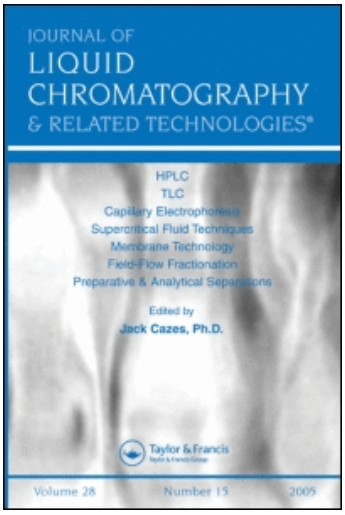

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

DEVELOPMENT OF A DERIVATIZATION METHOD, COUPLED WITH REVERSE PHASE HPLC, FOR MONITORING THE FORMATION OF AN ENOLATE INTERMEDIATE

A. Abrahim[a]; R. Hartman[a]; Z. Ge[a]; B. Mao[a]; J. Marcoux[a]
[a] Merck Research Laboratories, Rahway, NJ, U.S.A.

Online publication date: 05 August 2002

J. LIQ. CHROM. & REL. TECHNOL., 25(7), 1049–1062 (2002)

DEVELOPMENT OF A DERIVATIZATION METHOD, COUPLED WITH REVERSE PHASE HPLC, FOR MONITORING THE FORMATION OF AN ENOLATE INTERMEDIATE

A. Abrahim,* R. Hartman, Z. Ge, B. Mao, and J. Marcoux

Merck Research Laboratories, P. O. Box 2000, Rahway, NJ 07065-0914, USA

ABSTRACT

A sensitive liquid chromatographic method has been developed to monitor the formation of an enolate intermediate in a synthetic route to Etoricoxib, a drug candidate for the treatment of arthritis. The method requires the derivatization of the enolate with methyl iodide to form a stable methylketosulfone derivative followed by reverse phase HPLC analysis. Parameters affecting the derivatization, including the nature of derivatizing agent, reaction solvent, amount of derivatizing agent, reaction time, reaction temperature, and amount of excess base in the reaction were investigated. The derivatization reaction was shown to give selective C-alkylation. The linear range of the chromatographic method for the determination of the starting material, ketosulfone, and the derivative, methylketosulfone, was

*Corresponding author. E-mail: Ahmed_Abrahim@merck.com

determined. Finally, the accuracy of the method was established based on recovery experiments.

INTRODUCTION

A step in a synthetic route to Etoricoxib (COX-2 selective inhibitor), a drug candidate being developed for treatment of arthritis (1), involves the reaction of ketosulfone I with potassium tert-butoxide in THF to form potassium enolate intermediate II (Figure 1) (2). Accurate chromatographic analysis of this reaction is critical to the overall yield and quality of the final drug substance. Therefore, it was essential to develop a sensitive and accurate analytical procedure that can simultaneously determine the level of the starting material, ketosulfone I, and the enolate intermediate II during processing.

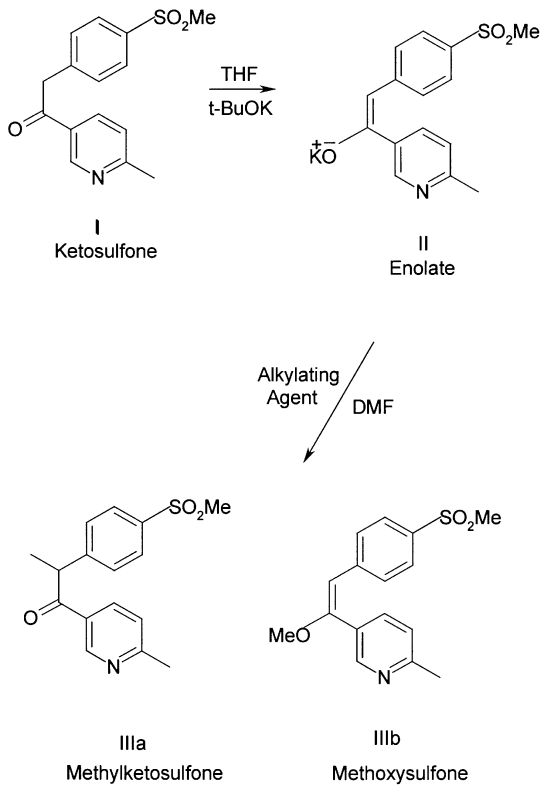

Figure 1. Schematic diagram of enolate derivatization procedure.

