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## Note

# High-pressure liquid chromatographic method for surveying the purity of tolmetin sodium dihydrate

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Since the introduction of indomethacin as a non-steroidal anti-inflammatory agent, a great deal of activity has been directed towards synthesizing safer and better tolerated aryl acetic acids for clinical use. Carson *et al.*[1] have reported the synthesis of several benzoylmethylpyrroleacetic acids that have been shown to be active as anti-inflammatory agents[1]. One member of the series, tolmetin sodium dihydrate (sodium 1-methyl-5-[4-methylbenzoyl]-1*H*-pyrrole-2-acetate dihydrate)[1], has recently been introduced for the treatment of rheumatoid arthritis.

In conjunction with the clinical development of tolmetin sodium dihydrate, a quantitative procedure for determining the purity as well as a means of monitoring chemical stability were needed. Since the compound undergoes rapid decomposition upon heating, neither differential scanning calorimetry nor direct analysis by gas chromatography (GC) was an applicable technique. In addition, combined derivatization–GC procedures, although suitable for urine and plasma analysis[2,3] were deemed unacceptable for evaluating compound purity. In order for a derivatization procedure to be acceptable, it would require similarity in extractability and reactivity between tolmetin sodium dihydrate and any possible impurities. Since impurities may not always be known, one would not totally be assured of detecting them.

Alternatively, since high-pressure liquid chromatography (HPLC) lends itself to the direct analysis of thermally labile compounds, it eliminates the potential problems in volatility and uncertainty in differences of extractability and reactivity between tolmetin sodium dihydrate and known or unknown impurities. In addition, an added advantage is rapid sample preparation requiring only a simple dissolution step in a suitable solvent.

In this paper, liquid chromatographic conditions are described for the identification and determination of nine related compounds which theoretically may be present as impurities in tolmetin sodium dihydrate. Using the method presented, a purity profile can be obtained. The chromatographic conditions are also potentially applicable for use in stability testing, dosage form analysis, and plasma and urine level determinations.

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## EXPERIMENTAL

### *Equipment*

All HPLC work was performed on a Waters Assoc. Model 202 liquid chromatograph equipped with dual 6000 p.s.i. pumps, a solvent programmer, and a 254-nm UV detector. Samples were introduced on-column with Precision Sampling syringes. Columns were maintained at ambient temperature.

### *Columns*

The 25 cm × 2.2 mm I.D. micro-silica columns were slurry packed, conditioned and evaluated as previously described[4]. All columns were packed with LiChrosorb SI-60 (average particle diameter 10 $\mu$m) obtained from EM Labs. (Elmsford, N.Y., U.S.A.).

### *Reagents*

Both the *n*-hexane and isopropanol used were distilled-in-glass quality solvents obtained from Burdick and Jackson Labs. (Muskegon, Mich., U.S.A.). The acetic acid (glacial) was reagent grade obtained from Mallinckrodt (St. Louis, Mo., U.S.A.).

### *HPLC method*

After having placed the micro-silica column in the chromatograph, solvent reservoir A was filled with 0.25% acetic acid in *n*-hexane, and solvent reservoir B with isopropanol–*n*-hexane (1:1, v/v) (containing 0.25% acetic acid). The flow-rate was set at 2 ml/min, the gradient programmer at 2% solvent B and the detector range control at the 0.08-absorbance units full scale (a.u.f.s.) position. Prior to use, all columns were conditioned at least twice by programming the solvent gradient from 2 to 60% solvent B over a 20-min time interval. A concave solvent gradient (No. 7) was used as shown in Fig. 1.

One hundred mg of tolmetin sodium dihydrate were dissolved in 2 ml of methanol (50 mg/ml) and 10 $\mu$l of this solution were injected on-column. After sample introduction, the solvent gradient was run as described above. Following each run, initial conditions were re-established through an identical reverse solvent program. In the case of the test mixtures containing the nine possible impurities, the samples were prepared by dissolving 100 mg of tolmetin sodium dihydrate in 2 ml of a methanol solution in which 0.1–0.2 mg of each of the various possible impurities had been dissolved.

### *Calibration curves*

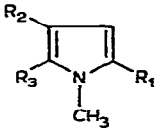
The calibration curves for each possible impurity were prepared in the following manner: 10 mg of a particular test compound were dissolved in 100 ml of methanol (0.1 mg/ml). A second solution was prepared by diluting the original solution 1:10 with methanol. Injections of 5, 4, 3 and 2 $\mu$l (0.5, 0.4, 0.3 and 0.2 $\mu$g) of the initial solution and 10 and 5 $\mu$l (0.1 and 0.05 $\mu$g) of the diluted solution were made. A calibration curve was established for each test compound using the isocratic conditions listed in Table III.

