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Note

High-performance liquid chromatographic determination of indomethacin serum concentrations

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Indomethacin has been used with varying success for closure of patent ductus arteriosis (PDA) in premature infants[1,2]. Successful PDA closure is particularly difficult in neonates below 1000 g, more than 14 days postnatal age, and with very large PDA[2]. Since the cause of the limited success may be due to inadequate indomethacin serum concentrations, the availability of a rapid, reliable indomethacin assay may be very helpful. We describe such an assay.

MATERIALS AND METHODS

Apparatus and chromatographic conditions

The high-performance liquid chromatography (HPLC) system (Waters Assoc., Milford, MA, U.S.A.) consisted of two Model 510 pumps controlled by an automated gradient controller (Model 680), a Model 441 fixed-wavelength ultraviolet detector (set at 254 nm and 0.01 a.u.f.s.), and a 710B WISP autosampler. The recorder used was a Model 740 data module and was set for a run time of 10 min. The analytical column used was a Nova Radial-Pak® C_{18} reversed-phase column (Waters) in a radial compression unit (Waters) with a C_{18} precolumn.

Chemicals

HPLC-grade acetonitrile was obtained from Burdick and Jackson (Muskegon, MI, U.S.A.) and indomethacin, flufenamic acid (the internal standard) and acetic acid were obtained from Sigma (St. Louis, MO, U.S.A.).

Standard solutions

Stock solutions of indomethacin (10 μg/ml) and the internal standard, flufenamic acid (40 μg/ml) were prepared in acetonitrile. Standards were prepared in drug-free serum using these solutions to formulate indomethacin concentrations of 0.25–5 μg/ml, each containing a final flufenamic concentration of 20 μg/ml. These

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standards were found to be stable for 5 months if refrigerated at 4°C. Serum-based standards were compared to standards prepared in acetonitrile and shown to be identical, allowing the less expensive acetonitrile-based preparations to serve as standards for the assay.

The mobile phase was acetonitrile–water–acetic acid (54.2:45.2:0.6). This was achieved by combining solution A [water–acetonitrile–acetic acid (44:950:6)] and solution B [acetonitrile–water–acetic acid (950:44:6)] in a ratio of solution A–solution B = 55:45. The pH of the mobile phase is 3.6.

Sample preparation

The extraction method was tested at three different injection volumes: 25, 50 and 100 μl. To the appropriate amount of serum, an equivalent volume of internal standard solution (flufenamic acid, 40 μg/ml) was added in a conical centrifuge tube. Protein was then precipitated by adding 1 ml of acetonitrile to the mixture, then agitating it on a Vortex mixer for 1 min. The samples were then centrifuged at 9500 *g* for 2 min. The supernatant was separated into a glass test-tube and evaporated to dryness under a stream of nitrogen at 37°C. The residue from the supernatant was reconstituted with 100 μl of mobile phase, and 75 μl were injected.

Analytical recovery and extraction

The assay recovery was assessed for both indomethacin and flufenamic acid. Indomethacin was studied at 0.1, 0.4, 1.5 and 4 μg/ml. Flufenamic acid was studied at 1.5 and 4 μg/ml. Six serum samples containing indomethacin and flufenamic acid were extracted and injected. In addition, 6 samples of each drug at concentrations of 1.5 and 4 μg/ml were prepared in mobile phase and injected directly. Recovery was calculated based on the peak heights for drugs extracted from serum (Peak Extr) and the drugs dissolved in mobile phase and injected directly (Peak Dir), using the formula:

$$\% \text{ recovery} = \frac{\text{Peak Extr}}{\text{Peak Dir}} \times 100$$

RESULTS

Representative chromatograms of extractions of blank serum and a serum sample spiked to contain 2 μg/ml flufenamic acid are depicted in Fig. 1. The response of the detector was linear from 0.1 to 5.3 μg/ml with a sensitivity of 0.1 μg/ml. There was very little between-run or within-run variability for serum samples sizes ranging from 25 μl to 100 μl (Table I). Recovery ranged from 99 to 107% for concentrations ranging from 0.1 to 4 μg/ml. The percent error of the assay ranged from a low of 0.5% at 2 μg/ml to a high of 9% at 0.1 μg/ml (Table II).

Other drugs which are commonly used concurrently with indomethacin are listed in Table III, along with retention times and capacity factors. These drugs do not interfere with the indomethacin assay.

