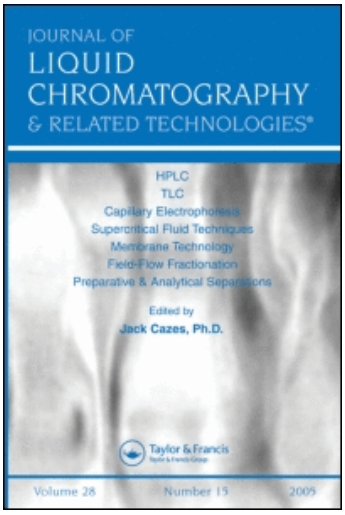

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Samuel B. Thomas[a]; Sargon J. Albazi[a]
[a] Department of Chemistry, Northeastern Illinois University, Chicago, Illinois

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SIMULTANEOUS DETERMINATION OF THE 5-LIPOXYGENASE INHIBITOR "ZILEUTON" AND ITS N-DEHYDROXYLATED METABOLITE IN UNTREATED RAT URINE BY MICELLAR LIQUID CHROMATOGRAPHY

Samuel B. Thomas,[†] Sargon J. Albazi*

Department of Chemistry
Northeastern Illinois University,
Chicago, Illinois 60625

ABSTRACT

A specific and sensitive HPLC procedure for the quantitative determination of Zileuton, (N- (1- (benzo-[b]-thien-2-yl)ethyl)-N-hydroxyurea), and its N-dehydroxylated metabolite in an untreated urine sample was developed. Separation of these compounds is achieved with micellar liquid chromatography, using sodium dodecyl sulfate (SDS) as the mobile phase and a CN-bonded silica column with UV detection at 262 nm. Because of the dissolving power of the micellar phase, urine samples were injected into the system without time-consuming protein precipitation and/or drug extraction steps.

[†]Present Address: Drug Metabolism Department, Pharmaceutical Products Department, Abbott Laboratories, Abbott Park, IL 60064.

Changes in mobile phase variables such as SDS concentration, pH and/or organic modifier concentration profoundly affected both chromatographic selectivity and drug retention. Linear calibration curves of the relative peak heights versus concentrations were obtained from 0.10 - 5.0 ppm for Zileuton and its metabolite. The minimum quantifiable concentration using this column was 0.08 - 0.10 ppm.

INTRODUCTION

Zileuton (Abbott-64077, Figure 1) is a 5-lipoxygenase inhibitor with potential clinical applications in the treatment of inflammatory diseases.[1,2] When Zileuton is metabolized, its major conversion is the N-hydroxylated form (Abbott-66193, Figure 1). Although an analytical method exists for the determination of Zileuton and its metabolites in biological fluids,[3] this method requires a lengthy solid phase extraction pretreatment process. A C_{18} stationary phase was their column of choice with a complex mobile phase of acetonitrile-methanol-tetrahydrofuran-water with phosphate buffers. Disposal and use of these toxic solvents poses danger not only to the individual working with them but also to the environment.

Several analytical problems exist in the assay of drugs in physiological fluids.[4] Often the drugs are present in low concentration, are strongly bound to proteins, and are in a complex matrix. Frequently the technique of choice for these types of analyses is high performance liquid chromatography (HPLC). However, due to interference from numerous endogenous compounds in the sample as well as very low analyte concentrations, direct HPLC assay of physiological fluids is usually unfeasible. The high-molecular-mass proteins found in these samples are particularly troublesome since they tend to precipitate within the column leading to rapid loss of chromatographic efficiency.

