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# DETERMINATION OF 2-(4-ISOBUTYLPHENYL)PROPIONIC ACID IN BULK DRUG AND COMPRESSED TABLETS BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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## SUMMARY

A reversed-phase liquid chromatographic method was developed for 2-(4-isobutylphenyl)propionic acid (IBPP) in bulk drug and compressed tablets containing 100–600 mg of drug per tablet. The chromatographic system consists of a microparticulate octadecylsilica column and a mobile phase of aqueous acetonitrile buffered with chloroacetic acid. Whole tablets are disintegrated, and the IBPP dissolved in a solution of the internal standard in mobile phase. The resulting suspension is then centrifuged, and an aliquot of the supernatant chromatographed. This system separates IBPP from its analogues and degradation products in less than 10 min. Recovery of the drug from spiked placebo preparations was quantitative. Precision of the assay was 0.3 and 0.7%, relative standard deviation, for bulk drug and Motrin® tablets, respectively.

## INTRODUCTION

The non-steroidal antiinflammatory drug 2-(4-isobutylphenyl)propionic acid (IBPP) is widely used in the treatment of rheumatoid arthritis. For quality control of this substance, an accurate, precise and simple assay method was needed. In addition, this assay was required to be stability indicating and specific for IBPP in the presence of process intermediates and impurities in the process intermediates. Quantitative assay methods for IBPP using gas chromatography[1–4] and high-performance liquid chromatography (HPLC)[5–12] have been described.

For simplicity, a liquid chromatographic assay method was desired. Of the published HPLC methods, only three use a buffered mobile phase[6,7,9]. Buffering of the mobile phase in reversed-phase liquid chromatography is necessary for an efficient and rugged assay method of an ionizable compound such as IBPP. These methods were not suitable for a simple measurement of IBPP in bulk drug and compressed tablet dosage forms. Therefore, a simple 'dissolve and shoot' assay was developed and is described in this report.

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## EXPERIMENTAL

### *Materials*

The structures, names, and abbreviated names of IBPP and related compounds are shown in Table I. Samples of these compounds were obtained from The Upjohn Company (Kalamazoo, MI, U.S.A.). Chloroacetic acid and the internal standard, valerophenone, were obtained from the Aldrich (Milwaukee, WI, U.S.A.). Acetonitrile was from Burdick and Jackson (Muskegon, MI, U.S.A.). All other chemicals were reagent grade and used as received.

### *Chromatography*

Experiments were conducted on a modular liquid chromatograph consisting of a Model 110A pump (Altex, Berkeley, CA, U.S.A.), an in-house designed and built autosampler[13] (Upjohn, Kalamazoo, MI, U.S.A.) containing an injection valve with a 10-$\mu$l loop (Valco, Austin, TX, U.S.A.), a LiChrosorb RP-18 guard column (Brownlee Labs., Santa Clara, CA, U.S.A.) and a fixed-wavelength UV detector at 254 nm (Model 1203, LDC, Rivera Beach, FL, U.S.A.). Chromatograms were recorded using an Omniscribe recorder (Houston Instrument, Austin, TX, U.S.A.). For quantitation, data was collected and processed by digital computer (PDP 11, Digital Equipment, Maynard, MA, U.S.A.).

The analytical column, a Zorbax ODS (25 cm $\times$ 4.6 mm I.D.) (DuPont, Wilmington, DE, U.S.A.), was operated at a flow-rate of 2 ml/min and ambient temperature. The mobile phase was prepared by dissolving 4.0 g of chloroacetic acid in 400 ml of distilled water and adjusting the pH to 3.0 with concentrated ammonium hydroxide. Stirring was continued and 600 ml of HPLC grade acetonitrile was added. The resulting mobile phase was filtered through a 0.45-$\mu$m nylon 66 filter (Pall Trinity Micro Corp., Cortland, NY, U.S.A.). When not in use, the column was washed with methanol–water (80:20, v/v) and stored in the same solvent.

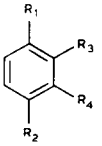
The volume of mobile phase in the column, $V_m$, which is equal to the retention volume of an unretained compound, was estimated by the retention volume of sodium nitrate (20 mg/ml in the mobile phase). The retention volumes of the compounds studied were measured using 12-mg/ml solutions of authentic standards. Capacity factors, $k'$, were determined using the equation: $k' = (V_R - V_m)/V_m$, where $V_R$ is the retention volume of the compound being measured. The separation factors, $\alpha$, were calculated by taking the ratio of the compound's capacity factor to ibuprofen's capacity factor.