Enolates are among the most useful intermediates for carbon−carbon bond formation (3). However, due to their ionic nature and instability in protic media, their chromatographic analysis may be challenging. A direct reverse phase HPLC analysis of the enolate intermediate is not possible due to the reversible nature of the enolate in the presence of a proton donor such as water. The ionic nature of the enolate and its very low solubility in nonpolar solvents makes the use of normal phase HPLC impractical as well. Therefore, in order to make use of these chromatographic techniques, chemical manipulation or derivatization of the enolate intermediate is desirable.

Derivatization procedures in combination with chromatographic separation have been reported for the analysis of enolate (4,5). Among the various derivatization techniques, alkylation with alkyl halides is the most popular procedure for liquid chromatographic analysis (6). While in most of the alkylation reactions the desired product is the main product, the competition between the carbon and oxygen atoms of the enolate anion for the alkylating agent generally reduces the yield and complicates the chromatographic separation (7,8). The reactivity and selectivity of the alkylation reaction depend on the structure of the enolate, the structure of the alkylating agent, and the solvent (9). For example, dialkyl sulfates and trialkyl oxonium salts are much more reactive than alkyl halides, and these hard alkylating agents may yield substantial amounts of O-alkylation products when reacted with metal enolates (10). The nature of the reaction solvent has a tremendous influence on the reactivity and selectivity of the metal enolate (11,12). It was shown that the reactivity of the enolate is particularly high in dipolar aprotic solvents, which favor the formation of solvent-separated ion pairs. Therefore, in order to achieve very selective mono alkylation products, the selection of derivatizing agent and reaction solvent is very crucial.

In this work, we have utilized two alkylating agents for derivatizing the enolate intermediate. Parameters affecting the derivatization, including the nature of derivatizing agent, reaction solvent, amount of derivatizing agent, reaction time, reaction temperature, and amount of excess base in the reaction were investigated. The linear range of the chromatographic method for the determination of ketosulfone I and the derivative III were assessed. Spiking and recovery experiments were performed in order to determine the accuracy of the method.

EXPERIMENTAL

Chemicals and Reagents

Potassium t-butoxide, methyl iodide, dimethylsulfate, tetrahydrofuran, dimethylformamide, dimethylacetamide, and dimethylsulfoxide were purchased

from Aldrich (Milwaukee, WI, USA). HPLC grade acetonitrile was purchased from Fisher Scientific (Pittsburgh, PA).

Synthesis of Enolate Intermediate (II)

Compound I (1.437 g, 4.97 mmol) and 5 mL THF were transferred into a 25 mL flask equipped with nitrogen inlet. The reaction mixture was then put on an ice bath and 5.93 mL (5.16 mmol) of potassium t-butoxide was added while continuously stirring the mixture. After 15 minutes of reaction in the ice bath, the reaction mixture was allowed to react at room temperature for an additional 45 minutes.

Derivatization of Intermediate (II)

In a 25 mL flask containing 1 mL of DMF and excess methyl iodide or dimethylsulfate, a 1 mL slurry of compound II was immediately added and stirred for 10 minutes at room temperature. A 100 μL aliquot of the resulting green solution was then pipetted into a 100 mL volumetric flask and diluted to volume with 50/50 acetonitrile/water. The sample was analyzed by reverse phase HPLC.

LC Conditions

HPLC

The separations were performed using either Thermo Separation Products P4000 solvent delivery system, AS3000 autosampler and UV1000 variable wavelength UV detector, or Agilent HP1100 HPLC System with variable wavelength detector. Chromatographic data were collected and analyzed by the Nelson Turbo*Chrom System. Separations were achieved using a Zorbax, Eclipse XDB C8 (5 μm particles), 250 cm × 4.6 mm i.d. column at room temperature. Injection volume of 10 μL and a flow rate of 1.5 mL/min were used. Initial gradient conditions began at 25% acetonitrile and 75% 10 mM KH_2PO_4 and were held at this composition for 7 minutes then were changed to 80% acetonitrile over 18 minutes, for a total of 25 minutes run time.

