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Note

Determination of bromfenac in plasma by high-performance liquid chromatography

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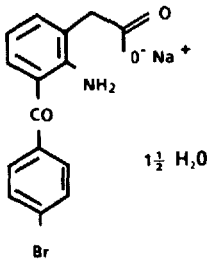
Bromfenac sodium, 2-amino-3-(4-bromobenzoyl)benzeneacetic acid sodium salt sesquihydrate, is a potent long-acting peripheral, analgesic compound with anti-inflammatory and antipyretic activities. It exhibits prostaglandin synthetase-inhibiting properties in the animal models [1, 2]. Because of the relatively narrow therapeutic indices of most non-steroidal anti-inflammatory compounds, it is important to monitor their concentrations in plasma in order to facilitate optimum dosage adjustment. Methods to assay non-steroidal anti-inflammatory drugs have been developed which range from direct spectrophotometric determination to the more selective gas chromatographic or high-performance liquid chromatographic (HPLC) procedures [3]. The following method was developed for the determination of bromfenac in plasma by HPLC. It is selective and utilizes a reasonable sample size (0.5 ml). The method is appropriate for samples generated from animal and human studies.

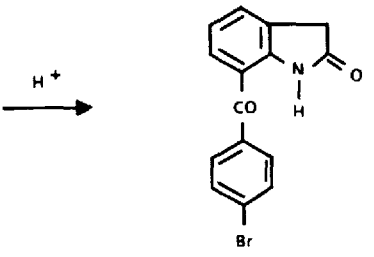
Bromfenac (I) is a very polar compound and freely ionizable. It is difficult to recover this compound from an aqueous matrix by the conventional solvent extraction method. The unchanged drug is rather stable in neutral or alkaline medium. But when exposed to acid, I will convert quantitatively (>99%) and rapidly to a cyclic compound, II (Fig. 1). The cyclic form of bromfenac, II, is readily extractable into organic solvents. Compound II has been detected in plasma and urine samples from animals dosed with bromfenac. The extraction scheme is based on extracting the cyclic form of bromfenac, II, first in alkaline medium and then converting the unchanged drug, I, to the cyclic form in acidic medium. The internal standard, III, used is the chloro analogue of bromfenac and it will also undergo quantitative ring closure in acidic medium.



Bromfenac Sodium

(2-amino-3-(4-bromobenzoyl) benzene acetic acid sodium salt sesquihydrate)

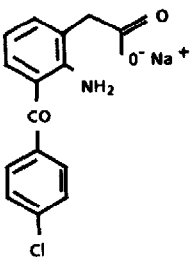
(I)



Metabolite

(7-(4-bromobenzoyl)-1,3-dihydro-2H-indole-2-one)

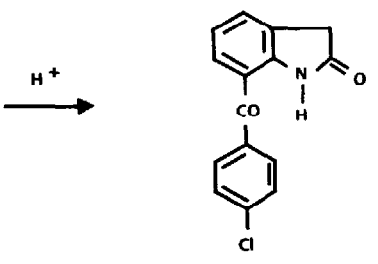
(II)



Internal Standard

(2-amino-3-(4-chlorobenzoyl) benzene acetic acid)

(III)



(7-(4-chlorobenzoyl)-1,3-dihydro-2H-indole-2-one)

(IV)

Fig. 1. Chemical structures of bromfenac, internal standard and their cyclic forms.

EXPERIMENTAL

Reagents

Sodium acetate, tetrahydrofuran, acetonitrile, concentrated ammonium hydroxide and concentrated hydrochloric acid were purchased from J.T. Baker (Phillipsburg, NJ, U.S.A.). Hexane was purchased from Burdick and Jackson Labs. (Muskegon, MI, U.S.A.). Bromfenac and internal standard were synthesized by A.H. Robins (Richmond, VA, U.S.A.).

