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STATISTICAL OPTIMIZATION OF A REVERSED-PHASE ION-PAIR LIQUID CHROMATOGRAPHIC METHOD FOR THE ANALYSIS OF TOLMETIN SODIUM IN DOSAGE FORMS

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SUMMARY

A quantitative liquid chromatographic method in which tolmetin sodium is separated from an internal standard on a C_{18} column with detection at 317 nm has been developed with the aid of two statistical optimization procedures. The effects of simultaneously varying the pH, methanol–water ratio, and the concentrations of buffer and ion-pair reagent in the mobile phase were studied. A two-level factorial design was used to investigate the interactions among the variables, and the sequential simplex procedure was used to optimize the separation. A novel quality criterion was developed for the simplex optimization. Using synthetic mixtures, the mean recovery value ± S.D. (n = 6) of tolmetin sodium was 98.7 ± 0.19% for tablets and 98.5 ± 0.12% for capsules. The assay results for commercial tablets and capsules were comparable to those obtained by the USP XXI procedure.

INTRODUCTION

Tolmetin sodium is a non-steroidal anti-inflammatory agent with analgesic and antipyretic activities. As the sodium salt dihydrate it is formulated in tablet and capsule forms, both of which are used for the treatment of rheumatoid arthritis and osteoarithritis[1].

Tolmetin has been determined in plasma samples by gas and liquid chromatographic procedures[2–7], and in solid dosage forms by spectrophotometry[8]. Its possible impurities have been quantitated by a normal-phase gradient high-performance liquid chromatography (HPLC) procedure[9]. The compendial assays for tablets and capsules entailed lengthy spectrophotometric procedures[10]. Because of the typically low recoveries of the plasma studies and the amount of time required for the spectrophotometric method, an alternate procedure suitable for the regulatory analysis of tolmetin in dosage forms was sought. This report describes the development of a rapid, accurate and selective LC method for the analysis of the drug in tablets and capsules.

The assay was developed using two mathematical statistical models–factorial

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design and simplex optimization[11–17]. These methods are used to assess the quality of a separation quantitatively. In a comparatively short time, a determination as to which variables have significant effects, and the relative importance, the degree of interaction, and the optimum levels of these variables was made.

Screening experiments were used to determine a reasonable first set of conditions, including the type of column, organic modifier, and sample concentration. These experiments also suggested the variables to be investigated. Two sets of two-level, full factorial experiments were carried out. The results were that pH, and the concentrations of buffer, ion-interaction reagent and methanol were significant and interactive. Peak shape and column retention could be closely controlled by adjusting these four variables.

The final step was to simultaneously optimize the values of the four variables using the simplex procedure. The simplex was terminated after the twelfth experiment and the best set of conditions was selected.

EXPERIMENTAL

Apparatus

A Tracor 950 chromatographic pump with 970A variable-wavelength detector and a TS-10 recorder were used (Tracor, Austin, TX, U.S.A.). Separations were performed on Zorbax ODS, 250 × 4.6 mm, 5-μm particles (DuPont, Wilmington, DE, U.S.A.), μBondapak C_{18}, 300 × 3.9 mm, 10-μm particles (Waters Assoc., Milford, MA, U.S.A.) and Ultrasphere ODS, 250 × 4.6 mm, 5-μm particles (Beckman Instruments, San Ramon, CA, U.S.A.) columns. Samples were introduced through a Rheodyne 7125 injection valve with a 20-μl sample loop (Rheodyne, Cotati, CA, U.S.A.).

Chemicals and reagents

Methanol was HPLC grade (Fisher Scientific, Fairlawn, NJ, U.S.A.). Other reagents were analytical-reagent grade. The tolmetin and zomepirac sodium dihydrates were kindly donated by McNeil Pharmaceutical (Spring House, PA, U.S.A.).

Procedure

Internal standard solution. A 420 μg/ml solution of zomepirac sodium dihydrate in methanol–water (1:1) was prepared.

Standard preparation. Approximately 22 mg of tolmetin sodium, previously dried at 105°C for 3 h was dissolved in methanol–water (1:1) in a 100-ml volumetric flask and 10.0-ml aliquots of this solution and the internal standard solution were combined and diluted to 100.0 ml with the same solvent.