### *Sample preparation*

Standards and bulk drug samples were prepared by accurately weighing approximately 1200 mg of sample and dissolving in 100 ml of internal standard solution (approximately 0.35 mg/ml valerophenone in mobile phase). For compressed tablet samples, the number of tablets equivalent to 1200 mg of IBPP were added to 100 ml of internal standard solution. The sample container was shaken for at least 30 min until the tablets were completely disintegrated, and the insoluble excipients were separated by centrifugation. The clear supernatant was separated for analysis.

TABLE I

NAMES, ABBREVIATIONS AND STRUCTURES OF COMPOUNDS STUDIED



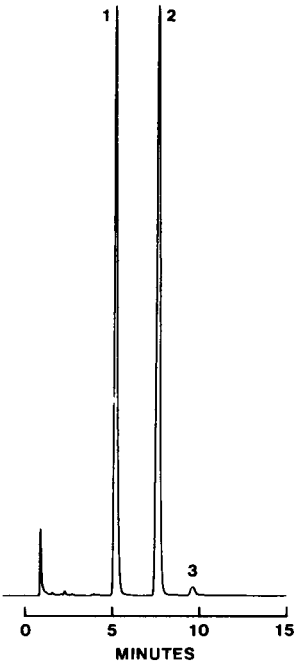

| *Name* | *Abbreviation* | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|---|
| 2-(4-Formylphenyl)-propionic acid | FPP | $-C(=O)-H$ | $-CH(CH_3)-COOH$ | H | H |
| 2-(4-Isobutyrylphenyl)-propionic acid | IBRPP | $-CH(CH_3)-C(=O)-CH_3$ | $-CH(CH_3)-COOH$ | H | H |
| 2-(4-*n*-Propylphenyl)-propionic acid | *n*-Propyl | $-CH_2CH_2CH_3$ | $-CH(CH_3)-COOH$ | H | H |
| 2-[4-(2-Methyl-1-propyl-1-ene)phenyl]propionic acid | Unsat | $-C(CH_3)=CH$ | $-CH(CH_3)-COOH$ | H | H |
| 2-(4-*n*-Butylphenyl)-propionic acid | *n*-Butyl | $-CH_2CH_2CH_2CH_3$ | $-CH(CH_3)-COOH$ | | |
| 2-(2-Isobutylphenyl)-propionic acid | Ortho | H | $-CH(CH_3)-COOH$ | $-CH(CH_3)-CH_2$ | H |
| 2-(3-Isobutylphenyl)-propionic acid | Meta | H | $-CH(CH_3)-COOH$ | H | $-CH(CH_3)-CH_2$ |
| 2-(4-Isobutylphenyl)-propionic acid | IBPP | $-CH(CH_3)-CH_2$ | $-CH(CH_3)-COOH$ | H | H |
| 2-(4-Isobutylphenyl)-2-hydroxypropionic acid | 2-OH | $-CH(CH_3)-CH_2$ | $-C(OH)(CH_3)-COOH$ | H | H |
| 2-(4-Isobutylphenyl)-propionamide | Amide | $-CH(CH_3)-CH_2$ | $-CH(CH_3)-C(=O)-NH_2$ | H | H |
| 4-Isobutylbenzoic acid | IBBA | $-CH(CH_3)-CH_2$ | $-COOH$ | H | H |
| 4-Isobutylacetophenone | IBAP | $-CH(CH_3)-CH_2$ | $-C(=O)-CH_3$ | H | H |

## RESULTS AND DISCUSSION

### *Chromatography development*

Fig. 1 shows a typical chromatogram of a preparation of Motrin® tablets. The drug and internal standard peaks are well resolved and both are symmetrical with very little tailing. The chromatogram is clean even though no cleanup steps were employed. Sample preparation is simply addition of 100 ml of the internal standard solution to tablets equivalent to 1200 mg of drug, then dissolution of the drug and centrifugation to separate insoluble excipients. It was found useful to shake compressed tablet sample preparations with 6-mm glass beads in order to help mechanically break the hard tablets.