LC-MS

The LC-MS experiments were performed with Agilent LC/MSD 1100 series. Separations were achieved using a Zorbax, Eclipse XDB C8 (5 μm

particles), 250 cm × 4.6 mm i.d. column at room temperature. Injection volume of 10 μL and a flow rate of 1.0 mL/min were used. Initial gradient conditions began at 25% acetonitrile and 75% of 0.1% formic acid (adjusted to pH = 5.0 with NH_4OH) and were held at this composition for 7 minutes then were changed to 80% acetonitrile over 18 minutes, for a total of 25 minutes run time. The ESI ionization mode was applied. The source was held at 350°C with nitrogen used as the nebulizer gas at a pressure of 40 psi. Drying gas flow was 13 L/min. The fragmentor voltage was optimized and set to 80 volts for the molecular ion and 200 volts to obtain fragmentation.

NMR Analysis

The proton and ^{13}C NMR spectra were collected on a Bruker-300 MHz NMR system (Bruker Instruments, Billerica, MA, USA) in $CDCl_3$.

RESULTS AND DISCUSSION

Assay Development

Selection of a Derivatizing Agent

The alkylation of enolate intermediate II can produce predominantly C-alkylation or O-alkylation, or a combination of the two, depending on the type of electrophile used, see Figure 1. The competition between C- and O-alkylation can be explained in terms of the Pearson–Klopman principle, which states that hard reagents attack the hard site of the anion and soft reagents attack the soft site (13). In an enolate anion the relatively small electronegative oxygen atom acts as the hard site, while the larger more polarizable carbon atom acts as the soft site. Therefore, two alkylating agents, methyl iodide (soft) and dimethylsulfate (hard), were investigated. Typical chromatograms of the enolate reaction with methyl iodide and dimethylsulfate are shown in Figures 2 and 3, respectively. The reaction of methyl iodide with the enolate intermediate produced one major component (rt = 12.3 minutes, 98.4% by area, peak-1) and a minor component (rt = 14.6 minutes, 1.6% by area, peak-2), see Figure 2 and Table 1. The reaction of dimethylsulfate with the enolate intermediate could not produce complete derivatization even when used in excess (10X). The reaction resulted in 38.8% of peak-1, 44.8% of peak-2, and 16.4% of the starting material, see Figure 3 and Table 1.

The identities of the derivatization products were characterized by LC-MS and proton and ^{13}C NMR. The LC-MS spectra of peak-1 and peak-2 are shown in Figures 4 and 5, respectively. The ionization of both components generated

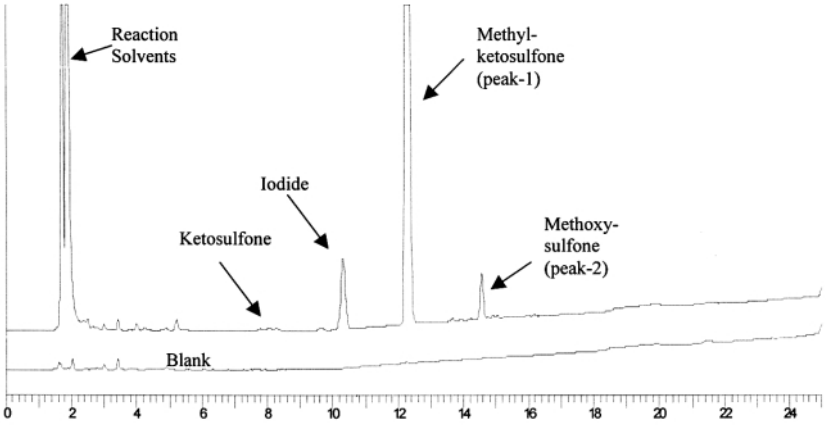

Figure 2. Typical chromatogram of the end of derivatization reaction using methyl iodide.

molecular ions $[M+H]^+$ with m/z of 304.3 for the alkylated product and two fragmentations with m/z of 225.4 and 93.2, corresponding to the cleavage of the sulfone groups, and methylpyridine ring, respectively. As shown in Figure 5, there were three additional fragmentations for peak-2 with the fragmentation of m/z of 289.2 of most significance. This fragment corresponds to the cleavage of the methyl

