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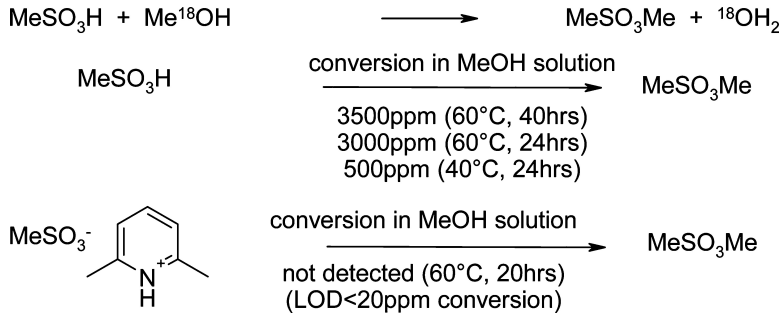

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Mechanism and Processing Parameters Affecting the Formation of Methyl Methanesulfonate from Methanol and Methanesulfonic Acid: An Illustrative Example for Sulfonate Ester Impurity Formation

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Abstract:

Sulfonate salts offer useful modification of physicochemical properties of active pharmaceutical ingredients (APIs) containing basic groups, but there are regulatory concerns over the presence of sulfonate esters as potential genotoxic impurities (PGIs). Whilst sulfonate esters could theoretically result from interaction between sulfonic acids and alcohols, literature on their formation is sparse. GC−MS analysis of reactions of methanesulfonic acid (MSA) and isotopically labeled methanol (^{18}O-label) confirm methanol C−O bond cleavage in the formation of the methyl methanesulfonate (MMS), consistent with reversal of well-established mechanisms for solvolysis of sulfonate esters. Studies of reaction profiles quantify methyl methanesulfonate formation under a range of conditions relevant to API processing. Maximum conversion to MMS in reaction mixtures was 0.35%, determined by analytical methods developed specifically for reaction mixture analysis. Sulfonate ester formation is dramatically reduced at lower temperatures, in the presence of small amounts of water, or when acid is partially neutralized by substoichiometric amounts of the weak base, 2,6-lutidine, used to mimic conversion of a basic API to a salt in pharmaceutical manufacture. In the presence of a slight excess of base, ester formation was not detected. These findings, particularly those involving an excess of base, are compelling and provide a scientific understanding to allow for the design of processing conditions to minimize and control sulfonate ester formation.

Introduction

Sulfonic acids are widely used for salt formation during the synthesis and production of drug substances. Sulfonic acids can react with low molecular weight alcohols such as methanol, ethanol, or isopropanol to form the corresponding sulfonate esters. These sulfonate esters have a demonstrated potential for genotoxicity, and therefore their potential presence in trace levels in active pharmaceutical ingredients (APIs) has recently raised concerns.[1,2] Such alcohols are commonly used as solvents during salt formation and in earlier steps of drug synthesis.

Whilst there is much literature on the solvolytic instability of sulfonate esters,[3−7] there is little information in the literature on the extent of their formation from these alcohols and sulfonic acids or potentially from sulfonate salts.[8] Synthetically useful yields of sulfonate esters from the relevant sulfonic acids have been reported under forcing conditions employing orthoformates[9] or orthoacetates,[10] but such sulfonate esters are normally prepared using strategies involving alternative sulfonate precursors, e.g. sulfonyl chlorides.

Given the paucity of literature on the formation of sulfonate esters from these alcohol/sulfonic acid systems, and the importance of the quantities formed from a product safety perspective, we endeavored to elucidate and understand the extent to which these substances may be formed under conditions that mimic the preparation of salts of APIs. To facilitate

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□ GlaxoSmithKline, U.K.
● GlaxoSmithKline, U.S.A.
△ Wyeth Research.
▼ F. Hoffmann-La Roche Ltd.