The rationale for the chromatographic conditions chosen for this separation is detailed in the following discussion: 1200 mg of drug is used for analysis to correspond with the current United States Pharmacopoeia gas chromatographic procedure[14]. In the newly developed liquid chromatographic assay the same mass of drug is taken for analysis in the standard and sample preparations so that automated sample preparation can easily be adapted. In addition, this is the simplest possible sample preparation and avoids operator errors such as incorrect dilutions. To minimize the concentration of drug injected on the column, 100 ml of solvent was chosen. A volume of 100 ml is the largest volume easily measured and dispensed. The re-



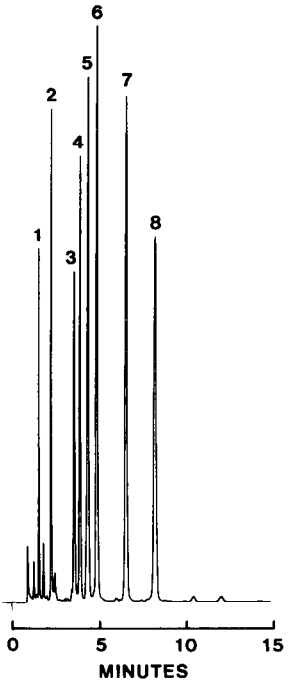




Fig. 1. Liquid chromatogram of a sample preparation of Motrin tablets. Peaks: 1 = IBPP; 2 = valerophenone (internal standard); 3 = IBAP.

Fig. 2. Liquid chromatograms of IBPP and possible process impurities and degradation products. Peaks: 1 = FPP; 2 = IBRPP; 3 = Amide; 4 = Unsat; 5 = Ortho; 6 = IBPP; 7 = valerophenone (internal standard); 8 = IBAP.

sulting IBPP concentration injected onto the column is 12 mg/ml. The acetonitrile concentration of the sample preparation solvent (which should also be the mobile phase) must be greater than 50% in order to dissolve this quantity of IBPP. Such a high concentration of IBPP places limits on the kind of packing material that can be used in the chromatographic column.

The column chosen for this assay, a Zorbax ODS, gives good retention of the drug when using a mobile phase containing 60% acetonitrile. A Waters μBondapak $C_{18}$ column, because of lower surface coverage of alkyl groups, requires a mobile phase with a lower acetonitrile concentration, and solubility problems result. A LiChrosorb RP-18 column produced results very similar to a μBondapak $C_{18}$ column.

For efficient and trouble free chromatography it is desirable that the IBPP be protonated in the mobile phase to suppress its ionization. For pH control the modifier used most often in previous methods is acetic acid[8,10,12]. IBPP has a $pK_a$ of 4.4 and therefore cannot be fully protonated by acetic acid, whose $pK_a$ is 4.75. Also, acetic acid–sodium acetate buffer does not have enough buffering capacity at the desired pH of 3.0. Chloroacetic acid–ammonium chloroacetate was chosen as the mobile phase buffer, because the $pK_a$ of chloroacetic acid is 2.85. The mobile phase could then be adequately buffered with 42 m*M* chloroacetate at pH 3, providing protonation of the IBPP while allowing a relatively long column lifetime. Valerophenone was selected as the internal standard because of retention time and, being a ketone, is chemically similar to many of the impurities.

Specificity of this chromatographic system was demonstrated by chromatographing a mixture of IBPP, structurally similar compounds and degradation products. Fig. 2 shows that all these compounds are easily separated from the drug peak. The degradation products, caused by oxidation of IBPP, are FPP, IBRPP, and IBAP. Table II lists retention data for these and related compounds. Chromatograms of a compressed tablet placebo (Fig. 3A), the internal standard (Fig. 3B) and Motrin tablet sample preparation (Fig. 3C) demonstrate that there are no peaks which interfere with the determination of IBPP.