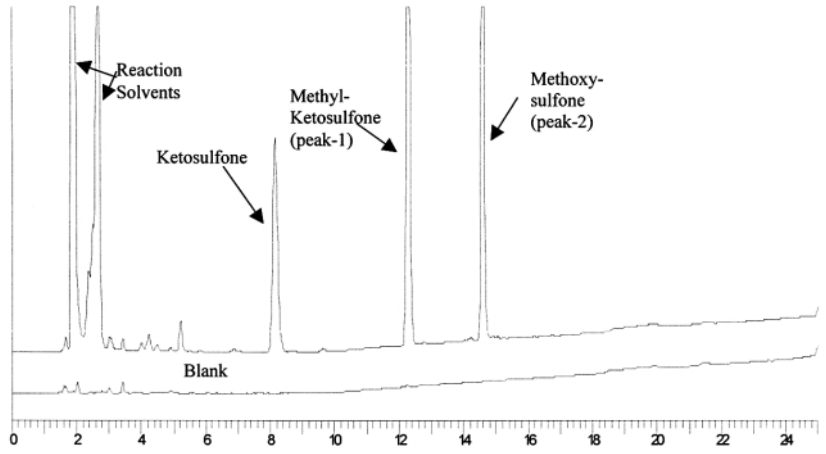

Figure 3. Typical chromatogram of the end of derivatization reaction using dimethylsulfate.

Table 1. The Effect of Derivatizing Agent

Derivatizing Agent	Reaction Time (min)	Area% Ketosulfone	Area% Methyl-ketosulfone	Area% Methoxy-sulfone
Methyl Iodide	10	< 0.02	98.4	1.6
Dimethylsulfate	10	16.4	38.8	44.8

group from the methoxy part of the molecule. The molecular ions and fragmentation patterns suggest that peak-1 is the C-alkylated product, and peak-2 is the O-alkylated product. The proton and ^{13}C NMR characterization of peak-1 also conformed with C-alkylated product (methylketosulfone) (14). Based on these LC-MS data and supporting NMR data for peak-1, we determined the two peaks (peak-1 and 2) to be methylketosulfone (Figure 1, IIIa) and methoxysulfone (Figure 1, IIIb), respectively. Since the reaction of methyl iodide with the enolate produced predominantly C-alkylated product (98%) and minimal side products, methyl iodide was chosen as the alkylating agent for the derivatization reaction.

The Effect of Reaction Solvents

The nature of the solvent has a tremendous influence on the reactivity and orientation of metal enolate alkylations (11,12). The effect could be due to anion solvation through hydrogen bonding in protic solvent and solvation of the cation

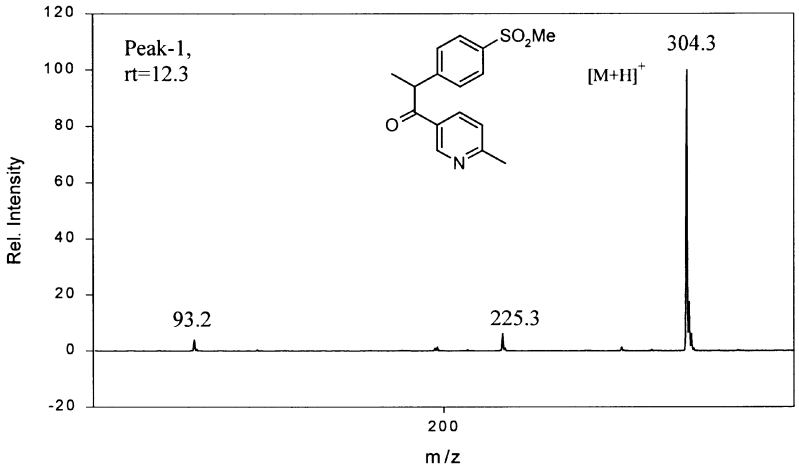

Figure 4. LC-MS spectrum of methylketosulfone.