High-performance liquid chromatography

The HPLC system consisted of a solvent delivery system (Waters Model 510, Waters Assoc., Milford, MA, U.S.A.), an autosampler (Waters, WISP Model

710B) and a variable-wavelength detector (Schoeffel F.S. 770 LCUV, Schoeffel Instruments, Westwood, NJ, U.S.A.) set at 270 nm.

The chromatographic separation was achieved on a 10-μm, 30 cm$\times$3.9 mm I.D. reversed-phase μBondapak C_{18} column (Waters). The mobile phase used was 0.05 *M* sodium acetate buffer (pH 6.5)–acetonitrile–tetrahydrofuran (55:39:6) delivered at 1.5 ml/min at ambient temperature. The eluted compounds were detected by UV absorption at 270 nm. The output signal generated by the detector was processed by a computer-automated laboratory system (Computer Inquiry Systems, Walwick, NJ, U.S.A.) and recorded on a 10-mV chart recorder (Hewlett Packard). The chromatographic data were processed by a Hewlett Packard computer (Model 1000, Hewlett Packard, Palo Alto, CA, U.S.A.).

Standard solution

A stock solution of the internal standard containing 100 μg/ml was prepared in methanol. This solution was diluted to 1 μg/ml with water daily to use in the assay.

A stock solution of bromfenac containing 1 mg/ml was prepared in water. This solution was then diluted to produce solutions at concentrations of 10 and 1 μg/ml in water. Different volumes of these solutions were added to drug-free plasma to obtain standards ranging from 0 to 1 μg/ml.

Extraction procedure

A 0.5-ml sample portion of either standard or unknown samples of plasma were transferred to 15-ml centrifuge tubes containing 0.2 ml of the internal standard solution. A 7-ml volume of hexane and 0.5 ml of 5% ammonium hydroxide solution were added to the tubes. The tubes were tightly stoppered and rapidly shaken for 10 min in a reciprocating shaker. Then they were centrifuged at 550 g for 5 min. The organic layer was removed and discarded. A 1-ml volume of 2 *M* hydrochloric acid solution was added to the aqueous phase, mixed briefly and allowed to stand for 20 min. At the end of the 20 min, 7 ml of hexane were added, the tubes stoppered tightly, rapidly shaken for 10 min and centrifuged for 5 min at 550 *g*. The organic layer was transferred to a clean centrifuge tube and blown to dryness under a gentle stream of nitrogen in a warm water bath (60°C). The residue was reconstituted with 0.2 ml of the mobile phase and 0.1 ml was injected into the HPLC system. Fig. 2 shows the summary of the extraction scheme.

Precision, reproducibility and accuracy studies

To test the precision and reproducibility of this method, six standard curves for bromfenac were run on consecutive working days. The range of the curves was 0–1 μg/ml. The coefficient of variation of the peak-height ratio was determined at each concentration level of the standard curve. The slope, intercept and the correlation coefficient of the daily standard curves were also calculated.

The accuracy of the method was determined by assaying thirty randomized samples prepared with various concentrations of bromfenac.

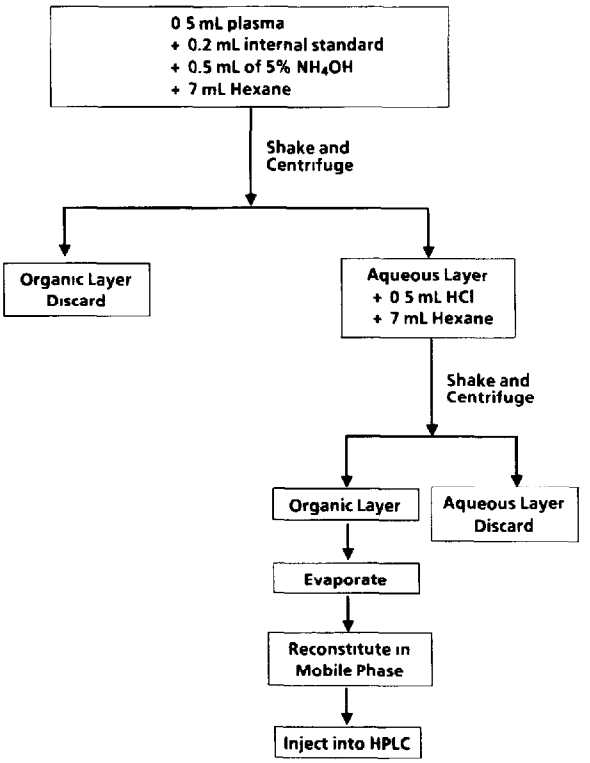


Fig. 2 Extraction scheme for bromfenac from plasma.