Sample preparation. An amount of ground tablet or capsule powder corresponding to 200 mg of tolmetin was weighed into a 100-ml volumetric flask and diluted to volume with methanol–water (1:1). This preparation was mixed and a portion filtered through medium porosity filter paper. A 10.0-ml aliquot of the filtrate was diluted to 100 ml with methanol–water. A 10.0-ml aliquot of the resulting solution and 10.0 ml of the internal standard solution were combined in a 100-ml volumetric flask and diluted to volume with methanol–water.

Chromatographic conditions. The mobile phase was prepared by dissolving 1.36

g of monobasic potassium phosphate and 3.39 g of tetrabutylammonium phosphate in 350 ml of water, then adding 650 ml of methanol and 1 ml of acetic acid, mixing, filtering through a membrane filter (0.45 μm porosity) and degassing. The methanol–water levels may be adjusted to obtain acceptable separations. The flow-rate was set at 1.0 ml/min, the detector wavelength at 317 nm and 20 μl portions of the preparations were injected.

Calculation. The quantity (in mg) of tolmetin in the portion of powder taken is equal to (257.29/279.27) (C) (R_u/R_s), in which 257.29 and 279.27 are the molecular weights of tolmetin and anhydrous tolmetin sodium respectively, C is the concentration, in mg per ml of tolmetin sodium in the standard preparation, and R_u and R_s are the peak response ratios of the analyte to the internal standard obtained from the sample preparation and the standard preparation respectively.

RESULTS AND DISCUSSION

The decision to apply experimental design techniques to the development of the method was made after a series of screening experiments revealed that peak shape and reproducibility could not adequately be controlled by a mobile phase composed only of solvents without solute conditioning reagents such as buffers, counter ions and acids. Mobile phase additives were needed but the possible combinations of mobile phase components and the proportions of each component were numerous. In order to rationalize the decision making process, two experimental models were chosen, factorial design and simplex optimization.

TABLE I

ELUENT COMPOSITIONS, k' VALUES, AND CALCULATED EFFECTS OF FIRST SERIES OF FACTORIAL DESIGN EXPERIMENTS

Exp. No.	*Methanol–water ratio*	*Apparent pH*	*Buffer conc. (mM)*	*k'*	
				Tolmetin	*Zomepirac*
1	55:45	6.0	5	2.82	4.68
2	55:45	6.0	0	0.10	0.15
3	55:45	4.0	5	4.30	6.72
4	55:45	4.0	0	0.10	0.15
5	45:55	6.0	5	7.28	13.28
6	45:55	6.0	0	0.71	1.34
7	45:55	4.0	5	14.72	24.83
8	45:55	4.0	0	1.29	2.40

Variable		*Effect*	
(1)	Methanol–water ratio	−4.17	−7.54
(2)	pH	−2.38	−3.66
(3)	Buffer concentration	6.73	11.37
	Interaction 1 × 2	−2.09	−3.13
	1 × 3	−3.27	−5.82
	2 × 3	1.64	2.64

TABLE II

CONDITIONS AND RESULTS OF SECOND SERIES OF FACTORIAL DESIGN EXPERIMENTS

Exp. No.	*Methanol–water ratio*	*Apparent pH*	*Buffer conc. (mM)*	*IIR conc. (mM)*	*k'*	
					Tolmetin	*Zomepirac*
1	65:35	7.0	75	10	–	–
2	65:35	7.0	75	5	–	–
3	65:35	7.0	25	10	1.95	2.80
4	65:35	7.0	25	5	2.00	3.00
5	65:35	5.0	75	10	–	–
6	65:35	5.0	75	5	–	–
7	65:35	5.0	25	10	2.33	3.48
8	65:35	5.0	25	5	3.92	5.82
9	60:40	7.0	75	10	4.00	6.16
10	60:40	7.0	75	5	3.15	5.00
11	60:40	7.0	25	10	3.24	5.03
12	60:40	7.0	25	5	2.49	3.88
13	60:40	5.0	75	10	5.02	7.42
14	60:40	5.0	75	5	4.82	7.31
15	60:40	5.0	25	10	5.61	8.43
16	60:40	5.0	25	5	3.64	5.49