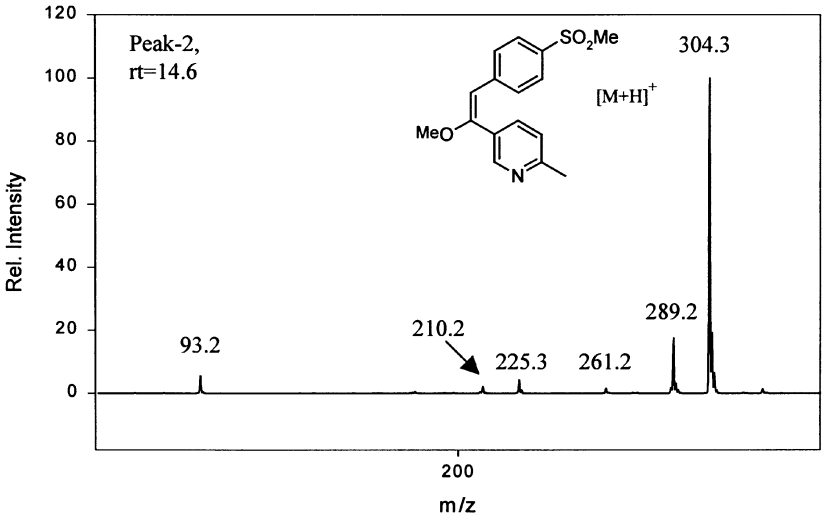

Figure 5. LC-MS spectrum of methoxysulfone.

by bulky aprotic media. Therefore, the effect of solvent on the reactivity and selectivity of the enolate intermediate was assessed by performing the derivatization reaction in polar protic and aprotic solvents. The derivatization reaction was completed within one minute when polar aprotic solvents, such as dimethylformamide (DMF), dimethysulfoxide (DMSO), and dimethylacetamide (DMAC) were used as reaction solvents. No significant changes in the reaction time and the alkylation products (C- versus O-alkylation) were observed when these solvents were used. As expected, in polar protic solvents such as methanol a much slower reaction rate was observed, see Table 2. When protic solvents, such as methanol are present, the enolate anion is stabilized through hydrogen bonding with the solvent molecules. For reactions to occur, these hydrogen bonds must be broken, adding an extra energy barrier (12). Consequently, the reaction of enolate

Table 2. The Effect of Solvent on the Derivatization Reaction

Reaction No.	Solvent	Time (min)	Area% Ketosulfone	Area% Methyl-ketosulfone	Area% Methoxy-sulfone
1	MeOH	60	3.1	96.2	0.6
2	DMF	1	0.1	98.4	0.9
3	DMSO	1	0.1	98.6	0.7
4	DMAC	1	0.2	98.2	0.5

is favored in polar aprotic solvents, such as dimethylformamide and dimethylacetamide.

Optimization of Derivatization Reaction

The Amount of Derivatizing Agent

To optimize the amount of derivatizing agent used for 1 mL ($\approx$ 1.5 mmol) of enolate slurry, different amounts of methyl iodide over a range of 10–300 μL (0.2–4.8 mmol) were examined. As shown in Figure 6, the formation of the derivative (methylketosulfone) increased with the increase in the amount of methyl iodide. The formation of the derivative leveled off at approximately 75 μL of methyl iodide ($\approx$ 1 mole equivalent). However, in order to compensate for reaction variability and ensure complete derivatization of the enolate, 200 μL (excess) of methyl iodide was used for further applications.

The Effect of Reaction Time and Reaction Temperature

The effect of reaction time on the derivatization reaction was assessed at −20°C and 23°C (ambient). As shown in Figure 7, the derivatization reaction