## RESULTS AND DISCUSSION

A HPLC method has been developed to survey qualitatively and evaluate quantitatively the purity of tolmetin sodium dihydrate. To demonstrate the selectivity of this procedure, nine structurally related and theoretically possible impurities have been run in conjunction with tolmetin sodium dihydrate. The structures of these compounds have been summarized in Table I. All test compounds have been run under both gradient and isocratic conditions.

TABLE I

HPLC RETENTION TIMES OF THE TEST COMPOUNDS BY GRADIENT ELUTION




| *Compound* | $R_1$ | $R_2$ | $R_3$ | *Retention time (min)* |
|---|---|---|---|---|
| I *p*-Toluic acid | — | — | — | 0.9 |
| II | $-CH_2COOC_2H_5$ | –H | $CH_3-C_6H_4-CO-$ | 1.2 |
| III | $-CH_2COOCH_3$ | –H | $CH_3-C_6H_4-CO-$ | 1.4 |
| IV | $-CH_2COOH$ | –H | –H | 3.4 |
| V | –COOH | $CH_3-C_6H_4-CO-$ | $-CH_3$ | 3.4 |
| VI (Tolmetin) | $-CH_2COOH$ | –H | $CH_3-C_6H_4-CO-$ | 8.6 |
| VII | $-CONH_2$ | $CH_3-C_6H_4-CO-$ | $-CH_3$ | 11.9 |
| VIII | $-CH_2COOH$ | $CH_3-C_6H_4-CO-$ | –H | 15.6 |
| IX | $-CH_2CONH_2$ | –H | $CH_3-C_6H_4-CO-$ | 17.8 |
| X | $-CH_2CONH_2$ | $CH_3-C_6H_4-CO-$ | –H | 21.8 |

A representative liquid chromatogram of a mixture of tolmetin sodium dihydrate and 0.1% of each of the test compounds is shown in Fig. 1. The chromatogram was obtained at a detector range of 0.08 a.u.f.s. under the previously described gradient conditions.

Gradient conditions were chosen to cover a wide range of solvent polarities. This permitted both very similar compounds such as the methyl and ethyl esters (compounds II and III listed in Table I) as well as dissimilar materials to be separated conveniently in a relatively short time. In addition, the possibility of detecting unknown impurities was greatly enhanced.

Retention times for tolmetin sodium dihydrate and the nine test compounds under gradient elution conditions are summarized in Table I for a typical micro-silica column. A chromatographic analysis under gradient conditions can be completed in approximately 30 min followed by re-establishment of initial mobile phase conditions in an additional 25–30 min.

As shown in Fig. 1, the survey separation was carried out in an overloaded condition with respect to tolmetin sodium dihydrate (500 $\mu$g). By employing this procedure, the increased sensitivities for each test compound were realized. Thus it was possible to detect easily trace levels of less than 0.1% of each test material. In addition, since this separation was carried at a detector setting of 0.08 a.u.f.s., further increases in sensitivity were possible by using lower detector settings.

