JPP
Journal of
Pharmacy and Pharmacology

JPP 2009, 61: 269–278

Received July 25, 2008
Accepted December 9, 2008
DOI 10.1211/jpp/61.03.0001
ISSN 0022-3573

Review Article

Drug substances presented as sulfonic acid salts: overview of utility, safety and regulation

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Abstract

Objectives Controlling genotoxic impurities represents a significant challenge to both industry and regulators. The potential for formation of genotoxic short-chain alkyl esters of sulfonic acids during synthesis of sulfonic acid salts is a long-standing regulatory concern. This review provides a general overview of the utility of sulfonic acids as salt-forming moieties and discusses strategies for effectively minimizing the potential for alkyl sulfonate formation during the synthesis and processing of sulfonate salt active pharmaceutical ingredients. The potential implications of the recent establishment of a substantial human threshold dose for ethyl methanesulfonate for the safety assessment of alkyl sulfonates in general are also discussed.

Key findings The formation of alkyl sulfonates requires highly acidic conditions, possibly combined with long reaction times and/or elevated temperatures, to generate significant amounts, and these conditions are most unlikely to be present in the synthesis of active pharmaceutical ingredient sulfonate salts. It is possible to design salt formation conditions, using a short-chain alcohol as solvent, to manufacture sulfonate salts that are essentially free of alkyl sulfonate impurities. Processes using non-acidic conditions such as ethanol recrystallization or wet granulation should not raise any concerns of alkyl sulfonate formation.

Summary An understanding of the mechanism of formation of alkyl sulfonates is critical in order to avoid restricting or over-controlling sulfonic acid salts, which have many technical advantages as pharmaceutical counterions. Recent regulatory acceptance of a human threshold limit dose of 2 mg/kg per day for ethyl methanesulfonate, indicating that its toxicological risks have previously been considerably overestimated, could signal the beginning of the end over safety concerns on alkyl sulfonate residues, thus removing a major constraint from the exploitation of sulfonic acid counterions.

Keywords active pharmaceutical ingredients; genotoxic impurities; sulfonate acid salts; Viracept

Introduction

Residual genotoxic impurities, and particularly alkyl esters of alkyl or aryl sulfonic acids, have been, and probably remain, a significant safety concern to drug regulators. Since the sulfonate moiety is readily displaced by a variety of nucleophiles, such esters can act as DNA alkylating agents in biological systems and have been shown to exert genotoxic effects in bacterial and mammalian cells.[1] Glowienke *et al.*[2] studied 19 sulfonic acid esters of methane-, benzene- and toluenesulfonic acid, and showed that the isopropyl esters were consistently the most potent mutagens (stronger than all primary alkyl esters and secondary butyl ester), either in the Ames test or micronucleus assay.

In 2000, the European Directorate for the Quality of Medicines and Healthcare requested additional information on the requirement for pharmacopoeial limit tests for alkyl mesylate impurities in mesylate salts (mesilates, methanesulfonic acids).[3] In 2005, the European Pharmacopoeia drafted a production statement for inclusion in the monographs of all mesylate-containing active pharmaceutical ingredients (APIs).[4] This statement indicated that: 'The production method must be evaluated to determine the potential for formation of alkyl mesylates, which is particularly likely to occur if the reaction medium contains lower alcohols. Where necessary, the production method is validated to demonstrate that alkyl mesylates are not detectable in the final product.'

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Production statements are used *in lieu* of routine testing and allow the use of fundamental scientific and mechanistic knowledge combined with process information concerning specific impurity formation[5] in order to support a risk assessment of the likely formation of these reactive impurities. In contrast, the other major pharmacopoeias rarely include tests for sulfonate esters in their monographs. The one obvious exception is the Atracurium Besylate (benzene sulfonic acid) monograph in the United States Pharmacopeia, which includes a test for residual methyl besylate, with an acceptance limit of 100 ppm;[6] the limit in the European Pharmacopoeia is 10 ppm.[7]

The European Directorate for the Quality of Medicines and Healthcare recently identified the need to develop a (draft) policy for dealing with potentially genotoxic impurities that can be applied during review and revision of monographs.[8] Adoption of a pragmatic approach is advocated; for existing monographs, in the absence of new data demonstrating genotoxicity of an impurity, then structural alerts alone are considered insufficient justification to trigger additional measures. Retrospective application of current EU regulatory guidance to marketed products is considered unnecessary except where there are data showing genotoxicity of an expected potentially genotoxic impurity. The United States Pharmacopeia has held similar discussions on specification issues related to genotoxic impurities.[9]

In parallel with this pharmacopoeial initiative, specific European guidance concerning safe limits for genotoxic impurities has been developed. The Committee for Medicinal Products for Human Use (CHMP) issued their finalized Guideline on the Limits of Genotoxic Impurities in 2006, and this has been in effect since 1 January 2007.[10] A Questions and Answers Supplement[11] to the guideline was issued in January 2008 and updated in June 2008 in order to clarify a number of remaining issues within the guideline, including 'cause for concern', 'as low as reasonably practicable', structural alerts, staged threshold of toxicological concern (TTC) and multiple genotoxic impurities.