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(2) Coordination Group for Mutual Recognition-Human committee (CMDh), Request to Assess the Risk of Occurrence of Contamination with Mesilate Esters and Other Related Compounds in Pharmaceutical, EMEA/CMDh/ 98694/2008, London, February 27, 2008.
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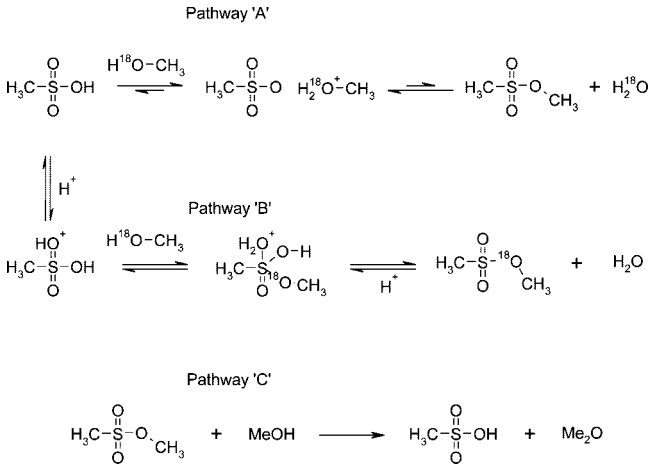

Figure 1

greater understanding of these systems, a number of example systems have been investigated. In this paper, the results of a study of the direct formation of methyl methanesulfonate (methyl mesylate, MMS) from methanesulfonic acid (MSA) and methanol are presented. Since sulfonic acids share common characteristics of extremely high acidity and low nucleophilicity, understanding of this model system is anticipated to be applicable to formation of sulfonate esters more generally.

Two mechanistic pathways for sulfonate ester formation are shown in Figure 1, using MSA and methanol as an example. Pathway A describes nucleophilic attack of sulfonate anion on protonated alcohol, to give sulfonate ester and water, with nucleophilic attack of water at carbon being the reverse reaction. Pathway B draws analogy from the $A_{AC}2$ mechanism for reversible acid-catalyzed formation of carboxylic esters. These pathways would be distinguished through a study of the fate of an oxygen-labeled alcohol. Pathway C represents further decomposition of sulfonate ester (irrespective of route of formation) through alcoholysis to generate sulfonic acid and an ether. This reaction, in conjunction with the reversible mechanisms for ester formation, will limit the extent of ester formation. Profiling MMS formation in appropriate reaction mixtures would provide understanding of the sensitivity of the dynamics of ester formation to process factors applicable to API salt formation. These include concentration, processing time, temperature, and solvent composition, particularly the presence of water. Furthermore, profiling in the presence of an organic model base (2,6-lutidine) would extend this knowledge to scenarios actually reflecting those present when making an API salt.

Experimental Section

Reagents. The following chemicals were used as supplied: methanesulfonic acid (MSA) from Sigma-Aldrich (Steinheim, Germany, ref 47,135-6), methanol (MeOH) from Biosolve (Valkenswaard, The Netherlands, ref 13680602), ^{18}O-labeled methanol (^{18}O-MeOH) from Isotec (Isotec Inc., Miamiburg, OH, U.S.A., ref 609889-19), and 2,6-lutidine (ReagentPlus grade, 98%) from Sigma-Aldrich (ref L390-0).

Isotopic Studies. Methanesulfonic acid and methanol (1:10 v/v) were placed in 2 mL analysis vials, and the vials were sealed with crimp-top closures. Reaction mixtures were made using either methanol or ^{18}O-labeled methanol.

GC−MS Analysis. For direct GC analysis using liquid injection, MSA (10 μL) was mixed with methanol (100 μL) in a 2 mL GC vial with a 200 μL glass insert. The vials were sealed with crimp-top closures and placed for 2 h at 78 °C, and then samples (1 μL) were analyzed by GC−MS. These analyses were performed on an Agilent 6890GC-5973MSD system (Agilent Technologies, Wilmington, DE, U.S.A.). Injection (1 μL) was performed in split mode (1/50 split ratio) using a split/splitless inlet at 250 °C. Separations were achieved on a 60 m × 0.25 mm i.d. × 1.4 μm df DB-VRX column (Agilent Technologies). The carrier gas was helium at 2.4 mL/min constant flow rate. The column was temperature programmed from 60 °C (1 min hold) at 10 °C/min to 200 °C and at 30 °C/min to 250 °C (1.33 min hold). Detection was performed in scan mode (scan range: 10−300 *m*/*z*) with a zero minute solvent delay.

In addition, analyses of reaction mixtures were performed using static headspace (SHS) injection in combination with GC−MS.