Micellar liquid chromatography (MLC) with normal micelles is an alternative to conventional reverse phase liquid chromatography (RPLC), which employs aqueous solutions of surfactants above the micellar critical concentration as the mobile phases, instead of hydro-organic mixtures.[5] One of the main advantages of MLC is the possibility of determining drugs in physiological fluids without the need of a separation of the proteins present in the samples.[6] Micellar chromatography is suited for direct injection because

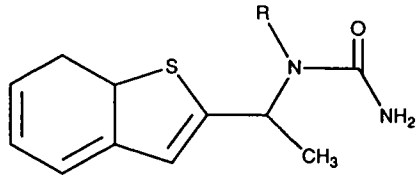

Figure 1: Structures of Zileuton, R = OH and Abbott-66193, R = H

the proteins are solubilized by the micellar aggregates in the mobile phase and are eluted with the void volume.[7]

The purpose of this study was to develop a specific and sensitive procedure for the simultaneous quantification of Zileuton and its N-dehydroxylated metabolite by direct injection of untreated urine sample into a micellar high performance liquid chromatographic system.

MATERIALS AND METHODS

Instrumentation

The chromatography mobile phase was delivered by an Applied Biosystems Model 400 isocratic pump. Samples were injected using a Rheodyne Model 7125 syringe-loading sample injector with a 200-µl loop. Peaks were detected with an Applied Biosystems Model 785A UV programmable detector set at 262 nm. The chromatograms were obtained using a Spectra-Physics chrom-jet integrator. A FlAtron CH-30 column heater controlled by a TC50 controller was used for the high temperature experiments. The column (25 cm long and 4.6 mm i.d.) was packed with 5 micron IB-sil CN-bonded silica (Phenomenex, Torrance, CA). The void volume of the system was calculated by using the peak of injected water. All pH measurements were performed with an Orion Model EA 920 Digital pH meter and an Orion combination glass electrode. The flow rate was set at 1.0 mL/min.

Chemicals and Reagents

2-Propanol, phosphoric acid (85%), sodium dodecylsulphate, SDS, (99%), and sodium phosphate dibasic heptahydrate were purchased from commercial vendors and used without purification. The water used was from a Millipore Milli-Q water purification system. Zileuton and its N-hydroxylated metabolite were gifts from Abbott Laboratories.

A stock solution of sodium dodecylsulphate was prepared in the Milli-Q water at a concentration of 0.2 M. Micellar mobile phases were prepared by adding the appropriate amounts of the stock SDS solution, sodium phosphate dibasic and 2-propanol. The mobile phases were diluted to the volume with the Milli-Q water to obtain different concentrations and adjusted to the appropriate pH with phosphoric acid. The mobile phases were vacuum filtered through a 0.45 μ nylon membrane.

Stock solutions of Zileuton and its metabolite were made by dissolving them in 2-propanol and then diluted to appropriate volume with 0.05 M SDS solution. These solutions were stored in a freezer and were stable for at least 2-weeks. Working solutions were prepared by dilution of the stock solutions.

Preparation of Standard in Rat Urine

Urine samples were obtained from female rats. Standard solutions were prepared by the addition of appropriate volumes of standard solutions to urine samples that had been diluted in half with water. Samples were then frozen and stored in the dark. Under such conditions spiked samples were stable for at least a week. The stability after one week was not investigated.

RESULTS AND DISCUSSION

Studies to determine the adequate composition of the mobile phase (pH, concentration of SDS, and concentration of modifier) for the separation and elution of Zileuton and its N-hydroxylated metabolite in both aqueous solutions and urine matrix were performed.

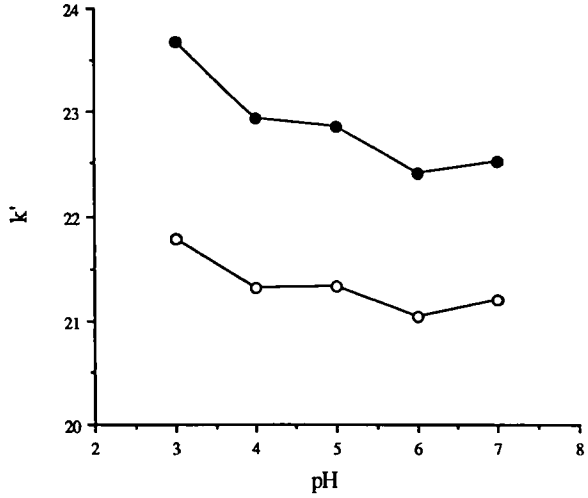

Figure 2: Dependence of capacity factor on pH of the mobile phase with 0.025 M SDS in 0.01 M phosphate buffer at room temperature. (o) = Zileuton and (•) = Abbott-66193.