Variable		*Effect*	
(1)	Methanol–water ratio	−1.20	−1.93
(2)	pH	−1.41	−2.02
(3)	Buffer concentration	0.50	0.76
(4)	IIR concentration	0.35	0.47
	Interaction 1 × 2	0.65	0.84
	1 × 3	0.35	0.59
	1 × 4	−0.91	−1.32
	2 × 3	0.53	0.82
	2 × 4	0.17	0.24
	3 × 4	−0.01	−0.04

While it is not strictly necessary to use two design techniques, the practice has been recommended by Deming and Morgan[18]. Further, This study was originally undertaken as part of a project, the goal of which was to revise drug monographs of the *United States Pharmacopeia*[10]. In view of this goal, it was useful to combine the greater information content and ruggedness testing inherent in a factorial design with the speed of the simplex optimization.

Factorial design

A three-variable, two-level, full factorial design was chosen in order to investigate the effects of each component and their interactions. The variables were the methanol-water ratio, pH, and buffer concentration (Table I). The main effects and interactions were calculated according to Box *et al.*[19]. The effect of a variable on retention is indicated by its sign (+ or −). The capacity factor, k', was selected as a rapid means of measuring the influence of a mobile phase component on the eluting peaks. Experiments were conducted in random order.

From the table it can be seen that each of the variables had a significant effect on the retention of the compounds with the buffer exerting the most profound effect. The interaction of the methanol–water ratio with the buffer concentration was greater than the other combined effects, which indicates that the alteration in retention caused by the presence of the buffer is dependent on the methanol–water ratio.

Peak shape and reproducibility for most of the mobile phases tested were unacceptable even with those in which resolution and analysis time were adequate. An ion-interaction reagent, tetabutylammonium phosphate, which can be used for the analysis of weak organic acids, was therefore, added to the mobile phase. To explore the effect of this addition, as well as the role of other components, a second set of factorial experiments was done. Different levels of methanol–water ratio, pH, and buffer concentration were selected in order to maximize the information that could be extracted from the experimental data (Table II).

Unfortunately, precipitation of the salts occurred in the eluents when the concentrations of methanol and buffer were at their highest levels. Nevertheless, an analysis of the data could be made. Calculation of the main effects and interactions show that they had markedly decreased from those of the first set of experiments. The tolmetin and internal standard were found to be less sensitive to variations in the levels of the components of the mobile phase. The wide fluctuations in k' apparent in the first grouping were eliminated by the regulating influences of the buffer and the ion-interaction reagent and is reflected in the calculated effects. Excellent peak shape and reproducibility were also achieved.

Simplex optimization

In order to identify the optimum conditions for the analysis, a five dimensional sequential simplex optimization was undertaken. The most difficult aspect of this technique is the selection of a quantitative criterion that will permit the evaluation of a set of chromatograms. A variety of approaches have been proposed[20,21], many requiring complex measurements and calculations, and frequently, the use of a computer. An alternate criterion was sought in which the quality assessment could be based on the capacity factor, k'. The capacity factor is easily obtained. It is preferred over such other simple parameters as retention time or retention volume because it is independent of the flow rate and column volume. Previously proposed criteria include requirements for the resolution of peaks and the maximum time of analysis. The capacity factor is well-suited as a means of expressing these requirements in a usable mathematical form. Optimal k' values can be selected to locate any pair of peaks so that sufficient resolution is achieved within preselected time restrictions. For this study, optimal k' values of 2 and 4 were chosen for tolmetin and the internal standard respectively. Based on these considerations, an empirical separation factor (ESF) was developed. Excellent concordance was found between the calculated and experimental values.

$$\mathrm{ESF} = (|k'\mathrm{optA} - k'\mathrm{actA}| + |k'\mathrm{optB} - k'\mathrm{actB}|) + \left|1 - \frac{(k'\mathrm{optB} - k'\mathrm{optA})}{(k'\mathrm{actB} - k'\mathrm{actA})}\right|$$

where k'optA,B and k'actA,B are the previously selected and experimentally determined capacity factors respectively for any pair of peaks, A and B. If the optimal