For headspace analysis, the same GC−MS system was used. SHS was performed using an MPS2 sampler (Gerstel GmbH, Mulheim, Germany) in headspace mode. MSA (10 μL) was mixed with methanol (100 μL) in a 20 mL headspace vial. The vial was sealed and placed at 78 °C for 2 h immediately prior to analysis. Additional static headspace equilibration was performed at 105 °C during 15 min, while shaking the vial at 600 rpm. Injection of 1 mL of headspace gas was performed using a heated (110 °C) gastight syringe (2.5 mL) in split mode (1/10 split ratio) at 250 °C (split/splitless inlet temperature). Separation was performed on a 60 m × 0.25 mm i.d. × 1.4 μm df DB-VRX column (Agilent Technologies) using the same analytical conditions as previously described.

Reaction Profiling. A methanolic solution of methanesulfonic acid was prepared (100 μL MSA/mL solution, ca. 1 M). Water contents were determined by Karl Fischer titration. Samples (1 mL) were sealed in 2 mL crimp-top vials, and incubated at constant temperature in a circulator-controlled block. For each time point, a fresh vial was sampled for determination of sulfonate ester content, by methods developed specifically for these reaction matrices.[11] This entailed addition of a known amount of d_3-methyl methanesulfonate as internal standard, derivatisation with pentafluorophenylthiolate, and analysis by headspace GC−MS.

This methodology was used for reaction profiling in the presence of added water, or in the presence of added 2,6-lutidine. The reverse reactions (involving solvolysis) were carried out in a similar manner, starting with solutions of methyl methanesulfonate (7 mM) in methanol, containing methanesulfonic acid, 2,6-lutidine, or water as appropriate.

Results

Pathways A and B can be distinguished by studying the fate of the oxygen-label when the reaction is carried out using ^{18}O-methanol. Two reaction mixtures (MSA/methanol and MSA/

(11) Jacq, K.; Delaney, E.; Teasdale, A.; Eyley, S.; Taylor-Worth, K.; Lipczynski, A.; Reif, D. D.; Elder, D. P.; Facchine, K. L.; Golec, S.; Schulte-Oestrich, R.; Sandra, P.; David, F. J. *Pharm. Biomed. Anal.* **2008**, In press. 10.1016/j.jpba.2008.09.028.

^{18}O-methanol, heated at 78 °C for two hours) were analysed by GC−MS using direct liquid injection. Peaks corresponding to excess methanol, water and MMS were detected. Mass spectra confirmed the peak assignments. The positive EI mass spectrum of methyl methanesulfonate (MMS) showed the molecular ion at *m/z* 110, and the fragments at M-1 (*m/z* 109), M-15 (M-CH_3, *m/z* 95) and M-31 (M-OCH_3, *m/z* 79) were also detected. The most abundant ion observed (*m/z* 80) corresponds to the SO_3^+ ion. The chromatogram obtained for the reaction mixture prepared using the isotopically labeled methanol was identical to the chromatogram obtained for reaction mixture prepared using unlabeled methanol. The same peaks were detected, with the largest peak now identified as unreacted (excess) ^{18}*O*-methanol. The mass spectrum of the MMS formed in this reaction was identical to the spectrum from the unlabeled reaction, demonstrating that the ^{18}O atom is not incorporated into the methyl methanesulfonate molecule, supporting ester formation via Pathway A.

Dimethyl ether was detected by both direct injection and by static headspace analysis (SHS), eluting just before methanol in the GC analysis. Structural assignment was indicated by the mass spectrum, which showed the molecular ion at *m/z* 46, and M-1 at *m/z* 45. An abundant ion at *m/z* 29, corresponding to CHO^+ was also present. The mass spectrum of the dimethyl ether formed from reaction in ^{18}O-labeled methanol clearly showed that the major ions in the mass spectrum had now shifted by 2 mass units, indicating that the ^{18}O atom is incorporated in the ether. Ion chromatograms for both reaction mixtures were extracted at *m/z* 18 (water) and *m/z* 20 ($^{18}OH_2$) obtained for both reaction mixtures. Overlays showed clearly that the peak in the ion trace at *m/z* 20 was only present in the labeled reaction, and was not detected in the unlabeled reaction. These experiments do not distinguish between ether formation via solvolysis of the sulfonate ester and acid-catalyzed decomposition of the alcohol.

Applying the principles of microscopic reversibility, the forward reaction of sulfonate ester formation deduced from these labeling experiments is in accord with well-established mechanistic pathways for sulfonate ester solvolyses (cleavage of the carbon−oxygen bond) and demonstrates a sound basis for understanding the balance between sulfonate ester formation and its decomposition by solvolytic pathways.