In cases where compound-specific carcinogenicity data are unavailable, the guideline advocates an approach based on the TTC for defining the acceptable patient intake of DNA-reactive genotoxic impurities. Exposure at the TTC is expected to incur an additional cancer risk of not greater than 1 in 100 000 over a human lifetime. Derived from linear extrapolations of rodent potency data for over 700 carcinogens, the TTC for the 'average' genotoxin is equated to a lifetime exposure of 1.5 μg/day. Highly potent genotoxins such as nitroso and azoxy compounds are excluded from this general limit. In the general case, however, based on the 'default' TTC of 1.5 μg/day, an appropriate concentration of the genotoxic impurity in the API can be then calculated by dividing the TTC by the maximum daily dose of the API. Genotoxins that cause chromosomal damage or other effects but are not DNA-reactive (called non-thresholded genotoxins in the guideline), are not subject to TTC limits; control is achieved in a similar way to solvent impurities using the permitted daily exposure (PDE) concept. Draft guidance from the US Food and Drug Administration (FDA) was released in mid-December 2008 and contains recommendations that are closely similar to those in the EU guideline.[12] Jacobson-Kram and McGovern[13] at the Center for Drug Evaluation and Research, FDA, maintained that while impurities should always be reduced to the lowest levels reasonably practicable, they did acknowledge that impurities cannot be totally eliminated and meaningful specifications for impurities need to be established.

The Question and Answers Supplement[14] to the EU guideline on genotoxic impurities indicates that multiple structurally similar genotoxic impurities will be subject to an overall limit of 1.5 μg/day. Such a provision may create significant difficulties in the analysis of sulfonic acid salts where there is often only a theoretical possibility for formation of several alkyl sulfonates (e.g. via contaminants in sulfonic acid reagent and use of one or more alcohol solvents in the overall process). Controlling multiple sulfonate esters to the implied low limits would represent a significant challenge to existing analytical methodologies, which would be aggravated for drug substances with a high daily dose. In cases where there is a theoretical potential for formation of multiple structurally related genotoxic impurities, an assessment based on process parameters could be used to focus effort on those impurities that are of actual risk. This could be augmented by evaluation of batch analytical data to enable appropriate specification limits to be set only for those impurities that are likely to be present, thus avoiding attrition of the overall TTC limit by allowing for the cumulative limit of quantitation for theoretical impurities. Recent developments suggest that the TTC concept as applied to genotoxic impurities is markedly overconservative and may not be ultimately sustainable in its present form.[15] The establishment of a default value significantly higher than 1.5 μg/day would clearly alleviate analytical challenges on multiple genotoxic impurities, but such a change seems unlikely to occur in the short-term.

In late 2007, the European Medicines Agency suspended the marketing authorization of Viracept (nelfinavir mesylate), an anti-viral medicinal product, owing to concerns over the presence of elevated levels of ethyl methanesulfonate (EMS), in the drug product.[16] Subsequently, the CHMP assessed the corrective and preventative measures that were put in place by the marketing authorization holder (MAH), which were verified by on-site inspections. CHMP were 'reassured that the contamination causes had been eliminated and that the future production of Viracept would meet the required quality standards' and recommended lifting the suspension of the marketing authorization only a few months after its imposition.[17]

Up to the time of the EMS in Viracept incident (see below), contamination of mesylate/sulfonate salt drug substances appears to have been a largely theoretical concern, but recently a more focused regulatory attitude has been evident. Set against this, significant benefits are provided by sulfonate counterions, particularly in the development of low solubility drug substances. Moreover, the Viracept incident appears to have been an unusual, and possibly unique, case involving good manufacturing practice (GMP) issues.

In late July 2008, CHMP announced that it endorsed a human PDE of 2 mg/kg/day EMS proposed by the Viracept MAH based on data from in-vivo studies in rodents. Since the highest level of EMS contamination of Viracept resulted

in an intake of 0.05 mg/kg/day, the CHMP considered that exposed patients should not be at increased risk of developing cancer.[18]

This review aims to provide a general overview of the utility of sulfonic acids as salt-forming moieties, and to evaluate whether more general lessons need to be learned from the Viracept recall, and whether strategies can be developed for effectively minimizing the potential for alkyl sulfonate formation during the synthesis and processing of sulfonate salt APIs. In addition, the potential implications of the recent establishment of a substantial human threshold dose for EMS for the safety assessment of alkyl sulfonates in general are discussed.