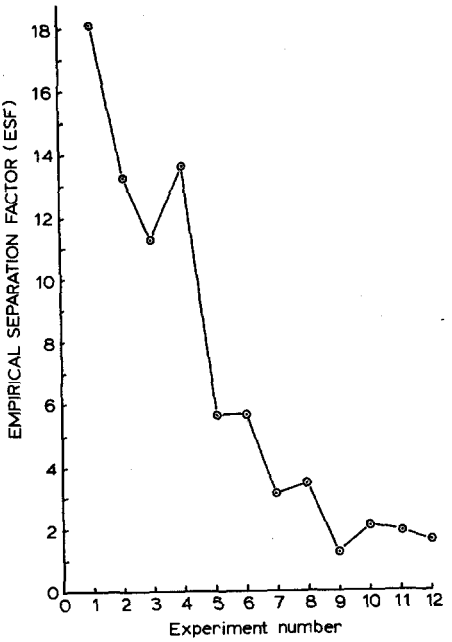

Fig. 1. Progress of the simplex.

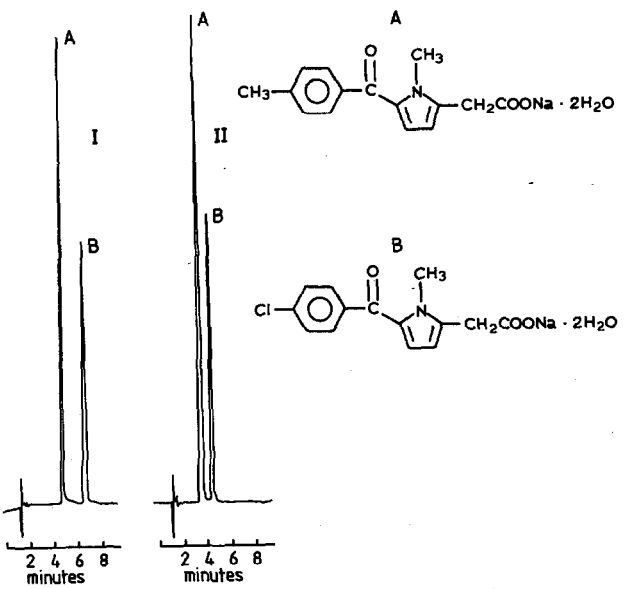

Fig. 2. HPLC separation of (A) tolmetin and (B) zomepirac, the internal standard, on two columns: I, Zorbax; II, new μBondapak.

conditions are achieved, ESF = 0. The first term of the equation sums the absolute differences between the desired and achieved capacity factors. Absolute values are needed to prevent the diminishment of the total if the differences for the two peaks are of opposite sign. Because absolute values are being used, however, the same ESF would result from a chromatogram in which resolution decreases as for one in which it increases to an equal degree. A second term was added to produce a higher less desirable ESF for the less well resolved pair. The numerator of this term is constant. The denominator decreases as resolution decreases, thus increasing the quotient and consequently the ESF. The quotient is subtracted from 1 so that the total ESF for an ideal situation is 0. The absolute value is again used in the second term to avoid the negative sum which would otherwise result when the k'act values are less than the k'opt values.

The initial conditions/step sizes for the simplex were (1) methanol–water ratio, 55:45/5%; (2) pH, 5.0/0.5; (3) buffer concentration, 10 mM/5 mM; and (4) ion-interaction reagent concentration, 10 mM/5 mM. The rapid progress of the simplex is shown in Fig. 1. The proposed method is based on the conditions of the ninth experiment. A chromatogram obtained with the optimized mobile phase is shown in Fig. 2.

Validation

The method was tested for specificity, linearity, precision, sensitivity and ruggedness according to the format proposed by Debesis *et al.*[22].