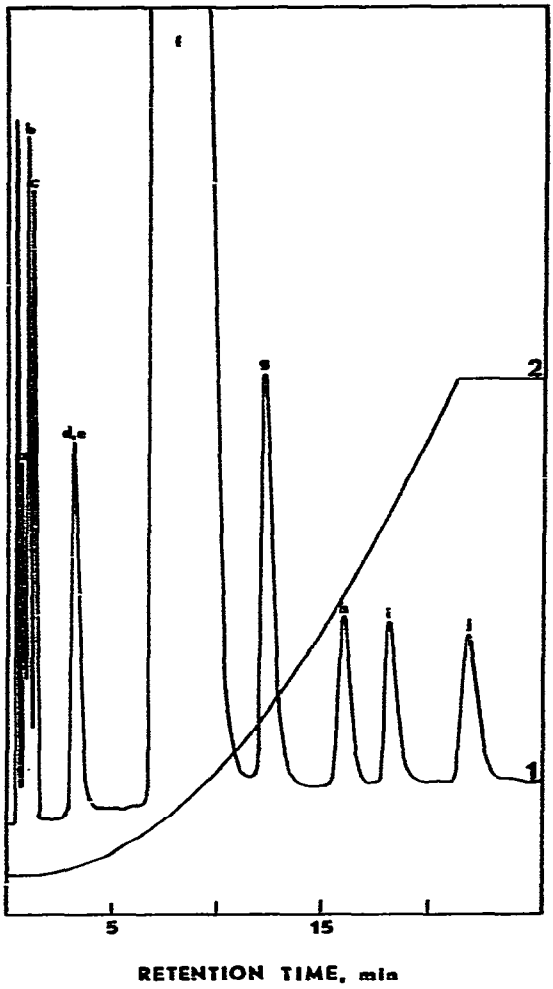



Fig. 1. Elution of compounds under gradient conditions. (1) Representative chromatogram of a mixture of 500 $\mu$g of tolmetin sodium dihydrate containing 0.1% of each of the test impurities (compounds listed in Table I, a–j correspond to compounds I–X respectively). (2) Representation of solvent gradient (concave gradient, setting No. 7).

During analysis, qualitative identifications of any impurities may be made by comparing retention behavior with that of a newly chromatographed mixture of the nine test compounds listed in Table I. To evaluate the reproducibility of compound retention under the listed gradient conditions, a test mixture containing 0.2% of each possible impurity in a 50 mg/ml methanol solution of tolmetin sodium dihydrate was prepared. Five consecutive injections of this test solution were made during a single day. Following each run, the initial mobile phase conditions were re-established as previously described. The coefficients of variation in retention and relative retention for each compound were calculated. The data obtained are summarized in Table II. These data demonstrate that the retention and relative retention

TABLE II

VARIATION IN COMPOUND RETENTION UNDER GRADIENT ELUTION CONDITIONS

| *Compound* | *Coefficient of variation (%)* | |
|---|---|---|
| | *Retention* | *Relative retention* |
| I | 3.83 | 2.60 |
| II | 2.53 | 0.94 |
| III | 1.41 | 0.80 |
| IV | 2.97 | 1.14 |
| V | 2.97 | 1.14 |
| VI (tolmetin) | 1.90 | — |
| VII | 0.47 | 1.63 |
| VIII | 0.78 | 1.14 |
| IX | 0.06 | 1.64 |
| X | 0.10 | 2.00 |

times are sufficiently reproducible within a single day to permit the qualitative identification of any of the test impurities. In addition, since no significant differences in coefficients of variation were observed between absolute and relative retention measurements, either seem sufficiently precise for qualitative use.

If any impurities are detected and after they are initially identified, quantitation of each observed compound is performed using the listed isocratic conditions (Table III) and an appropriate calibration curve for the impurity.

Standard calibration plots of peak area *vs.* concentration (0.05 to 0.5 $\mu$g) for each of the nine test impurities were constructed and found to be rectilinear over the sample range studied (0.05 to 0.5 $\mu$g). This corresponds to 0.01–0.1 % of each impurity for a 500-$\mu$g injection of tolmetin sodium dihydrate.

TABLE III

HPLC ISOCRATIC CONDITION FOR DETERMINATION OF THE TEST COMPOUNDS

25 cm × 2.2 mm I.D. micro silica column; UV detector, 254 nm.

| *Compound* | *Per cent isopropanol in n-hexane (containing 0.25% acetic acid)* | *Sensitivity (a.u.f.s.)** | *Flow-rate (ml/min)* | *Ret. time (min)* |
|---|---|---|---|---|
| I | 1 | 0.08 | 1.0 | 1.7 |
| II | 1 | 0.16 | 1.0 | 2.3 |
| III | 1 | 0.16 | 1.0 | 2.8 |
| IV | 3 | 0.04 | 2.0 | 2.1 |
| V | 3 | 0.08 | 2.0 | 1.8 |
| VI | 10 | — | 1.0 | 3.2 |
| VII | 10 | 0.08 | 1.0 | 5.4 |
| VIII | 20 | 0.08 | 2.0 | 2.6 |
| IX | 20 | 0.08 | 2.0 | 3.4 |
| X | 30 | 0.04 | 2.0 | 5.0 |

* Sensitivity needed to insure at least 0.1 $\mu$g detection.

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REFERENCES

1 J. R. Carson, D. N. McKinstry and S. Wong, *J. Med. Chem.*, 14 (1971) 646.
2 M. L. Selley, J. Thomas and E. J. Triggs, *J. Chromatogr.*, 94 (1974) 143.
3 W. A. Cressman, B. Lopez and D. Sumner, *J. Pharm. Sci.*, 64 (1975) 1965.
4 R. K. Gilpin, D. J. Camillo and C. A. Janicki, *J. Chromatogr.*, 121 (1976) 13.