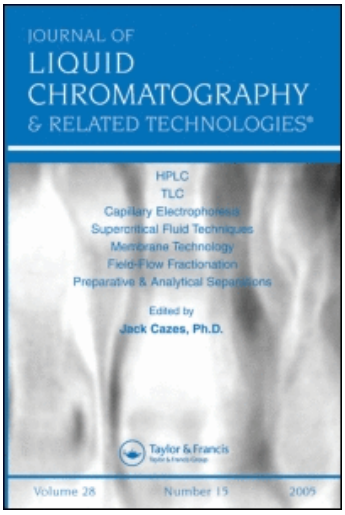

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HIGH PRESSURE LIQUID CHROMATOGRAPHIC MICROASSAY FOR SIMULTANEOUS MEASUREMENT OF DOXAPRAM AND ITS METABOLITES IN PREMATURE NEWBORN INFANTS

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ABSTRACT

A simple, specific, rapid and sensitive high pressure liquid chromatographic microassay is described for the simultaneous measurement of doxapram and its metabolites. Doxapram appears rapidly metabolized to ketodoxapram in premature newborn infants with apnea. Therapeutic drug monitoring for doxapram and its metabolites appears warranted during doxapram therapy of neonatal apnea.

INTRODUCTION

Doxapram (1-ethyl-4-4 (2-morpholino-ethyl) -3, 3-diphenyl - 2-pyrrolidinone, Fig 1) is a respiratory stimulant currently used

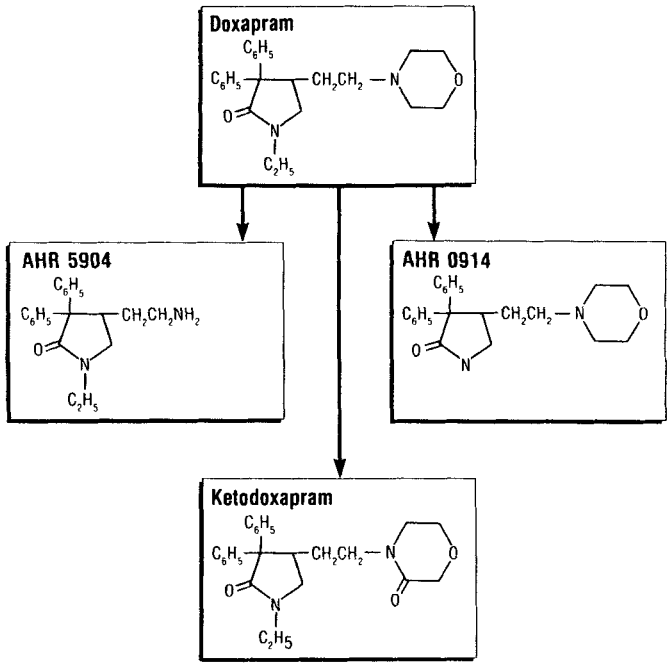

Figure 1. Structural formula of Doxapram and three of its metabolites.

to treat neonatal apnea unresponsive to methylxanthines (1-7), to facilitate weaning of neonates and young infants from mechanical ventilators and for central hypoventilation syndromes (8). Although doxapram is used often in neonatal apnea, rational dose regimens and therapeutic blood levels have not been clearly defined. Moreover , there is a significant discrepancy in the reported plasma half-life of the drug and its observed pharmacodynamic effects. Doses as low as 0.25 mg/kg/hr appear to produce significant pharmacologic effects (9). Currently used doses (2.5 mg/kg/h) and plasma concentrations exceeding 5 mg/l are associated with adverse effect such as blood pressure

changes (5, 6, 7). Thus, doxapram therapeutic drug monitoring in the neonatal period is highly advisable. Moreover, microassay techniques for doxapram are required for further studies on the kinetic behaviour of this drug in the newborn. Previous studies report the use of gas chromatographic and mass spectrophotometric techniques for the assay of this drug (10-15). This study describes a sensitive HPLC technique for the simultaneous measurement of doxapram and its metabolites using microsamples of blood.

MATERIALS AND METHODS:

PATIENTS:

Blood samples (0.3 ml/sample) from two premature neonates who received increasing doses of doxapram every 20 minutes were used in this study. Both neonates had apnea of prematurity which was unresponsive to theophylline. Infusion dose rate ranged from 0.1 mg/kg/h to 5 mg/kg/h intravenously. Blood samples were obtained from the heelstick and from the umbilical artery. The plasma was quickly separated and then kept frozen in -20 degrees C until the assay.