The specificity of the method was investigated by observing any interference by other compounds known to represent decomposition products and synthetic by-products of tolmetin, *i.e.*, *p*-toluic acid; 4-*p*-toluoyl-1-methylpyrrole-2-acetamide; ethyl 5-*p*-toluoyl-1-methylpyrrole-2-acetate; 1,5-dimethyl-4-(*p*-toluoyl)pyrrole-2-carboxamide; and methyl 5-*p*-toluoyl-1-methylpyrrole-2-acetate. The k' values were less than 1 for the first two compounds and greater than 3 for the others in a system in which the values for tolmetin and the internal standard were 1.5 and 2.1 respectively. Eluting sample and standard peaks were collected, and a complete ultraviolet spectrum of each peak was obtained. In all cases, sample and standard peaks were found to be identical.

The susceptibility of the method to alterations in the composition as well as in the ratio of the components of the mobile phase was evaluated using the optimization technique described earlier. The ideal method is one that will ensure that sensitive factors remain protected while those that are not (*e.g.* the methanol to water ratio) may be varied to suit column characteristics. It was verified that an increase of as much as 15% in either methanol or water, with no changes in the other components of the mobile phase, will not significantly affect the quality of the chromatograms.

Column-to-column variability was examined using three brands of octadecylsilane columns, namely Zorbax, Ultrasphere and μBondapak. For the μBondapak column, a comparison was also made between a new column and one that had been subjected to repeated use.

Zorbax columns have a high resolving power compared to most others. This method was developed using a Zorbax column with more resolution between tolmetin and the internal standard than is actually needed, on the theory that it could then be

TABLE III

ASSAY RESULTS FOR 200 mg TOLMETIN SODIUM TABLETS BY PROPOSED AND USP XXI METHODS

Composite No.	*% of declared*	
	Proposed method	*USP method*
1	100.2	100.8
2	99.2	99.9
3	99.3	100.0
4	99.7	–
5	99.7	–
6	99.4	–
7	99.6	–
8	99.7	–
9	99.7	–
10	99.4	–
Mean	99.6	100.2
S.D.	0.285	0.493
C.V. (%)	0.286	0.492

transferred to a column with less resolution and still give adequate separation. The Zorbax column had approximately three times the number of theoretical plates as the new μBondapak column, but the methods worked on both. On the old μBondapak column, the peaks were symmetrical, but not completely resolved. By decreasing the amount of methanol in the mobile phase, a good separation was achieved. Similarly, since the Zorbax column gave more separation than needed, the methanol concentration was increased to make the analysis more rapid, but with no loss in

TABLE IV

ASSAY RESULTS FOR 400 mg TOLMETIN SODIUM CAPSULES BY PROPOSED AND USP XXI METHODS

Composite No.	*% of declared*	
	Proposed method	*USP method*
1	101.2	101.8
2	101.4	101.5
3	101.2	101.1
4	101.9	–
5	101.6	–
6	101.8	–
7	101.0	–
8	101.0	–
9	101.8	–
10	101.2	–
Mean	101.3	101.5
S.D.	0.360	0.351
C.V. (%)	0.356	0.346

efficiency. The Ultrasphere column showed extreme tailing on a test mixture of methyl and ethyl parabens. This tailing was also seen in the tolmetin and internal standard peaks. These peaks were resolved, however, and the chromatogram could be used for quantitation.

Detector responses were linearly related to concentrations of tolmetin in the range 10–30 μg/ml (r = 0.99998), with a detection limit of about 1 μg/ml. The precision of the method, based on ten separate weighings of the same composites, resulted in coefficient of variation (C.V.) values of 0.286% for a tablet sample, 0.365% for a capsule sample, and 0.265% (n = 10) for reinjections of the same standard solution. The accuracy of the method was established on the basis of recoveries of the drug substance from synthetic formulations and commercial products. Recoveries (mean ± S.D. of the added amount) were: from the synthetic tablet formulation 98.7 ± 0.19% (n = 6); from the synthetic capsule formulation 98.5 ± 0.12% (n = 6); from commercial tablets 99.6 ± 0.29% (n = 3), and from commercial capsules 101.3 ± 0.36% (n = 3).

The proposed method was used to analyze commercial tablets and capsules of tolmetin sodium, and the results were compared with these obtained by the corresponding methods of USP XXI[10]. The results of these studies are presented in Tables III and IV. In general intermethod correspondence was excellent.

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