Utility of Sulfonic Acids as Salt-Forming Entities

Although a number of comprehensive reviews of pharmaceutical salts and their utility in the development of novel chemical entities have been published over the last few decades, no systematic evaluation of the opportunities proffered by specific classes, such as carboxylic or mineral acid salts, appears to be available. Consequently, a focused literature search and subsequent assessment has been undertaken on the utility of sulfonic acid salts in drug development. The preferred salt for a novel chemical entity needs to be assessed on an individual basis and will be chosen on many different, often conflicting, criteria, and it is acknowledged that sulfonic acid salts may not provide the best solution; in fact the free base may be the preferred form in some cases. Our key message is that sulfonic acid salts have many advantages and should not be discounted during the initial salt assessment due to perceived issues of safety (potential contamination with sulfonate esters) and thereby utilized only as a last resort.

Salt formation is a useful approach for optimizing the physicochemical, processing (formulation), biopharmaceutical or therapeutic properties of acid or basic APIs. Each of the individual salts of a particular API can be considered a unique chemical entity with its own distinct physicochemical and biopharmaceutical properties.[19,20] Due to the absence of any predictive relationships between the physicochemical properties of the free base or free acid and any of the resultant salts, selection of the best salt with the desired properties is a difficult, semi-empirical undertaking.[21,22]

Serajuddin evaluated salt usage over the last 10 years and indicated that the intrinsically low aqueous solubility of many APIs coupled with relatively low pH of maximum solubility values ensures that in some cases carboxylic acids are no longer capable of forming acceptable salts and would need to be excluded from the counterions available as salt-forming moieties.[23] As a result, stronger acids are required to form salts of such drug substances. Serajuddin also reviewed the trends in salt form usage for those medicinal products approved (120 in total) by the FDA over a recent 12-year period (1995–2006).[23] Of particular note was that use of mesylate salts had increased significantly to second in the order of ranking of anionic counterions and now comprised 10% of total usage.

In some instances, there can be significant advantages to selecting a mesylate salt over other strong acid counterions, particularly the more prevalent hydrochloride salt. Pharmaceutical salts frequently exist as hydrates[22] and this can be problematical during secondary processing, particularly wet granulation. In contrast to other salts of strong acids, mesylates tend not to form hydrates,[24] which makes them an attractive salt form for secondary processing, especially wet granulation.

The melting point of pharmaceutical salts can often impact on their physicochemical properties. In general, APIs with low melting points often exhibit plastic deformation during processing, which can cause both caking and aggregation, impacting on flow and compressibility characteristics.[25] The greater ionic content of strong acid salts (e.g. sulfonates, sulfates, hydrochlorides) usually ensures that the resultant salt is less plastic in nature, facilitating better secondary processing.[26]

Hydrochloride, and particularly dihydrochloride salts of weak bases, can undergo disproportionation.[20] The driving force for the reaction is the generation of volatile hydrogen chloride gas that is either lost from the system or reacts with other constituents of the formulation/processing equipment, leading to either de-stabilization (physical or chemical) or processing issues.[27,28] In contrast, disproportionation of mesylates (and other sulfonic acid salts) is much less common as by-products of the disproportionation reaction are stable and non-volatile and therefore the main driving force for disproportionation is significantly reduced.

Poor or inadequate solubility in aqueous and biorelevant media can often hinder and constrain development of oral and parenteral drug products.[29] Typically, increasing the melting point has an adverse effect on aqueous solubility owing to increasing crystal lattice energies.[30] Sulfonic acid salts tend to be an exception to this rule, exhibiting both high melting points and good solubility. Bighley *et al.*[20] reported that the increased prevalence of sulfonate salts was because of their influence on both dissolution rate and reactivity. They indicated that the mesylate salts of APIs tend to be highly soluble, leading to rapid dissolution.

Li *et al.*[31] demonstrated that strong acid salts, such as mesylates (or other sulfonic acid salts), which have higher aqueous solubilities than the corresponding hydrochloride salt, may also have additional in-vivo advantages. This was attributed to the common ion effect that can reduce the solubilities of hydrochloride salts in gastric environments. Li *et al.*[31] showed that the high solubility and high surface area of haloperidol mesylate resulted in enhanced dissolution rates (<2 min in pH 2 simulated gastric media), which were more rapid than the competing common ion formation (i.e. conversion to lower solubility hydrochloride salt).

Potential for Formation of Alkyl Esters of Sulfonic Acids During Sulfonic Acid Salt Synthesis

As already indicated, concerns over the potential for genotoxic alkyl mesylates to be formed during the synthesis of methanesulfonic acid (MSA) salt drug substances were

first formally raised by the European Directorate for the Quality of Medicines and Healthcare in 2000. Since that time, regulatory controls have extended to sulfonic acid salts in general and have been adopted by agencies in other jurisdictions, although the concerns remain hypothetical rather than evidence-based. The available evidence concerning the potential for formation of alkyl mesylates during MSA salt formation has recently been reviewed,[32] and any further relevant information is presented below.