APPARATUS:

The Water's HPLC equipment including the Water's Intelligent Sample processor (WISP) , M-45 solvent delivery pump, uBondapak C18 column with pre-column, a model 441 UV absorbance detector and a Water's data module were used in this study.

CHEMICALS:

The chemicals included ammonium phosphate buffer, acetonitrile and methanol (Fisher Laboratories), doxapram and 3 metabolites supplied by AH Robbins (Richmond, Virginia). Doxapram standard solutions ranged from 2.0 ug/ml to 30.0 ug/ml in buffer. Serum was then spiked with standard solutions to a

final concentration of 1.0 ug/ml to 15.0 ug/ml. The internal standard used was beta hydroxy phenyl theophylline (BHPT) (Sigma, St. Louis, Missouri) 1.5 ug/ml dissolved in buffer.

PROCEDURES:

Spiked serum standards or sample serum containing doxapram (50 ul) was added to 50 ul internal standard, 250 ul buffer and 3 mls of chloroform: isopropyl alcohol (90:10). The mixture was vortexed for 1 minute and then centrifuged for 10 minutes. The top aqueous layer was aspirated and the bottom organic layer was evaporated to dryness. The residue was re-dissolved in buffer (100 ul) and then 30 ul injected into the column.

CHROMATOGRAPHY CONDITIONS:

The mobile phase was ammonium phosphate buffer 0.01 M, pH 4.5 (55%), methanol (30%) and acetonitrile (15%). The column was a Bondapak C18 with a guard column. Flow rate was 1.3 ml/min, sensitivity was 0.02 absorbance units (aufs), wavelength was 214 nm and run time was 15 min.

RESULTS AND DISCUSSION:

Typical chromatograms are shown in Figure 2. A chromatogram using the plasma of a doxapram treated newborn infant is also shown in Figure 3. There was a good separation of the chromatographic peaks. The retention times are shown in Figure 2 and Table II; BHPT= 2.5 min, AHR 5904 = 4.45, AHR 0914=5.2, doxapram=7.29 and AHR 5955 ketodoxapram=10.8 minutes. Percent recovery is shown in Table I; recovery ranged from 70 to 100 % at the concentrations used. Coefficients of variation (%) for retention times are shown in Table II; variation ranged from 0% to 1.9%.

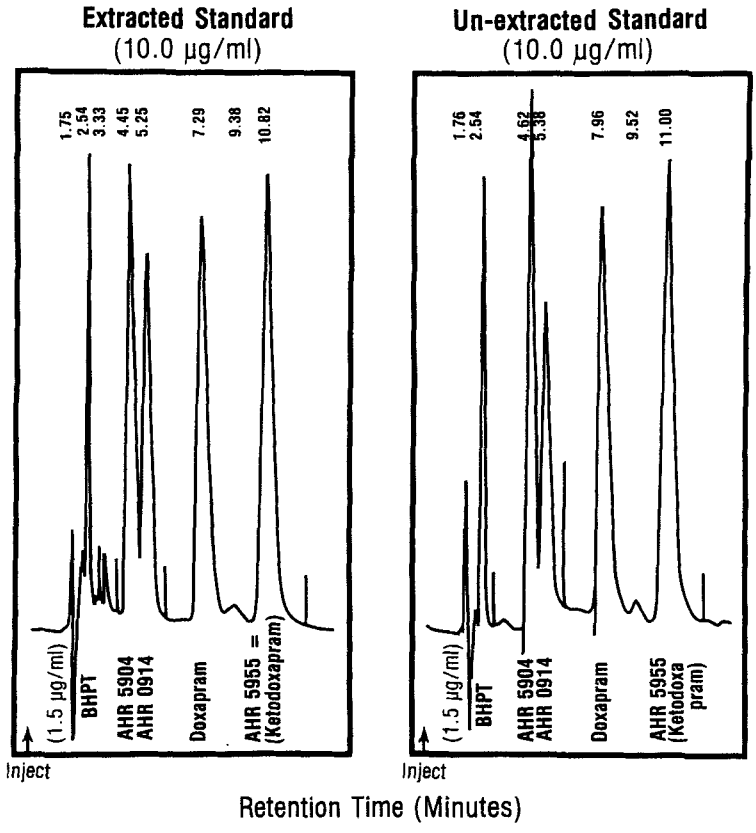

Figure 2. Typical chromatogram of doxapram and three of its metabolites. Identification and retention times noted in figure.