Separation of Zileuton and Abbott-66193

The effect of pH on the retention of Zileuton and its metabolite was examined in the pH range of 3.00 - 7.00. For Zileuton there was no significant effect on the capacity factor in the region studied (Figure 2). This is expected since the drug is neutral in this region. For Abbott-66193, the capacity factor decreased when the pH of the mobile phase was increased from 3.00 to 5.00 and remained constant at higher pH (Figure 2). In this pH region, the metabolite Abbott-66193 is protonated and therefore will interact with the adsorbed anionic surfactant on the stationary phase, thus shifting the equilibrium from the mobile phase to the stationary phase. The resolution of the solutes decreased as the pH increased from 3.00 to 7.00 (Figure 3).

Figure 4 shows the dependence of the capacity factor on the surfactant concentration in the range of 0.02 - 0.10 M at pH 3.00. Increasing the SDS concentration results in a decrease of the retention times and the capacity factor of the solutes. This is most likely due to an increase in the number of micelles in the mobile phase as the concentration of surfactant increases.[8] When the results were replotted as 1/k' against [SDS], a linear relationship was

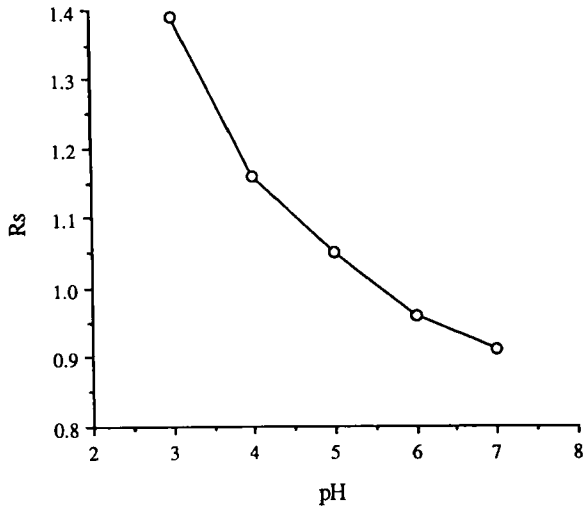

Figure 3: Effect of pH on the resolution of Zileuton and its metabolite. Conditions: 0.025 M SDS containing 0.01 M phosphate buffer.

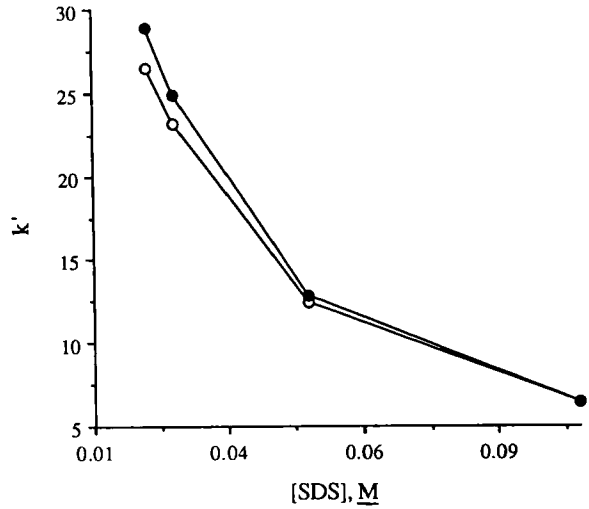

Figure 4: Dependence of capacity on [SDS] of the mobile phase pH 3.00 at room temperature. (o) = Zileuton and (•) = Abbott-66193