Alkyl sulfonates are normally synthesized by reacting the acid chloride form of the sulfonic acid with the appropriate alcohol. For example, EMS is readily prepared from methanesulfonyl chloride and ethanol. Alkyl sulfonates may however arise from the reaction between short-chain alcoholic solvents (e.g. methanol, ethanol, propanol and iso-propanol) and sulfonic acids. In this type of system, similar to carboxylic acid esters, sulfonic acid ester formation occurs in a two-stage equilibrium reaction (Figure 1): (1) protonation of the alcohol to form an oxonium ion; and (2) nucleophilic displacement of the hydroxonium moiety by sulfonate anion then produces an alkyl sulfonate.[32]

Significant concentrations of protonated alcohol are needed for the reaction to proceed by the established pathway. Alcohols are weakly acidic and so are not readily protonated. For simple aliphatic alcohols, based on ^{13}C-NMR shift data for the α-carbon atom to the hydroxyl group in the presence of different strengths of sulfuric acid, it is concluded that protonated alcohol formation will not occur until the acidity of the reaction medium falls below pH 0.5.[33] Moreover, the sulfonate anion is a poor nucleophile owing to the delocalization of negative charge over three oxygen atoms, and any water formed during the reaction has the potential to hydrolyse any ester to its constituent acid and alcohol. Overall, the formation of alkyl sulfonyl esters in this kind of system is kinetically slow and typically unfavoured ($K_{eq} < 1 : 100$). The above information relates to binary systems (alcohol and sulfonic acid), but the synthesis of a sulfonate salt of a basic (normally amine-containing) active substance requires, of necessity, the presence of the free-base form of the drug substance in the reaction medium. In this case, as the sulfonic acid is added to an alcoholic solution of the free base, only the basic drug substance, not the alcohol solvent will be protonated, provided that no more than a molar equivalent of sulfonic acid is introduced into the system.

Experimental studies at the Product Quality Research Institute have confirmed the various elements of the mechanism of formation of alkyl sulfonates described above.[34] For example, there was no incorporation of ^{18}O into methyl methanesulfonate (MMS) formed in a system containing MSA and $CH_3{}^{18}OH$, indicating that MMS formation proceeds by initial protonation of methanol. This mechanistic information suggests a range of simple precautionary measures (such as cooling, stirring, addition of water, avoidance of a molar excess of sulfonic acid) in order to avoid sulfonate ester formation. In addition, it is necessary to check the sulfonic acid starting material for the presence of impurities that may produce alkyl sulfonates under the conditions of the salt-formation reaction. For example, impurities in MSA such as pre-formed MMS, thiomesylates and methanesulfonyl chloride can react with alcohols in transesterification reactions to form traces of alkyl mesylates.[31]

Complete avoidance of alcoholic solvents as a reaction medium would circumvent the formation of alkyl esters of sulfonic acids. Suitable non-hydroxylic solvents could be acetonitrile,1,4-dioxane, anisole, *tert*-butylmethyl ether or tetrahydrofuran. Some potentially useful solvents (such as ethyl acetate and dichloromethane) may contain traces of ethanol. However, such solvents may not be particularly suitable for crystallization of the sulfonic acid salt and so use of a hydroxylic solvent, with suitable precautions, may be preferable.

Ethyl Methanesulfonate Contamination Incident

The drug product Viracept is indicated for antiretroviral combination treatment of patients infected with human immunodeficiency virus (HIV-1). The non-peptidic drug substance is nelfinavir mesylate and the drug product is presented as a film-coated tablet for oral administration containing 250 mg nelfinavir. The maximum human dose is 2500 mg/day.

On 5 June 2007, the MAH informed the EMEA that Viracept was being recalled from European Union markets with immediate effect since contamination of the product with a genotoxic substance (ethyl methane sulfonate; ethyl mesylate; EMS) had been detected.

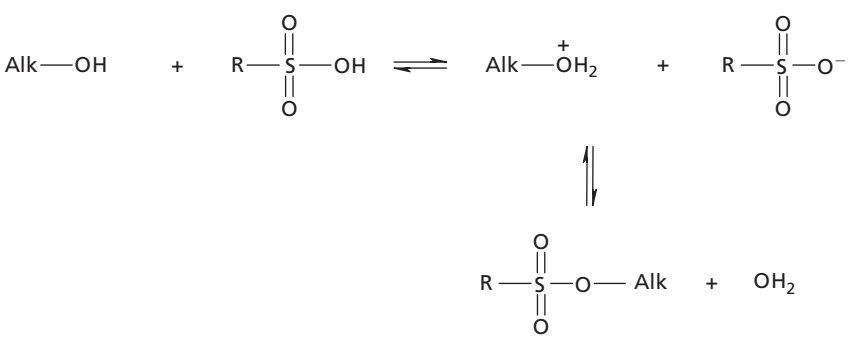


Figure 1 Formation of alkyl sulfonates from a sulfonic acid and a short-chain aliphatic alcohol. Alk = methyl, ethyl, propyl or *iso*propyl; R = methyl, ethyl, phenyl, 4-chlorophenyl (see Table 1).