DISCUSSION

Indomethacin is the treatment of choice for PDA closures[1,2]. Efficacy is felt to be related to maintaining adequate indomethacin serum concentrations[2–5]. While an

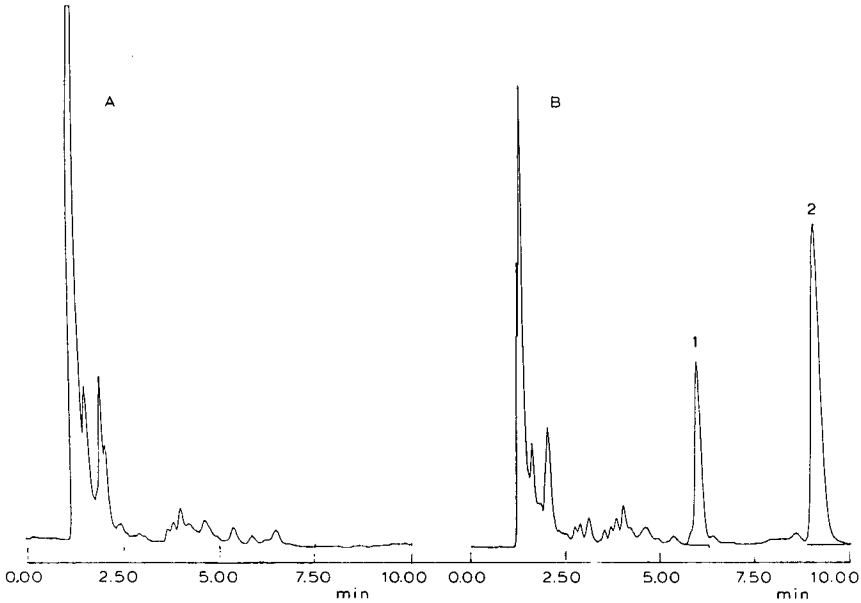

Fig. 1. Extracted blank serum (A) and serum spiked to contain 2.0 μg/ml indomethacin (1) and 20 μg/ml flufenamic acid (2) (B).

TABLE I

COMPARISON OF COEFFICIENTS OF VARIATION (C.V.) AND RECOVERIES USING DIFFERENT SERUM VOLUMES

	Volume (μl)		
	25	*50*	*100*
Within-day C.V. (%)	1.6	1.5	1.6
Between-day C.V. (%)	3.0	4.9	2.0
Recovery (%)	100	100	100

TABLE II

COMPARISON OF COEFFICIENTS OF VARIATION (C.V.) AND RECOVERIES USING 100 μl SERUM AT DIFFERENT INDOMETHACIN CONCENTRATIONS

Indomethacin concentration (μg/ml)	*C.V. (%)*	*Recovery (%)*
0.1	9.0	107
0.4	2.4	99
1.9	0.5	101
3.9	1.0	99

TABLE III

RETENTION TIMES AND CAPACITY FACTORS FOR OTHER DRUGS LIKELY TO BE PRESENT

Drug	*Retention time (min)*	*Capacity factor*
Indomethacin	6.07	3.82
Flufenamic acid	9.41	6.41
Ampicillin	Many peaks	<3.0
Dopamine HCl	3.15	1.47
Lorazepam	2.55	1.0
Gentamicin	3.15	1.47
Phenobarbital	2.78	1.18

optimal therapeutic range is unknown, several authors, using samples collected at times ranging from 2 to 24 h after a dose have cited[3–5] values from 0.25 to 3.5 μg/ml. Our approach has been to adjust each indomethacin dose based on available serum indomethacin concentrations and clinical response until PDA closure is achieved. Once PDA closure is achieved at a known indomethacin serum concentration, this "critical" concentration is maintained for 24 h. Using this approach, PDA closure was achieved in all 21 patients dosed to a critical concentration with one case of mild renal toxicity. This compared to only 64% PDA closure and up to 50% renal toxicity in two control groups[5].

The validity of this dosing approach depends on the rapid availability of the indomethacin concentration and limitation of the sample size required. This assay can be run on samples as small as 25 μl, although we have routinely used 100 μl. This compares to 200 μl or more for previously published assays[6–8]. The preparation and run time totals about 30 min, compared with 60 to 90 min for other methods we have utilized[6,7]. A final cost consideration is the reliability of standards prepared in acetonitrile rather than serum, and the stability of these standards for at least 5 months when refrigerated at 4°C.

CONCLUSION

The practicality of offering an indomethacin assay in the clinical laboratory depends on availability of an assay and relatively inexpensive assay which can be performed with speed and accuracy.

The assay we describe above meets these criteria. Most neonates require indomethacin serum concentrations of 0.5 to 2.5 μg/ml to close a PDA[9]. In this concentration range, the C.V. for our assay is below 2%. This new assay provides unique benefits making it particularly desirable for clinical laboratories wishing to provide this assay. Based on the increasing support for serum indomethacin concentration monitoring, we feel clinical laboratories will want to provide this assay.

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