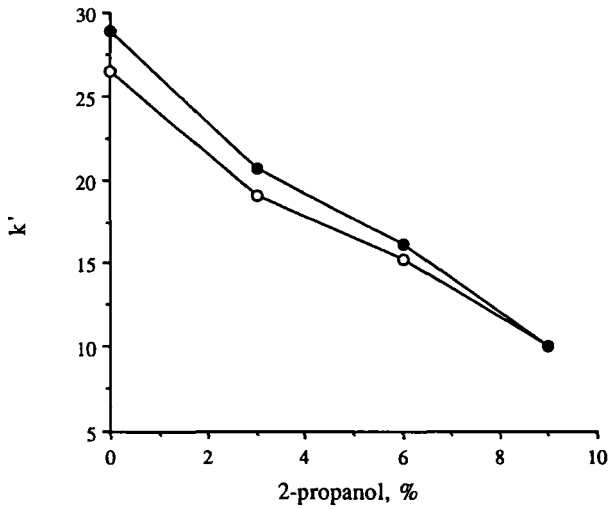

Figure 5: Dependence of capacity factor on the percentage of 2-propanol: 0.025 M SDS at pH 3.00. (o) = Zileuton and (•) = Abbott-66193

obtained. This behavior also is expected when hydrophobic interaction is the predominant factor contributing to the retention time.[9]

When 2-Propanol was added to the mobile phase in an effort to reduce the mass transfer of the solutes exiting out of the micelle and desorping from the column, both the capacity factor and resolution decreased (Figure 5). This may be related to a decrease in the polarity of the mobile phase and therefore a shift in the equilibrium of the lipophillic compounds from the stationary phase to the mobile phase.

In an attempt to improve the column efficiency as reported by other workers,[10] the effect of 2-propanol added to the mobile phase was studied. The number of theoretical plates (N) and the asymmetry ratios (B/A) from the observed peaks were compared. The values of N were calculated from the following equation which corrects for the asymmetry of skewed peaks:[10]

$$N = \frac{41.7\left(t_R / W_{0.1}\right)^2}{B/A + 1.25} \qquad (1)$$

where: t_R is the retention time,
$W_{0.1}$ is the peak width measured at 10% peak height,
B/A is the asymmetry ratio.

The results were shown in Table 1. The presence of 3% 2-propanol slightly increased the plate count and showed no significant effect on the asymmetry of both Zileuton and its metabolite, Abbott-66193.

The effect of column temperature on both the peak asymmetry and column efficiency was also studied. There were small improvements in the column efficiency as the temperature was increased from room temperature to 50°C (Table 2). However, we decided to carry out the remaining investigations at room temperature because of our experience that at higher temperatures, the column life, in particularly CN column, is shortened drastically.

Elution Studies on Urine Matrix

The chromatogram of the urine matrix shows a broad band due to the presence of the proteins at the solvent front and other smaller peaks due to the presence of endogenous compounds (Figure 6a and b). I. Pérez-Martinez et al.[11] indicated that in a purely micellar medium of SDS, the retention of the broad band was not large at pH 6.00 - 7.00 and was slightly affected by the SDS concentration. The retention of this band increased with decreasing pH.

Table 1

Variation of Efficiency and Asymmetry with 2-Propanol Concentration

	Zileuton, A64077		Metabolite, A66193	
2-Propanol, %	**N**	**B/A**	**N**	**B/A**
0	6960	1.02	6170	1.04
3	7167	1.00	6328	1.00
6	6061	1.07	6573	1.15
9		No Separation		

0.025 M SDS containing 0.01 M phosphate buffer and 3% 2-propanol pH 3.00 at 1.0 mL. min^{-1}; room temperature; 262 nm

Table 2

Variation of Efficiency and Assymmetry with Temperature

	Zileuton, A64077		Metabolite, A66193	
Temp,° C	**N**	**B/A**	**N**	**B/A**
Ambient	5095	1.12	5604	1.00
30	5993	1.14	5968	1.06
40	5877	1.25	6094	1.00
50	6269	1.18	5741	1.00

0.025 M SDS containing 0.01 M phosphate buffer and 3% 2-propanol pH 3.00 at 1.0 mL. min^{-1}; room temperature; 262 nm.

However, in order to obtain a satisfactory separation between Zileuton and its metabolite in the urine matrix (see Figure 3), the pH of the mobile phase was fixed at 3.00.