The root cause of the contamination was a GMP failure in respect of the holding tank for MSA, which is used in the final step of the synthesis to convert nelfinavir base to its mesylate salt. Following non-routine maintenance, the holding tank was cleaned according to the standard operating procedure (i.e. with ethanol), but, crucially, no tank drying was performed. On refilling of the tank with neat MSA, a reaction with the residual ethanol ensued, leading over time (several months) to the production of significant concentrations of EMS. This contaminated MSA was used for the October 2006 campaigns for the manufacture of nelfinavir mesylate API and subsequent drug product batches.

Following a series of activities including a manufacturing site inspection by Swissmedic, data review by ad-hoc expert groups and a presentation by the company to a plenary session of the CHMP in late June, a suspension of the marketing authorization was recommended by CHMP. The MAH agreed to follow a range of corrective and preventive actions (CAPA) and they were able to present the outcome of this programme to the CHMP in September. On 20 September 2007, the CHMP recommended a lifting of the suspension of the marketing authorization subject to additional commitments and follow-up measures.[35]

One part of the CAPA was to tighten the stoichiometric equivalents of MSA used in the process from the existing range of 0.97–1.03 to 0.97–0.995. The MAH also agreed to a corresponding tightening of the allowable working range of the pH in the reaction vessel from the existing 1.2–3.0 to 3.0–3.5 pH units. Finally, the MAH agreed to initially charging the reactor with only approximately 90% of the stoichiometric equivalents of MSA and then utilizing the final 10% of MSA to modify the pH into the desired range (3.0–3.5 pH units).

In parallel, the MAH agreed to restrict the levels of methanesulfonyl chloride in input batches of MSA to 0.1 ppm and the existing specification for residual MMS of 3 ppm was reduced to a total specification limit of 1 ppm for combined levels of MMS and EMS.

The MAH dispensed with the MSA holding tank (and used a disposable container), implemented a slower addition of the MSA into the suspension of nelfinavir base in ethanol and changed the location of the MSA inlet pipe to ensure optimized mixing conditions, thereby mitigating the potential for formation of high local concentrations of MSA. These measures, together with a specification limit of 0.5 ppm for residual MMS and EMS in nelfinavir mesylate API, were deemed to be acceptable by CHMP. Initially, a specification level of 0.6 ppm EMS was proposed (i.e. based on the TTC limit of 1.5 μg/day and 2.5 g/day drug substance), but the slightly lower combined limit for EMS and MMS was set, equivalent to ≤1.25 μg/day total genotoxic impurity.

The CHMP guideline indicates that a compound-specific assessment should be made in the case of genotoxic substances for which carcinogenicity bioassay data are available. Mouse bioassay data on MMS have been reported[36] and are sufficient to calculate the dose causing a carcinogenic response in 50% of test animals (TD50 value) (31.8 mg/kg/day) and derive a virtually safe dose based on a risk of 1 in 10^5 in a completely analogous way to the derivation of the default TTC. Linear extrapolation (i.e. dividing by 50 000) of the MMS TD50 gives a virtually safe dose of 0.64 μg/kg/day (equivalent to 38 μg/day in a 60 kg patient). Using more conservative methodology, the Office of Environmental Health Hazard Assessment has determined a no significant risk level for MMS of 7 μg/day.[37] The bioassay data on MMS are limited to a single study in male mice reported only as a short paper,[38] but it seems that the results were considered sufficiently robust to be included in the 730-compound dataset underpinning the TTC concept on carcinogens.[39,40] A compound-specific limit of 2.8 ppm (7 μg/day) or 15 ppm (38 μg/day) for MMS combined with a standard TTC limit for EMS (0.6 ppm, 1.5 μg/day) could have been set.

Follow-up Measures on Licensed Active Pharmaceutical Ingredients Presented as Sulfonic Acid Salts

Undoubtedly as a consequence of the Viracept situation, in late 2007 Swissmedic requested MAHs to perform risk assessments on preparations containing sulfonic acid salts (e.g. mesylates, besylates and tosylates) to check for the presence of sulfonate esters and, if required, to take appropriate measures to avoid them.[41] The legal basis for this requirement is the production statement of the European Pharmacopoeia and the allowable levels are based on the TTC limits.

Swissmedic recommends that the risk assessment should be based on eight key factors as follows. (1) If lower aliphatic alcohols are required to be used as solvents in the synthetic procedure, can the formation of alkyl sulfonates be minimized and an effective purification process implemented? (2) Are there appropriate specifications and validated methodologies developed in order to detect and control these impurities in the API using the TTC approach? (3) Is the quality of sulfonic acid starting materials adequate with respect to control of these impurities? Similarly, are the corresponding acid chlorides (potential precursors of the sulfonic acids) adequately controlled? (4) Has the supplier/MAH developed appropriate specifications and validated methods? Can the supplier/MAH guarantee that these impurities will not exceed the TTC in the final API? Swissmedic recommends that 'The cumulative risk linked to the presence of several alkyl-substituted or aryl-substituted sulfonic acid ester type impurities must be taken into account'. (5) If a sulfonic acid derivative is utilised as a reagent during one of the latter stages of API synthesis, it must also be included in the risk assessments. (6) If solvents are typically recycled, is the quality of these solvents controlled from the perspective of accumulation and contamination by sulfonate esters? (7) From a stability perspective, can the formation of these sulfonate esters be discounted during the storage of the API and in the resultant medicinal product? (8) From a secondary processing perspective, can the formation of these sulfonate esters be discounted during the manufacture of the drug product? Swissmedic noted that this is particularly applicable during alcoholic granulation and the regulatory agency also wanted assurances that sufficiently sensitive methods were developed to detect and control these impurities in the drug product using the TTC approach.

