyl alcohol, 25% acetonitrile in water (v/v) was prepared fresh daily. A flow rate of 60 ml/hr was used.

METHOD

To 1.0 ml of serum in a glass culture tube was added 1.5 ml of acetonitrile. After vortexing briefly, the sample was incubated for five minutes at 4°C and centrifuged for fifteen minutes at 4°C. The supernatant was decanted into a glass culture tube to which 1.0 ml of water was added. Following centrifugation for fifteen minutes at 4°C, the supernatant was pipetted off. A 1.0 ml aliquot of supernatant was added to 1.8 ml of water, injected directly into a 2.6 ml loop injector and chromatographed. The retention time of digoxin was established daily by injection and stepwise collection of nanogram quantities of digoxin spiked with tritiated digoxin. The eluent corresponding to the retention volume of digoxin was collected (1.5 ml). The dried residue was reconstituted with one ml of kit buffer. A 250 µl aliquot was evaluated in duplicate by using a ^{125}I digoxin radioimmuno-assay kit. Overnight storage of samples in sealed ampules, either after precipitation or after HPLC collection, has been shown to cause no change in results.

RESULTS

The present chromatographic system readily separates digoxin and its metabolites as well as potentially interfering compounds. The

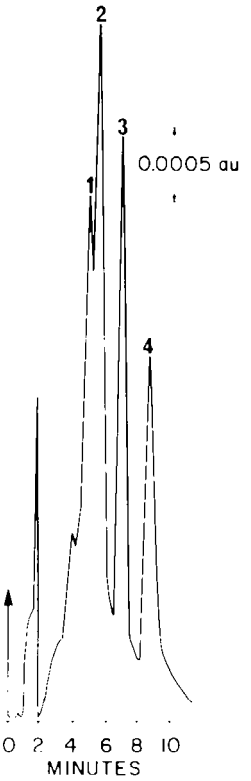

FIGURE 1. HPLC OF DIGOXIN: 1: DIGOXIGENIN; 2: DIGOXIGENIN MONODIGITOXOSIDE; 3: DIGOXIGENIN BISDIGITOXOSIDE; 4: DIGOXIN

retention times are given in Table I. Figure I illustrates a chromatogram of microgram quantities of digoxin and its metabolites.

Standard serum digoxin solutions were spiked with tritiated digoxin and used to assess the recovery and efficiency of the method. The precipitation step was found to yield a 95.3% recovery (C.V. = 1.0% for n = 10). Table II indicates the reproducibility of the column collection. The

TABLE 1

Retention Times of Digoxin, Metabolites and Compounds of Interest.

Compound	Retention Time
Digoxigenin	4.9 min
Digoxigenin-monodigitoxoside	5.4 min
Prednisolone	6.1 min
Digoxigenin-bisdigitoxide	6.8 min
Digoxin	8.6 min
Spironolactone	18.3 min
Canrenone	21.0 min
*Dihydrodigoxin	≈ 9.6 min

* Estimated by column collection, followed by use of an anisaldehyde spray after thin layer chromatography.

collection time (8.0-9.5 min) was established by a radioactive tracer which was chromatographed at the onset as well as in between several injections to ensure that the retention time remains constant during the course of a day.

TABLE 2

Reproducibility of Column Collection

Sample	% of ^{3}H Digoxin Collected in 1.5 ml eluant
1	79.2
2	85.2
3	80.2
4	81.6
5	75.4
6	82.6
	Mean = 80.7
	% C.V. = 4.1

TABLE 3

Estimation of Digoxin from Serum (3ng/ml) (n = 8)

Sample	ng/ml estimated	% Recovery
1	3.00	100.0
2	3.00	100.0
3	2.85	95.0
4	2.85	95.0
5	2.89	96.3
6	2.91	97.0
7	2.78	92.7
8	2.78	92.7
		Mean = 96.1%
		% C.V. = 3.0

The concentration of digoxin is estimated by the following equation:

$$\text{Digoxin (ng/ml of serum)} = \frac{100\ R}{P \times C}$$

where R = Digoxin estimated by RIA kit (ng/ml)

P = Percentage extraction from serum following denaturation

C = Percentage recovered from column

Both P and C are determined daily using ^{3}H digoxin.

The precision and accuracy were determined by the repeated assay of a 3 ng/ml digoxin serum solution. The results are shown in Table 3.

The method was applied in the analysis of digoxin serum concentration of digitalized patients. Table 4 summarizes the results obtained by using HPLC-R.I.A. and by direct R.I.A. It is noted that the latter method overestimates the digoxin plasma concentrations in these patients.

TABLE 4

Digoxin in Serum of Digitalized Patients

Patient	Digoxin Concentration ng/ml RIA	H.P.L.C. - R.I.A.	HPLC Digoxin / Serum RIA Digoxin
1	1.2	0.64	0.53
2	1.2	1.17	0.98
3	1.7	1.26	0.74
4	2.1	1.6	0.76
5	1.9	2.0	1.05
6	1.7	1.48	0.87
7	2.8	2.23	0.80
8	2.5	2.20	0.88
9	4.3	4.0	0.93
10	1.4	1.12	0.80
11	1.8	1.75	0.97
12	1.2	0.9	0.75
13	1.3	1.24	0.95
14	3.0	2.4	0.80
Mean ± SD	2.01 ± 0.89	1.71 ± 0.84	0.84 ± 0.13

DISCUSSION

Gibson et al. (4), using a combined HPLC-RIA technique, examined the plasma of 9 digitalized patients requiring maintenance renal dialysis and reported that the ratio, (digoxin estimated by HPLC-RIA/digoxin estimated by RIA), was 0.83 ± 0.12 (range 0.58 to 0.94). In the present study using 14 digitalized patients of unknown renal status, we obtained, surprisingly, a similar ratio of 0.84 ± 0.12 (range 0.53 to 1.05). The combined forward phase HPLC/RIA methods employed by Gibson et. al. and others (2,3) require tedious extraction techniques and a radioactive internal standard.

The present method involves the direct chromatography of diluted acetonitrile denatured plasma on a reversed phase column using a trace enrichment technique developed by Frei et al. (5) and thus eliminates the extraction step. The precision (C.V. < 4%) of the injection and collection step achieved by employing a full capacity constant volume loop technique obviates the need for an internal standard and thus further simplifies the assay.

About 15 to 20 assays can be performed per day by one technician and thus the procedure can conveniently be applied to the clinical monitoring of digoxin plasma concentration in digitalized patients.

ACKNOWLEDGEMENT

We would like to thank Dr. W. McLean, Drug Information Service, Ottawa General Hospital, for providing serum samples from digitalized patients.

REFERENCES

1. Gault, M.H., Charles, J.D., Sugden, D.L. and Kepkay, D.C., Hydrolysis of Digoxin by Acid, J. Pharm. Pharmacol., 29, 27, 1977.

2. Loo, J.C.K., McGilveray, I.J. and Jordan, N., Quantitation of Digoxigenin in Serum Following Oral Administration of Digoxin in Humans, Res. Commun. Chem. Pathol. Pharmacol., 17, 497, 1977.

3. McGilveray, I.J., Loo, J.C.K. and Jordan, N., Digoxin Dosage in Patients with Gastric Hyperacidity, CMA Journal, 121, 704, 1979.

4. Gibson, T.P. and Nelson, H.A., The Question of Cumulation of Digoxin Metabolites in Renal Failure, Clin. Pharmacol. Ther., 27, 219, 1980.

5. Frei, R.W. and Erni, F., Trace Enrichment Techniques in Reversed-Phase High-Performance Liquid Chromatography, J. Chromatog., 136, 63, 1977.