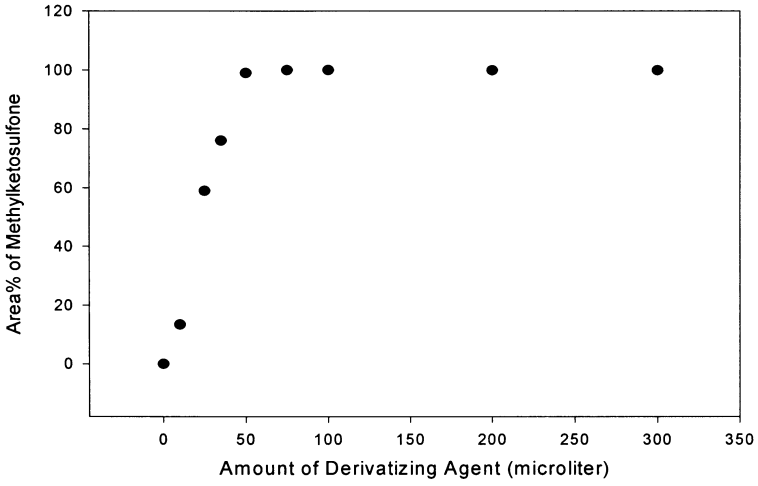

Figure 6. The effect of the amount of derivatizing agent on the formation of methylketosulfone (compound III).

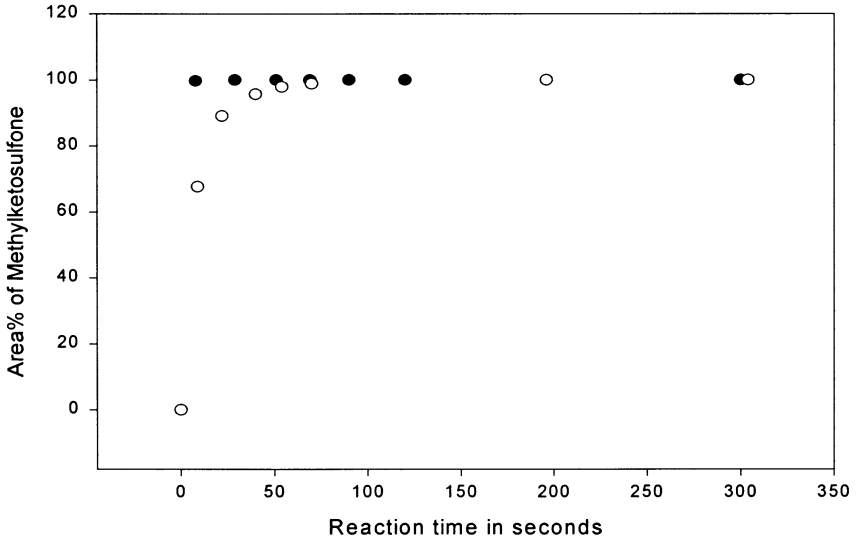

Figure 7. The effect of reaction temperature and reaction time on derivatization reaction. ● Reaction at 23°C (ambient). ○ Reaction at −20°C.

was completed in about three minutes and less than one minute when the reaction was performed at −20°C and ambient temperature, respectively. In order to simplify the derivatization procedure and offset any reaction variability, the reaction time of 5–10 minutes at ambient temperature was implemented.

Method Validation

Linearity of the Chromatographic Method

The linearity of the chromatographic method was determined by making triplicate injections of ketosulfone (compound I) and methylketosulfone (compound III) over the range of 0.02 to 123% of the target concentration (0.2 mg/mL). UV detector response was shown to be linear with coefficient of determination (r^2) of 1.0000 for both ketosulfone and methylketosulfone, respectively, see Figure 8 and Tables 3 and 4.

Based on the minimum signal to noise ratio of 3 : 1, a limit of detection of 0.04 μg/mL was established for the method.

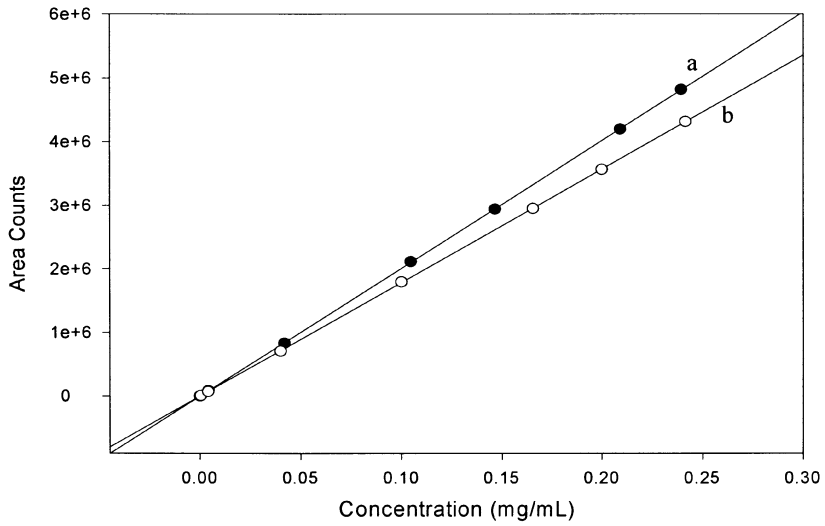

Figure 8. Linearity of detector response for ketosulfone (●) and methylketosulfone (○) at 220 nm.