The dynamics of sulfonate ester formation were monitored using highly sensitive and specific methods developed for this purpose[11] derived from methodologies for determination of alkylating agents in APIs.[12] As water is an important component in this reaction mechanism, Karl Fischer determinations were carried out on initial reaction mixtures, providing experimental values for water content to facilitate improved reaction understanding and characterization.

The formation of MMS in methanol solutions of MSA was initially studied in the temperature range between 40 and 60 °C, a range within the capabilities of the assay, and that encompasses common upper temperatures for API salt crystallizations from methanol. Profiles were determined over the course of up to 60 h, a reaction period longer than typical API processing times. The results are shown in Figure 2. The duplicate experiments at 50 °C show the excellent reproducibility of the derivatisation and analytical methodologies. As anticipated, the molar conversion to MMS was very low (approximately 0.35% at the highest temperature after 50 h), and the extent of sulfonate ester formation was significantly reduced at lower temperatures. The slowing of ester formation results from the balance of the forward reaction (formation) and the reverse reactions (hydrolysis and solvolysis) under the reaction conditions described.

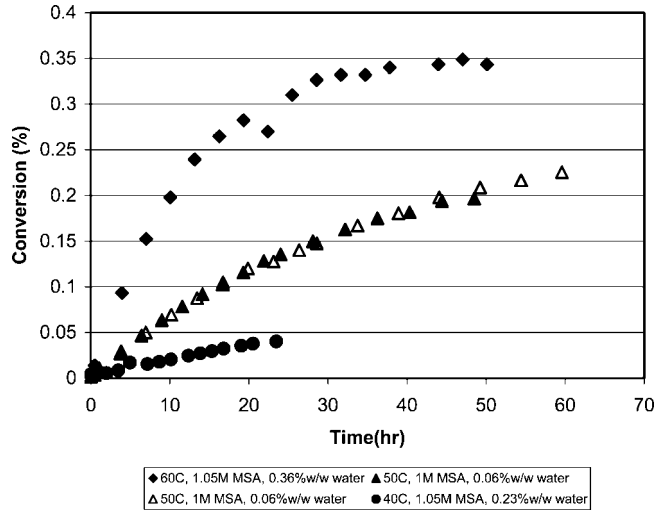

Figure 2. **Formation of methyl methanesulfonate from methanesulfonic acid in methanol, as a function of temperature.**

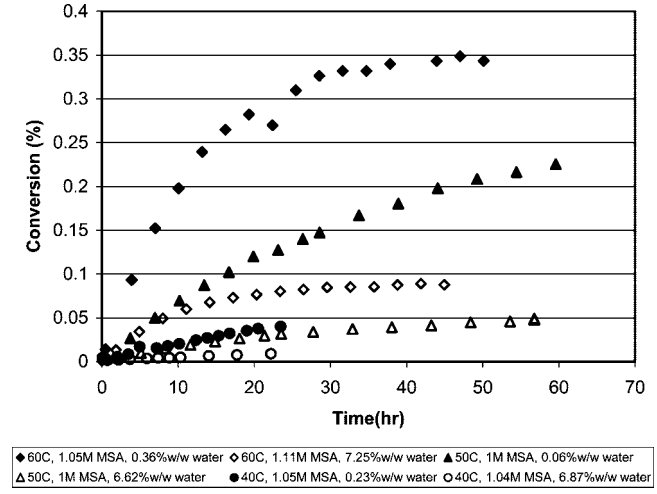

Figure 3. **Effect of water on the formation of methyl methanesulfonate from methanesulfonic acid in methanol, as a function of temperature.**

As aqueous alcohols are common solvent systems for API formations and crystallizations, the effects of water content on the dynamics of formation of MMS formation were also studied. Corresponding reaction profiles in the presence of added water are shown in Figure 3, where open data points denote experiments with added water. The water content in each reaction mixture was measured by Karl Fischer titration and expressed as % w/w. The presence of water at levels of about 7% w/w reduced the levels of MMS to approximately one-third, to below 1000 ppm molar conversion at 60 °C.