Direct Sample Injection

Based on these studies a mobile phase consisting of a solution containing 0.025M SDS and 3% 2-propanol at a pH of 3.00 were chosen as the optimum conditions for quantitation of Zileuton and its metabolite, Abbott-66193, in untreated urine.

Under these conditions, retention times for Zileuton and its metabolite were: (26.19 ± 0.20 minutes) and (27.71 ± 0.25 minutes) respectively, and all components in the urine sample eluted within 35 minutes (Figure 7). There were no significant changes in the retention times, pressure, or carry over peaks from one injection to another during this study.

The recovery of Zileuton and its metabolite from spiked urine was studied by adding different amount of these species to control (blank) urine samples. The results are shown in Table 3. The average percent recovery for Zileuton was 99.8 ± 3.6 over a 0.50 - 5.00 ppm concentration range and the recovery for Abbott-66193 was 101.1 ± 1.6 over the same concentration range as Zileuton. On the basis of on these results, both compounds can be quantitatively recovered.

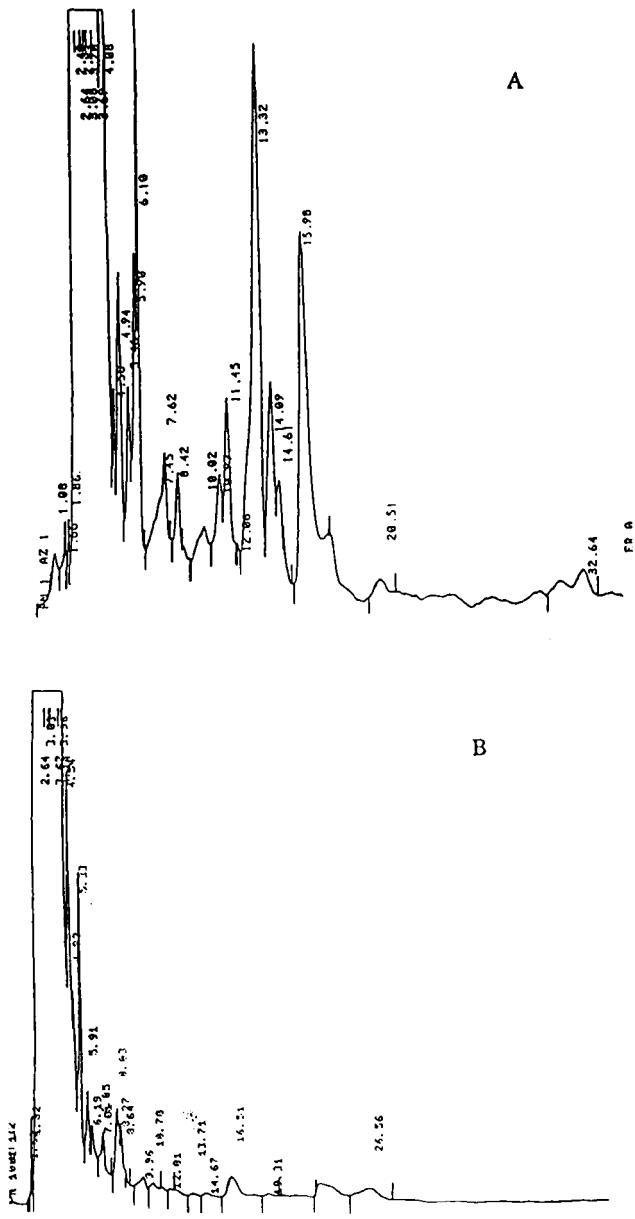

Figure 6: Experimental chromatograms: (a) urine sample (mobile phase 0.025 M SDS- 3% 2-propanol at pH 3.00); (b) urine sample (mobile phase 0.025 M SDS - 3% 2-propanol pH 7.00)