TABLE II

RETENTION DATA FOR IBPP AND RELATED COMPOUNDS

| *Compound* | $t_R$ *(min)* | $k'$ | $\alpha$ |
|---|---|---|---|
| FPP | 1.6 | 0.23 | 0.08 |
| IBRPP | 2.3 | 0.77 | 0.26 |
| 2-OH | 2.7 | 1.08 | 0.36 |
| Amide | 3.8 | 1.92 | 0.64 |
| *n*-Propyl | 4.0 | 2.08 | 0.70 |
| Unsat | 4.2 | 2.23 | 0.75 |
| IBBA | 4.2 | 2.23 | 0.75 |
| Ortho | 4.7 | 2.62 | 0.88 |
| Meta | 5.1 | 2.92 | 0.98 |
| IBPP | 5.2 | 3.00 | 1.00 |
| *n*-Butyl | 5.7 | 3.38 | 1.13 |
| Valerophenone | 7.1 | 4.46 | 1.49 |
| IBAP | 9.1 | 6.00 | 2.01 |

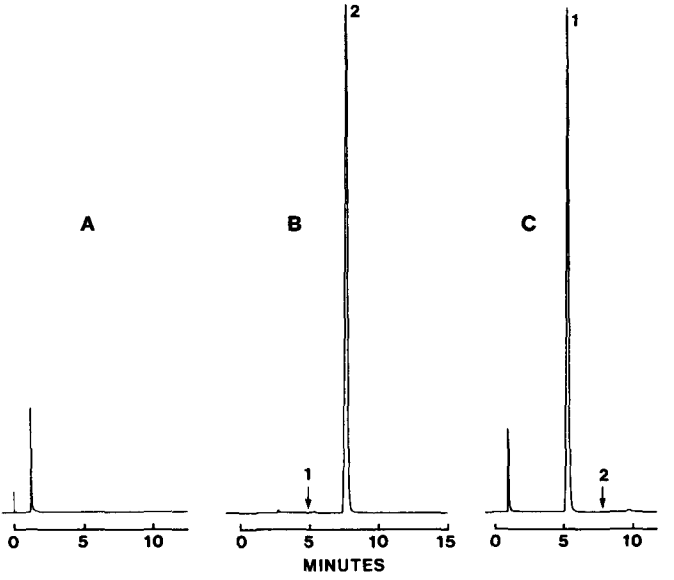



Fig. 3. Liquid chromatograms of coated compressed tablet placebo (A), internal standard valerophenone (B) and preparation of Motrin tablets without internal standard (C). Peaks: 1 = IBPP; 2 = valerophenone.

TABLE III

SPIKE RECOVERY STUDY

| *Added (mg)* | *Found (mg)* | *Recovered (%)* |
|---|---|---|
| 554 | 559 | 100.9 |
| 817 | 822 | 100.6 |
| 1112 | 1115 | 100.3 |
| 1375 | 1368 | 99.5 |
| 1649 | 1638 | 99.3 |
| 1914 | 1900 | 99.3 |
| | | $\bar{x}$ = 100.0 |
| | | R.S.D. = 0.7 |

TABLE IV

ASSAY PRECISION

FCT = film-coated tablet. CCT = coated compressed tablet.

| *Bulk drug (%)* | *Motrin tablets (mg/tab)* | | |
|---|---|---|---|
| | *FCT (100 mg/tab)* | *CCT (400 mg/tab)* | *FCT (600 mg/tab)* |
| 99.9 | 98.7 | 402 | 607 |
| 99.7 | 99.8 | 397 | 612 |
| 99.6 | 99.4 | 394 | 604 |
| 100.4 | 99.9 | 395 | 601 |
| 99.7 | 99.0 | 390 | 606 |
| 100.2 | 99.2 | 394 | |
| $\bar{x}$ 99.9 | 99.3 | 395 | 606 |
| $s$ 0.32 | 0.47 | 4.0 | 4.0 |
| R.S.D. (%) 0.32 | 0.47 | 1.0 | 0.66 |

*Assay validation*

The linearity of the method was demonstrated by assaying various amounts of a bulk drug sample according to the procedure. The concentrations of drug in the solutions analyzed ranged from 6 to 18 mg/ml, which corresponds to 50–150% of that called for in the procedure. The regression equation obtained was:

$$\text{drug conc. (mg/ml)} = \frac{\text{peak height ratio} - (0.002 \pm 0.004)}{0.0695 \pm 0.0003}$$

The correlation coefficient was 0.99999 and the intercept was zero to 95% confidence. Linearity data was generated using a fixed-wavelength (mercury line source) detector. When a variable-wavelength grating monochromator detector or a selectable-wavelength filter detector was used, both of which have a deuterium lamp

TABLE V

ASSAY RESULTS FOR BULK DRUG, COMPRESSED TABLET AND STABILITY SAMPLES

Average of two replications unless otherwise noted. FCT = filmcoated tablet; CCT = coated compressed tablet.