In addition, the Coordination Group for Mutual Recognition-Human committee (CMDh) made a similar request to MAHs to perform risk assessments on preparations containing sulfonic acid salts (e.g. mesylates, (di)isetionates, besylates and tosylates) to check for the presence of sulfonate esters and, if required, to take appropriate measures to avoid them.[42] This request has also been issued by the UK Medicines and Healthcare Products Regulatory Agency[43] and others. The legal premise for this requirement was again the production statement of the European Pharmacopoeia and the allowable levels are based on TTC limits. The risk assessments required by MAHs were similar to those requested by Swissmedic. MAHs were informed that any such changes requiring amendment to the method of manufacture or control of API or drug product must be submitted to the competent authorities using the established procedures, along with a timetable for the various submissions of each of the variations that would be required. CMDh indicated that the risk assessment should be made available upon request from any competent authority.

Swissmedic's point 5 (see above) has caused some confusion on the part of both regulators and industry. Typically, industry sponsors impurity fate mapping studies as part of their general risk assessment and often performs testing and control at an intermediate stage as part of this strategy. In these circumstances, provided sulfonic acid esters are controlled to an acceptable low level in an isolated intermediate, the need for setting levels in the API specification should be precluded.[44]

Comments on the Ethyl Methanesulfonate Contamination Incident and Follow-up Measures

The Viracept EMS contamination problem arose as a result of allowing residues of ethanol to remain in the MSA holding tank following cleaning. Levels of EMS in the resultant drug substance were initially extremely low (<1–8 ppm), reflecting the slow formation rate in the holding tank. However, over time, levels of EMS in the drug substance increased signi-ficantly up to 2300 ppm (3 months later).

Within the holding tank there was clearly an overwhelming molar excess of concentrated MSA in comparison with ethanol, which, in combination with the extended reaction period, drove an essentially unfavoured reaction (i.e. formation of EMS) essentially to completion. In contrast, during API salt formation there are two competing equilibria to consider: (1) API salt formation involving the rapid protonation of the API base; and (2) the much slower and less favoured formation of the sulfonyl ester(s). In the synthesis of sulfonic acid salts, the reaction times will be much shorter (minutes/hours) whereas, in the case of nelfinavir mesylate, EMS levels were allowed to build up over several months. Overall, the root cause of the Viracept contamination incident was largely a result of a GMP oversight, and the remediation measures (e.g. avoidance of a molar excess of sulfonic acid combined with strict pH control, careful addition of the sulfonic acid and adequate mixing in the reaction medium, control of sulfonic acid impurities) do provide useful insight towards minimizing the potential for alkyl sulfonate formation.

Concerns expressed by Swissmedic over the formation of alkyl sulfonates during storage of the drug substance/drug product probably do not represent actual risk given the crucial requirement for a highly acidic environment ($pH < 0.5$) in order to enable oxonion ion generation and subsequent ester formation. Given that a minimum pH of 3.0 is deemed acceptable in the CAPA for nelfinavir mesylate, concerns over alkyl mesylate formation during wet granulation at more neutral pHs also seem misplaced. Moreover, Miller *et al*.[45] detected no formation of EMS in storage trials of a mesylate salt in aqueous ethanol.

Both Swissmedic and CMDh have provided recent guidance to industry on the specific sulfonate salts of concern, but in both cases the information appears inconsistent and incomplete. The CMDh letter to MAHs covered mesilates, di-isetionates, tosilates or besilates, whereas the Swissmedic letter referred to mesilates, tosilates or besilates. The isetionates and di-isetionates are not commonly used salt forms and are not mentioned in the two extensive reviews of salt usage by Berge *et al*.[19] A summary of the acids used to form anionic salts, focusing on sulfonic acids, is provided in Table 1.

Only three of the top nine sulfonate salts shown in Table 1 have been identified for special consideration by the Swissmedic/CMDh regulatory communications. Although, there has been no recent update of the frequency of total usage of salts, Serajuddin[23] indicated that mesylate salt usage had increased significantly to second in the order of ranking of anionic counterions and now comprised 10% of total usage. He did not describe the usage of any other sulfonic acid salts, but noted that there had been a general upsurge in the use of strong inorganic counterions (e.g. hydrochlorides, mesylates, hydrobromides/bromides, and sulphate/bisulphates) and these had increased to just over three quarters of the total (79%). On the FDA website[46] 46 drug products are listed as containing mesylate salt APIs.

