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Note

Determination of some anti-inflammatory drugs in serum by high-performance liquid chromatography

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A number of methods have been published for the individual determination of some anti-inflammatory drugs in serum[1–11]. These include colorimetry, spectrofluorimetry and gas chromatography. Some of these methods are non-specific, others involve complex extraction procedures and derivatization steps. Although some methods have been presented for the analysis of these drugs in serum using high-performance liquid chromatography (HPLC)[12,13], they require at least 1 ml of serum and are only applicable to a specific drug.

The method outlined in this paper uses a small sample of serum and an identical protein precipitation extraction procedure with acetonitrile, followed by concentration of the extract for analysis by HPLC. The method can also be used for screening in cases of overdosage of these drugs.

EXPERIMENTAL AND RESULTS

Reagents

Acetonitrile (Nanograde; Mallinckrodt, St. Louis, Mo., U.S.A.).

High-performance liquid chromatography

A Waters Assoc. (Milford. Mass., U.S.A.) high-performance liquid chromatograph equipped with a Waters Assoc-450 variable wavelength UV detector was used. The column was a 30 cm × 3.9 mm I.D. tube packed with μBondapak C_{18} (Waters Assoc.). Samples were introduced by a syringe into a variable loop injector (Waters Assoc. Model U6K). The elution solvent was 60% acetonitrile in 45 m*M* KH_2PO_4 adjusted to pH 3.0 with orthophosphoric acid. The conditions for the individual analyses are shown in Table I. For the separation of the mixture of the anti-inflammatory drugs, a flow-rate of 0.8 ml/min and a wavelength of 225 nm was used. Under these conditions the elution times of naproxen, oxyphenbutazone, indomethacin, ibuprofen, phenylbutazone, mefenamic acid and flufenamic acid were 5.7, 5.8, 8.0, 8.6, 9.6, 10.3 and 10.5 min, respectively (Fig. 1.).

Extraction procedure

To 100 μl of serum in a pointed glass tube was slowly added 1.0 ml of acetonitrile. The tube was shaken vigorously by hand for 2 min, then centrifuged. An

TABLE I

HPLC CONDITIONS USING 60% ACETONITRILE IN 45 m*M* KH_2PO_4 FOR THE INDIVIDUAL ANALYSIS OF THE ANTI-INFLAMMATORY DRUGS

Compound	*Wavelength (nm)*	*Flow-rate (ml/min)*	*Sensitivity (a.u.f.s.)*
Naproxen	235	1.0	0.04
Oxyphenbutazone	240	2.0	0.04
Phenylbutazone	240	2.0	0.04
Indomethacin	260	2.0	0.02
Ibuprofen	225	1.5	0.02
Mefenamic acid	282	2.0	0.02
Flufenamic acid	282	2.0	0.02

0.5-ml aliquot of the supernatant was transferred to another tube and taken to dryness at 50° under a stream of nitrogen. The residue was redissolved in 100 μl of the eluting solvent and an aliquot, 10–20 μl, injected in the high-performance liquid chromatograph.

Quantitation

Peak heights obtained were compared to those of a series of injections from a standard solution in the range 20 to 200 ng. The use of an internal standard was not considered essential for the determination of these drugs at the therapeutic and overdose level, due to the reproducibility of the method. For sub-therapeutic analyses, any of the above drugs could serve as an internal standard for the other.

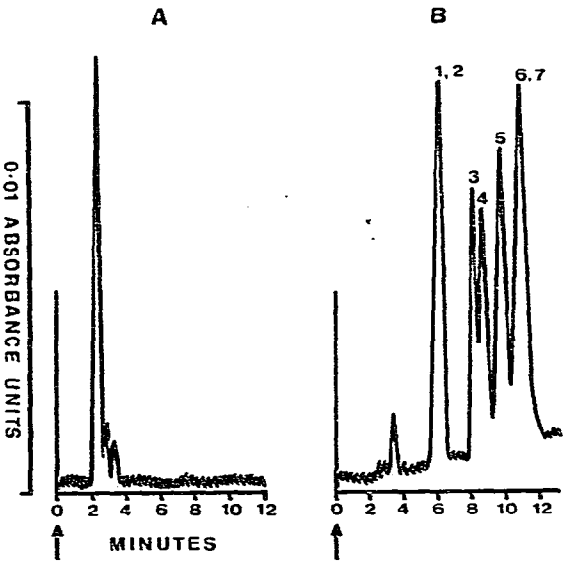

Fig. 1. A, HPLC trace of a blank plasma extract. B, Separation of the anti-inflammatory drugs by HPLC using 60% acetonitrile in 45 m*M* KH_2PO_4 adjusted to pH 3.0 at a flow-rate of 0.8 ml/min and wavelength of 225 nm. Peaks: 1 and 2 = naproxen (5 ng) and oxyphenbutazone (50 ng); 3 = indomethacin (50 ng); 4 = ibuprofen (200 ng); 5 = phenylbutazone (100 ng); 6 and 7 = mefenamic acid (70 ng) and flufenamic acid (70 ng).