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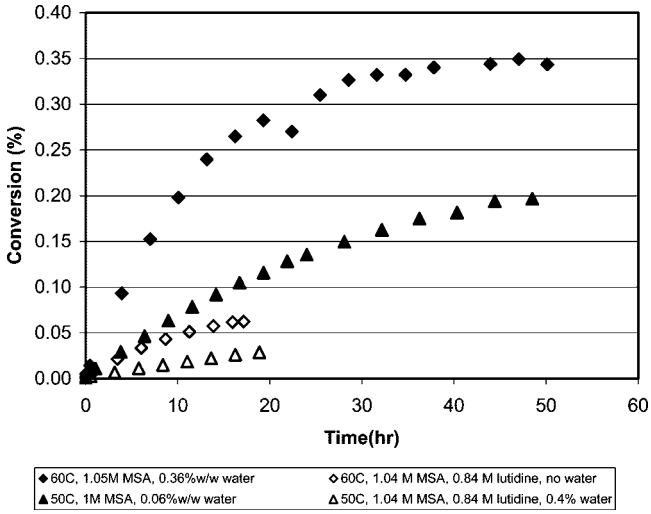

Figure 4. **Effect of partial neutralisation of methanesulfonic acid on the formation of methyl methanesulfonate from the acid in methanol, as a function of temperature.**

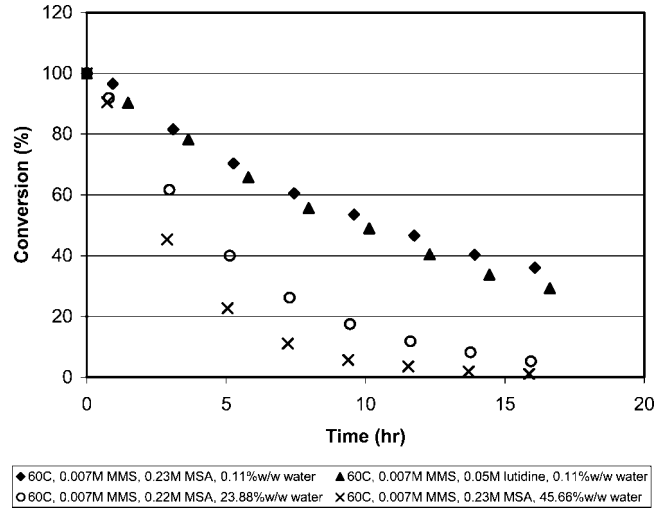

Figure 5. **Solvolysis of methyl methanesulfonate in methanolic solution.**

These results indicate that some sulfonate ester formation does occur under these strongly acidic conditions, approximately 1 M MSA in methanol. However, salt formations using sulfonic acid counterions often employ only small excesses of acid, leading to correspondingly low excesses of proton over the sulfonate anion present.

The formation of MMS under conditions more relevant to a salt formation (i.e., with added base) was tested using the weak base 2,6-lutidine (pK_a 6.76 in MeOH[16]). This base was selected because the conjugate acid would have a lower pK_a (hence give a slightly more acidic solution) than most API salts. In addition, the resultant methanesulfonate salt has sufficient solubility in methanol to provide homogeneous reaction mixtures at the high (ca. 1 M) concentrations used in the MSA reactions.

Experiments were performed to determine MMS formation using a slight molar excess of base (ca. 0.08 equiv) over MSA at temperatures of 40, 50, 60, and 70 °C over 20 h. In all samples, NO formation of MMS could be detected above background levels observed in blank samples. At the concentrations of sulfonate ion present, the method would readily have detected molar conversions of approximately 20 ppm.

In the presence of a significant excess of MSA (MSA:base 1:0.8, corresponding to salt formation using 25% excess acid, appreciably greater than typically employed in salt formation processes), MMS formation could be profiled. Comparative data are shown in Figure 4, where open data points denote lower proton concentrations. In the 20-h period studied, the molar conversion to the sulfonate ester amounted to ca. 0.06% at the highest temperature (compared with levels of 0.26% in the absence of base at a similar time-point).

With acidic conditions appearing necessary for sulfonate ester formation to be observed, it was of interest to determine whether acids weaker than MSA, for example orthophosphoric acid, might catalyse ester formation. Experiments using the methanesulfonate salt of 2,6-lutidine (1 M solution in methanol) were performed in the temperature range 40−70 °C in the presence of 0.66 M orthophosphoric acid. No formation of sulfonate ester could be detected above background levels.