| *Sample* | *Potency* | |
|---|---|---|
| | *Assay result* | *% of label* |
| Bulk drug 1 | 100.1% | 100.1 |
| Bulk drug 2 | 100.2% | 100.2 |
| Bulk drug 3 | 99.5% | 99.5 |
| 100 mg FCT 1 | 99.7 mg/tab | 99.7 |
| 100 mg FCT 2 | 99.8 mg/tab | 99.8 |
| 100 mg FCT 3 | 100.2 mg/tab | 100.2 |
| 200 mg FCT 1 | 200.3 mg/tab | 100.2 |
| 200 mg FCT 2 | 198.5 mg/tab | 99.3 |
| 200 mg FCT 3 | 201.0 mg/tab | 100.5 |
| 300 mg CCT 1 | 302 mg/tab | 100.7 |
| 300 mg CCT 2 | 300 mg/tab | 100.0 |
| 300 mg CCT 3 | 297 mg/tab | 99.0 |
| 400 mg CCT 1 | 399 mg/tab* | 99.7 |
| 400 mg CCT 2 | 401 mg/tab | 100.2 |
| 400 mg CCT 3 | 396 mg/tab | 99.0 |
| 600 mg FCT 1 | 600 mg/tab | 100.0 |
| 600 mg FCT 2 | 603 mg/tab | 100.5 |
| 600 mg FCT 3 | 606 mg/tab | 101.0 |
| 400 mg CCT stability 1** | 400 mg/tab | 100.0 |
| 400 mg CCT stability 2*** | 402 mg/tab | 100.5 |
| 600 mg FCT stability 3§ | 605 mg/tab | 100.8 |

* Number of replications = 6.
** Ambient temperature for 36 months.
*** For 3 months at 47°C.
§ For 3 months at 25°C and 93% humidity.

source, the standard curve was not linear. When the data from these two detectors were fitted to a linear model, the intercept was positive. The reason for this behavior is not known, but may be due to the bandpass of the detectors.

Recovery of the drug from a compressed tablet formulation was demonstrated by combining a constant portion of placebo to weighed portions of drug over the range 50–150% of label and analyzing according to the procedure. The results are listed in Table III. The recovery averaged 100.0% with a relative standard deviation of 0.7%.

Precision of the method was measured by replicate assay of several lots of Motrin tablets and bulk drug (Table IV). The overall precision for bulk drug was 0.4% and for Motrin tablets it was 0.7%. A summary of the assay results for various lots of bulk drug, compressed tablet dosage forms and stasbility lots is listed in Table V. The average concentration of IBPP is 100.0% with an R.S.D. of 0.5%. This indicates that the assay method is accurate and also that the content uniformity of Motrin tablets is constant.

## REFERENCES

1 D. G. Kaiser and G. J. Vangiessen, *J. Pharm. Sci.*, 63 (1974) 219.
2 D. J. Hoffman, *J. Pharm. Sci.*, 66 (1977) 749.
3 D. G. Kaiser and R. S. Martin, *J. Pharm. Sci.*, 67 (1978) 627.
4 L. Heikkinen, *J. Chromatogr.*, 307 (1984) 206.
5 D. Pitre and M. Grandi, *J. Chromatogr.*, 170 (1979) 278.
6 L. J. Dusci and L. P. Hackett, *J. Chromatogr.*, 172 (1979) 516.
7 J. K. Baker and E. K. Fifer, *J. Pharm. Sci.*, 69 (1980) 590.
8 G. L. Kearns and J. T. Wilson, *J. Chromatogr.*, 226 (1981) 183.
9 J. L. Shimek, N. G. S. Rao and S. K. Wahba Khalil, *J. Pharm. Sci.*, 70 (1981) 514.
10 A. Ali, S. Kazmi and F. M. Plakagiannis, *J. Pharm. Sci.*, 70 (1981) 944.
11 G. F. Lockwood and J. G. Wagner, *J. Chromatogr.*, 232 (1982) 335.
12 D. J. Greenblatt, R. M. Arendt and A. Locniskar, *Arzneim.-Forsch.*, 33 (1983) 1671.
13 W. F. Beyer and D. D. Gleason, *J. Pharm. Sci.*, 64 (1975) 1557.
14 *United States Pharmacopoeia (XX)*, Supplement 3a, Mack Publishing Co., Easton, PA, 1980, p. 534.