Pilot study in rats

A pilot study was conducted in rats to estimate the pharmacokinetic parameters. After an overnight fast, twenty rats were dosed orally with 0.6 mg/kg bromfenac dissolved in water. Blood samples were collected over 24 h after dosing. The plasma samples were assayed for bromfenac.

The conversion of bromfenac, I, and the internal standard, III, to their respectively cyclic forms were confirmed by mass spectrometry. Both cyclic compounds were also synthesized and used to develop the chromatographic separation. The conversion efficiency is better than 99% within the concentration range tested. The reaction in the acidic medium is almost instantaneous and reproducible.

The plasma concentration of the cyclic form of bromfenac, II, was generally less than 1% of the concentration of the unchanged drug in the same sample. The amounts of the unchanged drug and compound II recovered in the urine was usually less than 3% of the administered dose. As a result, the procedure to determine compound II first can almost be eliminated.

Even with conversion and double extraction procedure, the recoveries of bromfenac and internal standard added to plasma samples were over 90%. Fig. 3A and B show typical chromatograms of drug-free plasma, drug-free plasma spiked with the internal standard and drug-free plasma spiked with internal standard and bromfenac. The chromatograms of drug-free plasma show no interference peaks at the retention time of bromfenac or the internal standard.

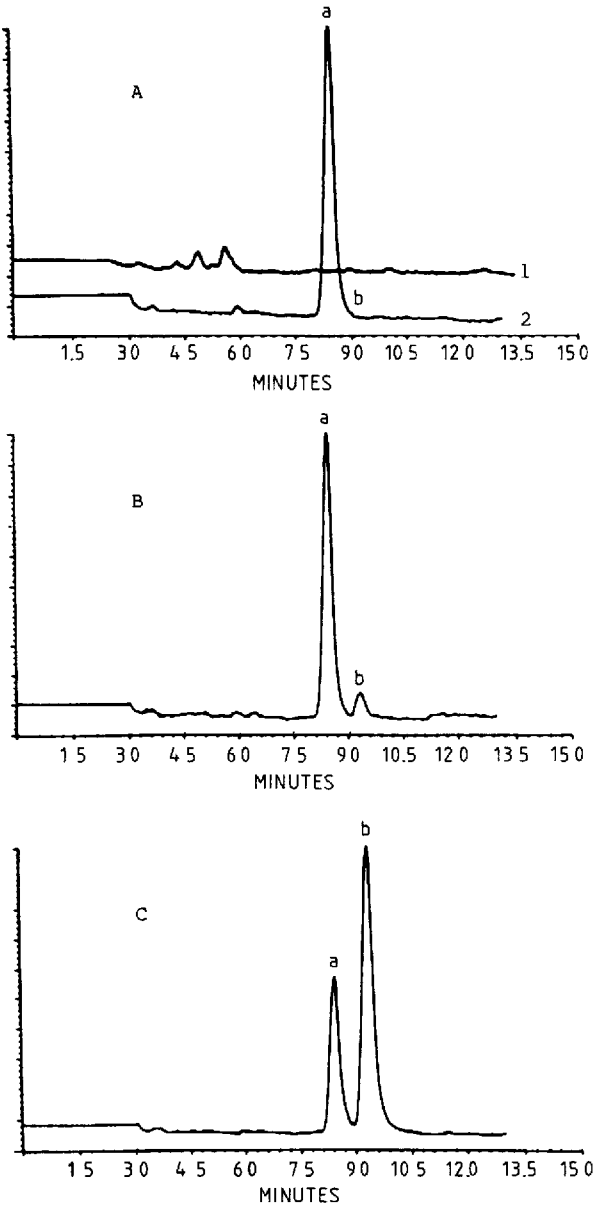


Fig. 3. (A) Chromatograms of (1) control plasma extract and (2) control plasma extract spiked with 0.4 μg/ml internal standard (a). (B) Chromatogram of control plasma extracts spiked with 0.4 μg/ml internal standard (a) and 0.05 μg/ml bromfenac (b). (C) Chromatogram of extract of plasma obtained at 12 h after oral dosing of rats with 0.6 mg/kg bromfenac.