As the production of the sulfonate ester is a balance of the rates of its formation and decomposition, the observed reductions in net formation could be due to decreased formation rate, increased decomposition rates, or a combination of these. The methodology developed for study of sulfonate ester formation was equally applicable to study of solvolytic reactions. Representative effects of added 2,6-lutidine, MSA, and water on MMS solvolysis in methanol were assessed at 60 °C. The observed rates of solvolysis were very similar (Figure 5) in the presence of either MSA or 2,6-lutidine, but increased with increasing water content.

Discussion

Applying the principles of microscopic reversibility, the forward reaction of sulfonate ester formation deduced from the labeling experiments is in accord with well-established mechanistic pathways for sulfonate ester solvolyses *via* cleavage of the carbon−oxygen bond.[5,6] In contrast, esters of carboxylic acids are normally formed and solvolysed through acyl-oxygen bond cleavage.[13,14] The differences in mechanistic pathways are well illustrated in a recent paper showing how, through selective manipulation of pH, selective decomposition of a sulfonate ester in the presence of a carboxylate ester could be accomplished.[15] With the analogy to carboxylate esters disproved, this provides a sound basis for understanding the balance between sulfonate ester formation and its limitation by solvolytic pathways. As methanesulfonic acid is a strong acid, it will typically be significantly ionized in methanolic solutions to form the mesylate anion and a methanolium cation, either as separate ions or as ion pairs, and the presence of excess sulfonic acid leads to sulfonate ester formation. However, the nondetection of ester in reactions using slight excesses of 2,6-lutine or in the presence of phosphoric acid indicates that acids comparable in strength to that of the 2,6-lutidinium ion or phosphoric acid do

(13) Smith, M. B.; March, J. *March's Advanced Organic Chemistry*; Wiley: New York, 2001; p 424.
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not catalyze a meaningful rate of ester formation at this level of detection. These data therefore provide some quantitative refinement to a qualitative discussion of the likelihood of sulfonate ester formation.[8]

The overall rates of sulfonate ester formation, and hence amounts of sulfonate ester formed, have been shown to be reduced in the presence of water. This can be rationalized by the competing solvation of proton by water compared with methanol thereby reducing the rate of the forward reaction, and by enhanced rate of hydrolysis of methyl methanesulfonate, as shown by the solvolysis data. The solvolytic data suggests that the solvolysis may be neither acid-catalyzed nor base-induced at the concentrations studied, (or that, by chance, the effects are equal under the conditions studied).

These experimental data relate directly to the formation of MMS ester in reaction mixtures, and not to isolated salts. They therefore give guidance on upper limits anticipated for this sulfonate ester prior to API salt isolation. Crystallization processes to isolate API sulfonate salts upgrade purity through rejection of impurities from the growing crystals. Consequently, these experimental data also provide estimates of the upper limits for sulfonate ester expected in crystallization liquors from API salt formation processes. This understanding can serve as the basis for planning experiments to demonstrate the efficiencies of discrimination against sulfonate esters during isolation procedures for particular APIs. Selection of reaction conditions to minimize ester formation and purification during isolation can ensure the development of robust processes that will provide material to meet API quality attributes relating to PGIs.

Conclusions

Evidence for the mechanism of formation of methyl methanesulfonate from methanesulfonic acid and methanol was attained. Studies of reaction profiles have quantified the levels of sulfonate ester formed under conditions relevant to the formation of methanesulfonate salts of pharmaceutically active bases. These studies demonstrate a clear scope to select conditions for the preparation of sulfonate salts in alcoholic solutions to minimize formation of sulfonate esters in reaction mixtures relevant to API salt formation, by the following:

- reducing time−temperature envelopes for solutions of sulfonic acids in alcohols
- incorporation of water into the process
- reducing or eliminating the excesses of sulfonic acid used in API salt formation

Of these, the most significant finding relates to the control that can be achieved through the stoichiometric level of acid used. When a slight excess of base is present, there is no discernible reaction rate to form the sulfonate ester and no mechanistic pathway to their formation.

An extended evaluation of the formation of other pharmaceutically relevant sulfonate esters from representative alcohols and sulfonic acids will be discussed in a future publication, which will also address the observed kinetics in greater detail.

Acknowledgment

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Supporting Information Available

GC−MS analyses of reactions of methanesulfonic acid (MSA) with methanol to form methyl methanesulfonate (MMS). This material is available free of charge via the Internet at http://pubs.acs.org.

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