Table 3

Recovery of Zileuton and Abbott-66193 from Urine Samples

	Zileuton		Abbot-66193	
Concentration (ppm)	**% Recovery**	**Average Recovery**	**% Recovery**	**Average Recovery**
0.50	103 101 107	103.7 ± 3.0	104 106 95	101.7 ± 5.8
1.0	93 102 90	95.0 ± 6.2	96 105 95	98.7 ± 5.5
2.0	100 103 98	100.3 ± 2.5	103 102 102	102.3 ± 0.6
5.0	101 101 99	100.3 ± 1.2	100 102 103	101.7 ± 1.5

0.025 M SDS containing 0.01 M phosphate buffer and 3% 2-propanol pH 3.00 at 1.0 mL. min^{-1}; room temperature; 262 nm.

The sensitivity of the method was investigated by preparing calibration plots of 50 μL each of Zileuton and Abbott-66193 in urine. Linear calibration curves of relative peak heights versus concentrations ($r > 0.9999$) were obtained from 0.25 to 5.00 ppm for Zileuton and Abbott-66193.

The typical linear relationship for the calibration curve can be expressed by the following regression equations:

$$\text{Peak Height} = 1850.7\,[\text{Zileuton}] + 234.12 \quad (2)$$

$$\text{Peak Height} = 1844.7\,[\text{Abbott-66193}] + 261.6 \quad (3)$$

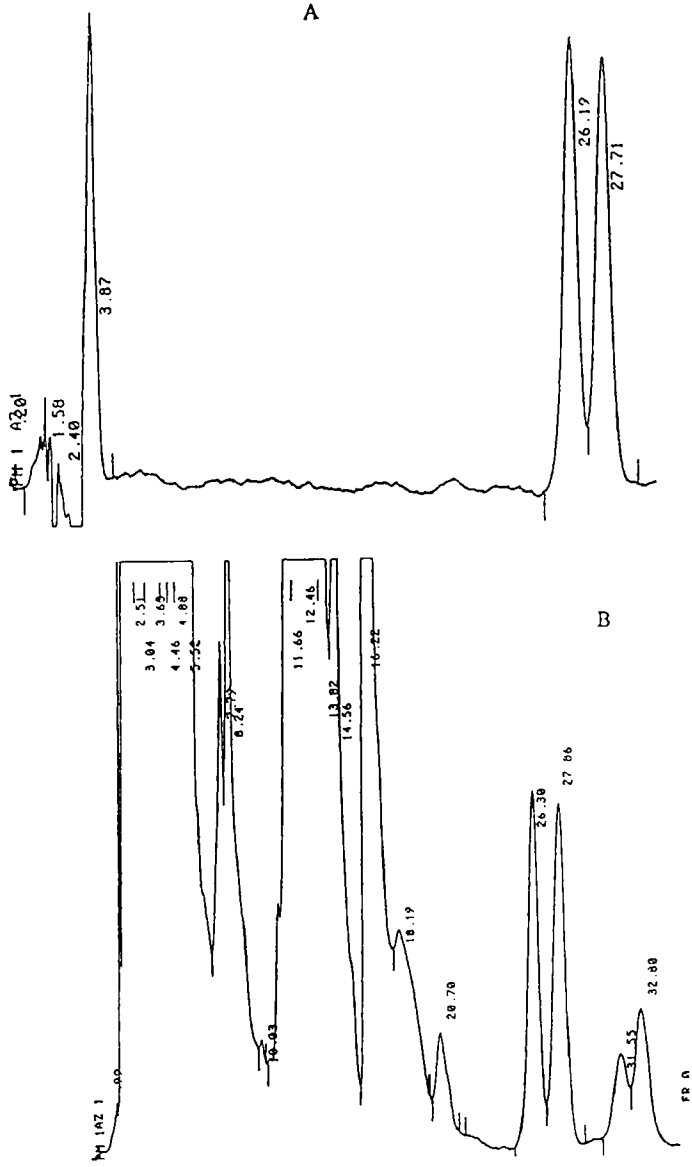

Figure 7: Typical chromatograms of (A) Zileuton and Abbott-66193 in surfactant (B) Zileuton and Abbott-66193 spiked into control (blank) rat urine. Conditions: 0.025 M SDS containing 0.01 M phosphate buffer pH 3.00, and 3% 2-propanol at room temperature.

