

a serum level of 1 ng/mL digoxin could easily be misidentified, leading to a possible overdose (or underdose) of the drug. Since digoxin is not effective at concentrations below 1 ng/mL and is toxic at concentrations much above that, the consequences of such an error in measurement could be severe. For this reason, use of a ouabain column in lieu of a digoxin column forms the basis of the present invention.

TABLE IA

Amounts of Ouabain and TETA Used in Resin Synthesis		
Lot No.	Ouabain (mg)	TETA (mg)
OU-A	325	125
OU-B	195	75
OU-C	130	50
OU-D	125	125
OU-E	75	75
OU-F	50	50
OU-G	50	125
OU-H	30	75
OU-I	20	50

TABLE IB

Amounts of Digoxin and TETA Used in Resin Synthesis		
Lot No.	Digoxin (mg)	TETA (mg)
DG-A	325	125
DG-B	195	75
DG-C	130	50
DG-D	125	125
DG-E	75	75
DG-F	50	50
DG-G	50	125
DG-H	30	75
DG-I	20	50

TABLE IIA

Comparison of Ouabain and Digoxin Resins: Background		
Lot No.	Background (A/min)	
	Ouabain (mg)	Digoxin
A	.234	.042
B	.254	.108
C	.261	.318
D	.111	.044
E	.121	.206
F	.124	.311
G	.164	.137
H	.166	.224
I	.159	.268

TABLE IIB

Comparison of Ouabain and Digoxin Resins: Sensitivity		
Lot No.	Sensitivity (A/min/5 ng/mL)	
	Ouabain (mg)	Digoxin
A	.030	.008
B	.019	—
C	.015	.013
D	.158*	.012
E	.148	.011
F	.148	.047
G	.106	.033
H	.108	.087
I	.112	.110*

TABLE III

Comparison of Resins OU-D and DG-I: Precision		
	OU-D	DG-I
	1 ng/mL Digoxin	
\bar{X} (ng/mL)	0.83	0.96
S.D. (ng/mL)	0.14	0.53
% C.V. (ng/mL)	17	55

TABLE III-continued

Comparison of Resins OU-D and DG-I: Precision		
	OU-D	DG-I
	3.4 ng/mL Digoxin	
\bar{X} (ng/mL)	3.4	3.4
S.D. (ng/mL)	0.09	0.37
% C.V. (ng/mL)	2.5	11

EXAMPLE 2

A. Synthesis of Divalent (F(ab')₂) Antibody Enzyme Conjugate

One milliliter of affinity purified antidigoxin F(ab')₂-fragments [preparation described in Example 1] (2.85 mg/mL protein in 0.015M sodium phosphate, 0.15M NaCl, 1 mM EDTA, pH 7.0) was mixed at 23°-25° C. with 9.1 μ L of a 60 mM solution of succinimidyl 4-(N-maleimido-methyl)cyclohexane-1-carboxylate (SMCC) dissolved in dimethylformamide. After 60 minutes the reaction was stopped by desalting the solution on a Sephadex G-25 column (1.5 cm \times 30 cm) equilibrated in the same sodium phosphate-NaCl-EDTA solution. The protein which eluted in the void volume was collected and concentrated to 1 mL using an Amicon stirred-cell concentrator (PM-30 membrane). Twenty-four mg of β -galactosidase dissolved in 1 mL of 0.05M Tris \cdot HCl, 0.15M NaCl, 1 mM MgCl₂, pH 7.5 was added to the F(ab')₂-SMCC adduct and allowed to react for 20 hours at 4° C. The reaction was quenched by the addition of 10 μ L of a 0.1M solution of 2-mercaptoethanol for 1 hour at 4° C. The F(ab')₂- β -galactosidase conjugate was separated from the unreacted β -galactosidase by chromatography on a Sepharose 4B column (1.5 cm \times 90 cm) equilibrated in 0.05M Tris \cdot HCl, 0.15M NaCl, 1 mM MgCl₂, pH 7.5 at 4° C.

B. Synthesis of Ouabain and Digoxin Resins

Ouabain and digoxin were each coupled separately to Sephadex G-10 at optimized ratios via bovine serum albumin (BSA).

(1) Ouabain-BSA was prepared by dissolving 5 g of ouabain-octahydrate in 500 mL of hot distilled water (70° C.). After the solution was allowed to cool to 25° C., 7.3 g of sodium metaperiodate was added followed by continuous mixing for 2 hours at 25° C. in the dark. The oxidation was then stopped by passing the mixture through a 250 mL bed of Dowex (1-X8) anion exchange resin. The eluate was collected and combined with a solution of bovine serum albumin (10 gm/500 mL) dissolved in 1M sodium phosphate buffer, pH 7.0. After 1 hour at 25° C., 0.64 g of sodium cyanoborohydride was added with stirring and the mixture was allowed to incubate for 72 hours at 25° C. The uncoupled ouabain was removed from the mixture by dialyzing the ouabain-BSA conjugate solution against running distilled water for 24 hours and then against 20 volumes of 0.015M sodium phosphate buffer, pH 7.0 at 4° C. The final ionic strength of the conjugate solution was adjusted to 0.25M by adding 14.6 g of NaCl prior to coupling to the Sephadex resin.

(2) Coupling of Ouabain-BSA to Sephadex G-10

Sephadex G-10 (420 g) (Pharmacia Fine Chemicals) was allowed to swell in 2000 mL of distilled water for >1 hour. Resin fines were removed by decanting and resuspension with 3 \times 2000 mL of water. The resin was then oxidized by resuspension in 1000 mL of water containing 20 g of dissolved sodium metaperiodate. After 10 minutes, the resin was washed with 5 \times 2000