TABLE II

RECOVERIES OF THE ANTI-INFLAMMATORY DRUGS ADDED TO SERUM

Compound	*Range (μg/ml)*	*No. of recoveries*	*Recovery (%)*
Oxyphenbutazone	10 –140	10	94 ± 3%
Indomethacin	1.3– 12	10	102 ± 2%
Phenylbutazone	10 –150	10	97 ± 6%
Mefenamic acid	1.0– 30	10	90 ± 5%
Flufenamic acid	1.0– 20	10	92 ± 3%
Ibuprofen	5.0– 80	7	80 ± 5%
Naproxen	1.0– 40	8	91 ± 3%

Recovery studies

The recoveries obtained are outlined in Table II.

Specificity

In all plasma samples examined, the chromatograms have been free from interfering peaks. Salicylate, at a level of 100 μg/ml, did not interfere with the assay.

DISCUSSION

A number of the common anti-inflammatory drugs were examined in serum using the outlined extraction procedure. The recoveries were good, indicating that the method could be used satisfactorily at the therapeutic or overdose level. The individual analyses were performed using the 60% acetonitrile in pH 3.0 buffer solution but both naproxen and oxyphenbutazone as well as mefenamic and flufenamic acid were not separated on this column under these conditions. Lowering the acetonitrile concentration to 45% did not give improved resolution. However, by changing the elution solvent to 35% acetonitrile in 0.7% NH_4Cl buffered to pH 7.8 with ammonia, resulted in a separation of all the drugs investigated. The order of elution was changed and the following times were observed using a flow-rate of 1.0 ml/min: oxyphenbutazone 3.0 min, naproxen 3.5 min, phenylbutazone 4.4 min, ibuprofen 5.4 min, indomethacin 7.2 min, mefanamic acid 7.8 min, flufenamic acid 10.2 min. This is an ideal system for the screening of unknown anti-inflammatory drugs as they are all well resolved (Fig. 2).

Sulindac (Clinoril), a new non-steroid anti-inflammatory agent was tried on the μBondapak column using the 60% acetonitrile pH 3.0 buffer system at a flow-rate of 0.8 ml/min. Sulindac eluted first (4.8 min) and was well separated from the other drugs. Work is continuing on the extraction of this drug and its metabolites, as the sulphide metabolite has been proposed as a pharmacologically active species[14].

The advantages of the method are its simplicity, rapidity and small serum sample volume. The same extraction technique can be used for each drug eliminating the need for complex extraction procedures and derivatisation steps necessary for analysis by gas–liquid chromatography. The method can also serve as a screening technique in cases of overdosage.

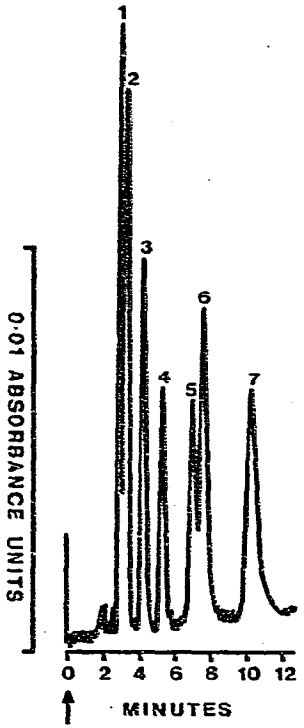

Fig. 2. Separation of the anti-inflammatory drugs by HPLC using 35% acetonitrile in 0.7% NH_4Cl buffered to pH 7.8 at a flow-rate of 1.0 ml/min and wavelength 225 nm. Peaks: 1 = oxyphenbutazone (100 ng); 2 = naproxen (5 ng); 3 = phenylbutazone (100 ng); 4 = ibuprofen (200 ng); 5 = indomethacin (50 ng); 6 = mefenamic acid (100 ng); 7 = flufenamic acid (100 ng).

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