Recovery Experiment

In order to establish the accuracy of the derivatization method, a series of enolate samples were synthesized by intentionally undercharging potassium tert-butoxide. The undercharge of base ranged 2–9% by weight. The samples were then derivatized with methyl iodide and analyzed by reverse phase HPLC. The

Table 3. Linearity of UV Detector Response for Ketosulfone

Concentration (mg/mL)	Average Area Counts	Response Factor	% RSD
0.00004182	969	23170732	6.0
0.00008364	1802	21544715	0.5
0.0004182	8564	20478240	0.6
0.004182	84307	20159493	0.1
0.04182	832173	19898924	0.2
0.10455	2112789	20208407	0.1
0.1466	2941753	20066528	0.0
0.2091	4195864	20066303	0.0
0.2394	4817493	20123195	0.3

Table 4. Linearity of UV Detector Response for Methylketosulfone

Concentration (mg/mL)	Average Area Counts	Response Factor	% RSD
0.00003998	808	20210105	8.1
0.00007996	1509	18871936	6.2
0.0003998	7189	17981491	1.0
0.003998	71658	17923462	0.1
0.03998	708186	17713507	0.2
0.09995	1796450	17973487	0.1
0.1655	2950746	17829281	0.0
0.1999	3564871	17833272	0.1
0.2415	4314298	17864588	0.3

observed and expected area % (corrected for response factor) of unreacted ketosulfone in the samples and the corresponding % recovery are shown in Table 5. The percent recovery is between 81 to 112%. The method was judged to be accurate based on this recovery experiment.

Applications

During a process development of Etoricoxib, potassium tert-butoxide was determined to be one of the reaction parameters that has an impact on the quality of the drug substance. An undercharge of this base leads to incomplete enolization of ketosulfone, which could substantially affect the overall yield of the drug substance. An overcharge of the base generates undesirable impurities in the drug substance. Therefore, it is critical to have a method that can simultaneously detect both the undercharge and overcharge of potassium tert-

Table 5. Recovery

Reaction No.	Molar Ratio of Ketosulfone to t-BuOK	Expected Ketosulfone *Area%	Observed Ketosulfone *Area%	% Recovery
1	1.00 : 1.00	< 0.02	1.50	
2	1.00 : 0.98	1.89	1.53 (3.03 − 1.50)	81
3	1.00 : 0.95	4.97	5.59 (7.09 − 1.50)	112
4	1.00 : 0.91	8.92	8.13 (9.63 − 1.50)	91

*corrected for response factor.

Table 6. The Effect of Excess Potassium Tert-Butoxide on the Derivatization Reaction

Mole Equivalent Ketosulfone : t-BuOK	Area% Methylketosulfone	Area% Methoxysulfone
1.00 : 1.04	98.4	1.6
1.00 : 1.20	86.7	13.0
1.00 : 1.50	68.5	31.5
1.00 : 2.00	40.7	59.3

butoxide at the end of enolate reaction. In the validation section of this report, it was demonstrated that the proposed method was accurate enough to determine the undercharge of potassium tert-butoxide. An attempt was made to utilize the same method for detecting the overcharge as well. Normally, 1.04 equivalent of potassium tert-butoxide is required for complete enolization of ketosulfone (I).

The derivatization of this sample resulted in 98.4% C-alkylated (methylketosulfone) and 1.6% O-alkylated (methoxysulfone) products, see Table 6. The effect of excess base on the derivatization reaction was investigated. Several enolate samples were synthesized by varying the amount of potassium tert-butoxide ranging from 1.2 to 2.0 mole equivalent. Subsequently, the samples were then derivatized with methyl iodide. As shown in Table 6, the selectivity (C- or O-alkylation) was drastically reduced as the amount of base increased in the reaction. When the amount of base was increased to 1.2, 1.5, and 2.0 mole equivalent, the O-alkylated derivative levels increased to 13.0, 31.5, and 59.3%, respectively. The increase in the O- versus C-alkylation ratio is a clear indication for an overcharge of potassium tert-butoxide. This is an important indication for the process, because corrective action could be taken prior to proceeding to the next step of the process.