TABLE I

Recovery of Doxapram and Its Metabolites (%)

Conc ug/ml	AHR 5904	AHR 0914	DOXAPRAM	AHR 5955
2.5	70%	100%	84%	80%
5.0	70%	100%	85%	80%
10.0	80%	100%	82%	80%

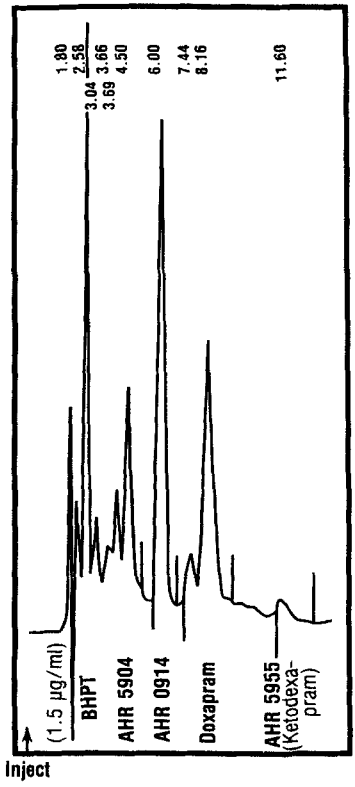

Figure 3. Chromatogram of doxapram and its metabolite from the plasma of a newborn baby receiving doxapram. Note a distinct peak of ketodoxapram.

The assay sensitivity was 0.5 ug/ml and the standard curves at concentrations ranging from 1.0 to 15.0 ug/ml were linear for AHR 5904 (r=0.99), AHR 0914 (r=0.99), AHR 5955 (r=0.99) and doxapram (r=0.99). Drugs at concentrations within therapeutic levels were used to spike samples for the studies on specificity: caffeine (10 ug/ml), theophylline (10 ug/ml), ampicillin (5 ug/ml), phenobarb (20 ug/ml), furosemide (2 ug/ml)

and gentamicin (5 ug/ml). No interference in the assay was found for each drug.

The plasma concentrations of doxapram and its metabolites from the neonates studied are shown in Table III. The plasma concentrations of doxapram remained relatively constant despite increasing doses of doxapram. In contrast, the plasma concentration of ketodoxapram increased directly proportional to the dose. This suggest that doxapram is probably metabolized rapidly at the morpholine ring (see Figure 1). Preliminary data suggest that ketodoxapram is an active metabolite (16) partly responsible for the respiratory stimulant effect of doxapram.

In summary, this simple HPLC microassay is rapid, specific, sensitive, and can be adapted for clinical drug monitoring and research studies for doxapram in the newborn infant.

TABLE II
Retention Times for Doxapram and Its Metabolites Coefficient of Variation (%)

Conc ug/ml	AHR 5904	AHR 0914	DOXAPRAM	AHR 5955 (ketodoxapram)	BHPT
2.5	4.51 ±0.06 (±1.48%)	5.27 ±0.05 (±1.0%)	7.87 ±0.06 (±0.76%)	10.94 ±0.12 (±1.11%)	2.54 ±0.00 (0%)
5.0	4.50 ±0.06 (±1.29%)	5.29 ±0.04 (±0.89%)	7.88 ±0.08 (±1.08%)	10.92 ±0.13 (±1.11%)	2.54 ±0.00 (0%)
10.0	4.53 ±0.09 (±1.9%)	5.29 ±0.03 (±0.54%)	7.88 ±0.08 (±1.06%)	10.93 ±0.14 (±1.20%)	2.55 ±0.01 (±0.32%)

Values are expressed in minutes as mean ± SEM. Numbers in parenthesis denote coefficient of variation.

TABLE III

Serum Concentration of Doxapram and Metabolites in Premature Newborn Infants with Apnea

DOSE (mg/kg/h)	Baby	AHR5904 1	AHR5904 2	AHR0914 1	AHR0914 2	AHR5955 1	AHR5955 2	DOXAPRAM 1	DOXAPRAM 2
0		-	-	-	-	-	-	-	-
0.1		-	0.3	-	-	1.4	1.2	0.8	0.9
0.5		-	0.2	-	0.6	1.4	1.2	0.8	0.9
1.0		0.4	1.2	0.6	0.7	2.4	1.1	0.9	1.0
2.5		-	2.0	0.6	0.9	3.2	3.5	0.9	1.1
5.0		-	2.0	0.6	0.6	4.5	9.5	1.1	1.0

Values are individual values for doxapram and each metabolite
Blank indicates non-detectable

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