Table 4

Precision and Accuracy of Concentration Measurements of Zileuton and Abbott-66193 in Rat Urine Samples

	Zileuton			Abbott-66193		
Conc. (ppm)	**Average ± SD**	**CV**	**Er, %**	**Average ± SD**	**CV**	**Er, %**
0.25	0.20 ± 0.02	10	-0.20	0.20 ± 0.01	5.0	-0.20
0.50	0.49 ± 0.03	6.1	-0.02	0.52 ± 0.05	9.6	0.04
1.00	1.11 ± 0.05	4.5	0.11	1.11 ± 0.03	2.7	0.11
2.00	2.48 ± 0.04	1.6	0.24	2.42 ± 0.03	1.2	0.21
5.00	5.20 ± 0.59	11	0.04	4.96 ± 0.07	1.4	-0.01

0.025 M SDS containing 0.01 M phosphate buffer and 3% 2-propanol pH 3.00 at 1.0 mL. min^{-1}; room temperature; 262 nm. Three replicates at each concentration.

The minimum detectable concentrations were 0.1 ppm for Zileuton and 0.08 ppm for Abbott-66193 based on a signal-to-noise ratio of 3 with an injection of 50 µL.

The precision and accuracy of this method was determined by making replicate measurements of five different standards within a concentration range of 0.25 to 5.0 ppm each of Zileuton and Abbott-66193. Very low relative standard deviations with low relative errors were obtained (Table 4).

Analytical Applications

The amount of Zileuton and Abbott-66193 in a pooled 24-hr female rat urine using the optimal conditions was determined. For a 50 µL sample, using the regression equation from the calibration curve and recovery values, the amount of Zileuton and Abbott-66193 was determined to be: 8.87 µg Zileuton / mL and 1.39 µg Abbott-66193/mL. These results are within the expected range from the dose amounts in the recovery studies by Abbott Laboratories.

CONCLUSIONS

The procedure reported allows the determination of Zileuton and its metabolite, Abbott-66193, in urine samples. Owing to the minimum handling time required, large series of samples can be processed without pretreatment. The results also show that the procedure could be a satisfactory choice to determine those compounds in other matrices. In addition, it utilizes a mobile phase which is nontoxic, nonflammable and relatively inexpensive . The sensitivity of the assay described is currently lower than the conventional assay employed by Abbott Laboratories.[3] Further investigations to increase the sensitivity of the assay are underway.

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REFERENCES

1. G. W. Carter, P. R. Young, D. H. Albert, J. Bouska, R. Dyer, R. L. Bell, J. B. Summers, D. W. Brooks, J. Pharmacol. Exp. Ther., **256**, 929 (1991).

2. R. L. Bell, P. R. Young, D. H. Albert, C. Lanni, J. B. Summers, D. W. Brooks, P. Rubin, G. W. Carter, Int. J. Immunopharm., **14**, 505 (1992).

3. R. A. Braeckman, K. A. Erdman, G. R. Granneman, AAPS, Las Vegas, Nov.,1990.

4. M. J. Koenigbauer, M. A. Curtis, J. of Chromatoogr., **427**, 277 (1988).

5. M. J. Medina-Hernandez, M. C. Garcia Alvarez-Coque, Analyst, **117**, 831 (1992).

6. M. J. Koenigbauer, J. Chromatogr., **531**, 79(1990).

7. J. Reynolds, S. J. Albazi, J. of Liqd. Chromatogr., **18(3)**, 537 (1995).

8. P. Yarmchuk, R. Weinberger, R. Hirsch, L. Cline-Love, Anal. Chem., **54**, 2233 (1982).

9. J. Dorsey, M. DeEchegaray, J. Landy, Anal. Chem., **55**, 924 (1983).

10. J. Foley and J. Dorsey, Anal. Chem., **55**, 730(1983).

11. I. Pérez-Martinez, S. Sagrado, M. J. Medina-Hernandez, Anal Chimi Acta, **304**, 195 (1995).

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