CONCLUSIONS

A very fast and selective derivatization method coupled with reversed phase HPLC has been developed and optimized for the determination of an enolate intermediate. The derivatization of the enolate with methyl iodide was shown to give selective C-alkylation. The ratio of O/C-alkylation, however, increased drastically as the amount of base in the enolate reaction was increased. The increase in the ratio of O/C-alkylation could be used as an indicator for the overcharge of potassium tert-butoxide in the reaction. Therefore, the proposed derivatization method could simultaneously be used for monitoring the formation of enolate intermediate, as well as to detect excess base in the enolate reaction.

ACKNOWLEDGMENT

The authors would like to thank Dr. Richard Thompson for his valuable suggestions on this work.

REFERENCES

1. Friesen, R.W.; Brideau, C.; Chan, C.C.; Charleson, S.; Deschenes, D.; Dube, D.; Ethier, D.; Fortin, R.; Gauthier, J.Y.; Girard, Y.; Gordon, R.; Greig, G.M.; Riendeau, D.; Savoie, C.; Wang, Z.; Wong, E.; Visco, D.; Xu, J.L.; Young, R.N. Bioorg. Med. Lett. **1998**, *8*, 2777.
2. Davies, I.W.; Marcoux, J.-F.; Corley, E.G.; Journet, M.; Palucki, D.-W.M.; Wu, J.; Larsen, R.D.; Rossen, K.; Pye, P.; DiMichele, L.; Dormer, P.; Reider, P. J. Org. Chem. **2000**, *65*, 8415.
3. Larock, R.C. *Comprehensive Organic Transformation*; V.C.H. Publishers: New York, 1989; 737–784; d'Angelo, J. Tetrahedron **1976**, *32*, 2979.
4. Hulshoff, A.; Forch, A.D. J. Chromatogr. **1981**, *220*, 275.
5. Wells, R.J. J. Chromatogr. A. **1999**, *843*, 1.
6. Lingeman, H.; Underberg, W.J.M. *Detection-Oriented Derivatization Techniques in Liquid Chromatography*, Chromatogr. Sci. Series; Marcel Dekker, Inc.: New York, 1990; Vol. 48, 1–46.
7. Bates, R.B.; Taylor, S.R. J. Org. Chem. **1993**, *58*, 4469.
8. Augustine, R.L. Tech. Appl. Org. Synth. **1979**, *1*, 86–329.
9. Pearson, R.G. J. Chem. Educ. **1968**, *45*, 581.
10. Heiszwolf, G.J.; Koosterziel, H. Rec. Trav. Chim. **1970**, *89*, 1153.
11. Zaugg, H.E.; Ratajczyk, J.F.; Leonard, J.E.; Schaeffer, A.D. J. Org. Chem. **1972**, *37*, 2249.
12. Zook, H.D.; Russo, T.J.; Ferrand, E.F.; Stotz, D.S. J. Org. Chem. **1968**, *33*, 2222.
13. Klopman, G. J. Amer. Chem. Soc. **1968**, *90*, 223.
14. NMR Characterization of Methylketosulfone: ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.27 (bs, 1H), 8.05 (dd, $J = 8$, 2 Hz, 1H), 7.83 (d, $J = 8$ Hz, 2H), 7.44 (d, $J = 8$ Hz, 2H), 7.17 (d, $J = 8$ Hz, 1H), 4.70 (q, $J = 7$ Hz, 1H), 2.97 (s, 3H), 2.53 (s, 3H), 1.52 (d, $J = 7$ Hz, 3H); ^{13}C NMR (75 MHz $CDCl_3$) δ 197.9, 163.6, 149.7, 147.0, 139.5, 136.4, 128.9, 128.8, 128.2, 123.4, 47.9, 44.4, 24.7, 19.1.

Received December 8, 2001
Accepted December 11, 2001
Manuscript 5697