The results of the six consecutive standard curves for bromfenac were linear between 0.03 and 1.0 μg/ml with a slope of 2.208 ± 0.08 and a correlation coefficient of 0.999. The intercept values of the regression line were always negligible. The coefficient of variation of the average peak-height ratio in the six standard curves was less than 9%.

TABLE I

DETERMINATION OF UNKNOWN AMOUNTS OF BROMFENAC ADDED TO PLASMA

Concentration added (μg/ml)	Number of samples	Concentration found (mean ± S.D.) (μg/ml)	Coefficient of variation (%)	Found (%)
0	4	BQL[a]	–	–
0.03	6	0.03 ± 0.005	16.7	100.0
0.06	6	0.06 ± 0.003	5.0	100.0
0.30	4	0.32 ± 0.007	2.4	105.6
0.60	4	0.63 ± 0.015	2.3	105.2
0.80	6	0.83 ± 0.023	2.8	104.0

[a]BQL = below quantifiable limit, i.e., concentration <0.03 μg/ml.

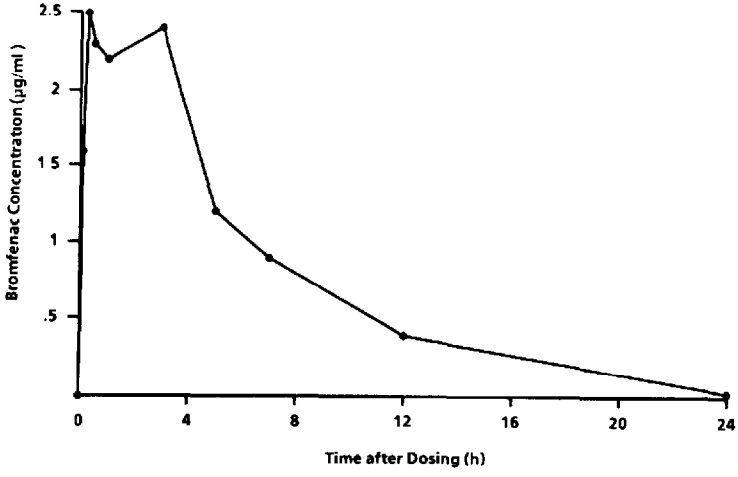


Fig. 4. Plasma concentration–time curve of bromfenac after a single oral dose of 0.6 mg/kg was given to rats.

Table I shows the recovery values for the thirty spiked samples. The percentage of bromfenac found was within 6% of the theoretical concentration added.

Plasma samples collected from rats after oral administration of bromfenac were analyzed by the method described above. Fig. 3C shows a chromatogram of a plasma sample collected at 12 h after dosing. A few samples from the early time intervals would need dilution in order to bring the assay concentrations within the standard curve. No other drug-related peaks were observed in the chromatograms. Fig. 4 shows the drug concentration–time profile for bromfenac in rats.

CONCLUSION

A sensitive and selective HPLC method was developed for determination of bromfenac in plasma. It has a linear range from 0.03 to 1.0 μg/ml when 0.5 ml of plasma was used for the assay. This method shows good precision and accuracy.

It was utilized successfully in determining bromfenac in plasma collected from animal and human pharmacokinetic, bioavailability and toxicology studies.

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