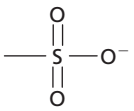
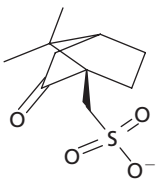
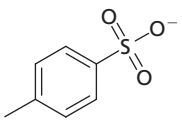
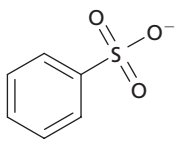
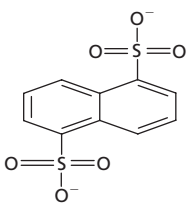
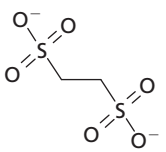
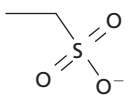
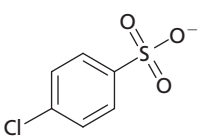
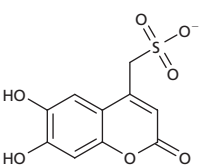
Minimizing the Potential for Alkyl Mesylate Formation

Given the information provided in the CAPA for nelfinavir mesylate and applying mechanistic considerations, including the results of the Product Quality Research Institute programme,[34] some general principles can be proposed that should lead to the minimization or indeed elimination of alkyl sulfonate formation during the synthesis of a sulfonic acid salt API.

Choice of reaction solvent

Although ethanol and isopropanol appear to be the most commonly used hydroxylic solvents, it is possible to employ several others. Table 2 shows potentially suitable hydroxylic solvents that are listed in the ICH Q3C (R3) guideline.[47] The PDE for all of these solvents is 50 mg/day (class 3), with the exception of methanol, which is controlled at 30 mg/day (class 2). Two other criteria that may impact on choice of solvent are cost and volatility (boiling point), and these are also shown in Table 2. In view of the recent establishment of a human threshold dose for EMS equivalent to 120 mg/day, ethanol is now most likely to be the solvent of choice when synthesizing APIs as mesylate salts. For example, EMS in

Table 1 Frequency of usage of sulfonic acid salts

Anion	Alternative name	Structure	Percent[a]
Methanesulfonate	Mesylate		3.20
Camphorsulfonate	Camyslate		0.59
Toluene 4-sulfonate	Tosylate		0.39
Benzenesulfonate	Besylate		0.26
1,5-Naphthalenedisulfonate	Napsylate, Napadisylate		0.20
1,2-Ethanedisulfonate	Edisylate		0.20
Ethanesulfonate	Esylate		0.13
4-Chlorobenzenesulfonate	Closylate		0.07
6,7-Dihydroxycoumarin-4-methanesulfonate	Cromesilate		0.07

Based on Berge *et al.*[19] and Bighley *et al.*[20]

[a]Based on the total number of anionic salts in clinical use up to 1993 (total 5.11%).

nelfinavir mesylate (up to 2500 mg/day) could be as high as 4.8% and still be within the threshold dose, although it would be virtually impossible to achieve this level of contamination, and the drug product would be organoleptically unacceptable. It is unclear whether the previous extremely low specification limit for EMS has been increased.

Table 2 Data on relevant hydroxylic solvents

Solvent	Permitted daily exposure (mg/day)	Boiling point (°C)	Relative cost[a]
Methanol	30	64.7	100
Ethanol	50	78.3	443
1-Propanol	50	97.2	198
2-Propanol	50	82.3	135
1-Butanol	50	117.7	168
2-Butanol	50	98	184
2-Methyl-1-propanol	50	107.7	150
1-Pentanol	50	138	290
3-Methyl-1-butanol	50	132	N/A

[a]Based on a 20-L volume purchased from various chemical laboratory suppliers.

Purity of sulfonic acid

In view of the potential for the presence of preformed alkyl sulfonates and/or acid chlorides in sulfonic acid starting material, the sulfonic acid purity may be critical in allowing the synthesis of a sulfonate salt API that is essentially free of alkyl sulfonates. However, concerns over the presence of preformed alkyl sulfonates or acid chlorides should be much reduced for less volatile sulfonic acids that can be crystallized from aqueous solvents and/or are available as hydrates (e.g. tosic acid monohydrate).

Reaction conditions

Suitable reaction conditions (e.g. control of pH, adequate stirring and cooling) have already been described for the synthesis of mesylate salts and should be generally applicable to all sulfonate salts.

Discussion

Controlling genotoxic and potentially genotoxic impurities in novel drug substances represents a significant challenge. The short-chain alkyl esters of sulfonic acids have received particular regulatory focus, especially in the aftermath of the Viracept recall in Europe, prompted by the presence of high levels of preformed EMS. This review has attempted to summarize the GMP issues that arose during the Viracept manufacture and the subsequent remedial actions that were implemented by the MAH, endorsed by the responsible regulatory agencies and which led to the rapid re-introduction of the product to the EU market.

Regulators have initiated an assessment of risk mitigation strategies to ensure that the alkyl sulfonates are appropriately controlled (e.g. on the basis of TTC limits). Only a few of the sulfonate salts that have been utilized in medicinal products have been specifically identified and the inclusion of the isetionate and di-isetionate salt forms is difficult to understand as their historical usage appears to be minimal.[19,20] In addition, an understanding of the mechanism of formation of alkyl sulfonates appears to be lacking in these documents.

Caution is required with regard to over-controlling the use of sulfonic acid salts since these can be readily synthesized to high purity standards in a short-chain alcohol reaction medium. Formation of alkyl sulfonates is kinetically unfavoured and is in competition with rapid and efficient base protonation, which severely restricts the amount of sulfonic acid available to partake in the unfavoured side-reaction. The addition of pre-formed EMS (in the MSA reagent) during the synthesis of nelfinavir mesylate is considered to be an inappropriate precedent on which to base concerns over alkyl sulfonate production during the manufacture of sulfonic acid salts. In parallel, the recent introduction of a revised manufacturing process for MSA avoids the formation of the sulfonyl chloride intermediate, which significantly reduces intrinsic levels of MMS in this key starting material.[48]

The regulatory implications of the establishment of a PDE of 2 mg/kg/day for EMS (nearly 5 orders of magnitude greater than the standard TTC of 1.5 μg/day), based on a threshold dose of 25 mg/kg/day for DNA damage in mice, are difficult to predict. On the one hand, regulators may insist that toxicological data must be provided to set threshold doses for every different alkyl sulfonate. On the other hand, a more enlightened approach may prevail involving 'reading across' from the EMS data to other alkyl sulfonates taking into account chemical and biological structure–activity relationships[1,2,49] and following a safety evaluation paradigm similar to that applied to food flavourings.[50,51] For example, MMS appears to be around 5 times more active than EMS in terms of DNA alkylation,[52,53] and so a suitable limit for MMS might be 200 μg/kg/day. This would be over 300 times higher than the virtually safe dose calculated by linear extrapolation of the TD50, clearly illustrating how simplistic high-to-low-dose extrapolation techniques fail to take into account the impact of detoxication reactions (particularly error-free DNA repair in the case of EMS) and often lead to vastly overconservative risk assessments. A further possible approach is based on the work of Vogel and Nivard,[54] who reported that the genetic activity profiles of monofunctional alkylating agents can be predicted on the basis of chemical reactivity parameters such as the Swain-Scott s constant. The latter is a measure of the selectivity of alkylation with different nucleophiles; a low s value (e.g. <0.5) indicates an undiscriminating akylating agent with high carcinogenic potency such as *N*-ethyl-*N*-nitrosourea that will react with water or O- and N-nucleophiles at a similar rate. In contrast, a relatively low carcinogenic potential is associated with alkylating agents with high Swain-Scott s constants. This is because high-s alkylating agents react selectively with the more nucleophilic N-atoms rather than O-atoms in DNA bases and efficient error-free repair of DNA-alkyation damage ensues. Only when this repair process is saturated will lasting DNA damage occur. Since N-alkylation damage to DNA by EMS (s = 0.67) has been shown to be highly efficiently repaired, even more selective alkylating agents should also exhibit a threshold. These include MMS and glycidaldehyde (s = 0.83), dimethyl sulfate (s = 0.86), epichlorohydrin (s = 0.93) and ethylene oxide (s = 0.96). Isopropyl mesylate (s = 0.29) is a more reactive and less selective alkylating agent and so may require a slightly more conservative risk assessment.

Conclusions

Sulfonic acid salts possess a range of properties that are useful to the synthetic and process chemist, allowing insoluble APIs with low pH of maximum solubility values to be readily crystallized and isolated. While not a universal panacea to the problem of salt formation, they do offer significant advantages as alternatives to conventional anions such as chloride or acetate. It seems likely that the full utility of sulfonic acid salts has not been realized owing to the perceived issue of potential contamination with genotoxic alkyl sulfonates. The recent Viracept (nelfinavir mesylate) recall due to contamination with EMS seemed initially to confirm long-standing regulatory concerns over such contamination. However, the investigation into this incident exonerated the use of the mesylate anion as such and emphasized the need for strong GMP control and a mechanistic understanding of sulfonate ester formation. It can be concluded that, as long as certain straightforward procedures and precautions are followed, all of the evidence suggests that it is eminently possible, using a short-chain alcohol as solvent, to synthesize a sulfonic acid salt of an amine-containing drug substance that is essentially free of alkyl sulfonate contamination. Moreover, the demonstration of highly effective DNA repair mechanisms as part of the establishment of a high (2 mg/kg/day) PDE for EMS, a classic DNA-reactive genotoxin, has the potential to significantly modify the risk assessment of numerous alkylating agent impurities, and so may signal the beginning of the end of the intense regulatory focus on alkyl sulfonates, thus removing many of the constraints on the exploitation of sulfonic acid counterions.

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

This review received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

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