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Low level determination of *p*-toluenesulfonate and benzenesulfonate esters in drug substance by high performance liquid chromatography/mass spectrometry

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Abstract

GC/FID and HPLC/MS single ion monitoring methods have been evaluated for the determination of trace levels of methyl, ethyl and isopropyl esters of *p*-toluenesulfonic acid and methyl, ethyl, isopropyl and *n*-butyl esters of benzenesulfonic acid in drug substances. These sulfonate esters have been highlighted as potential genotoxins. HPLC/MS was found to be more promising and limits of quantification were between 2.5 and 5 ng/mL, which enabled detection limits in drug substance at 0.01–0.1 ppm for a 50 mg/mL solution. For one drug substance excellent recoveries of 94–95% were obtained at the 1.0 ppm level, however, with a second drug substance, a besylate salt, recoveries ranged from 86% to 100% and were dependant on the sample preparation. Limited stability of the sulfonate esters in various potential sample solutions indicated that samples may need to be prepared immediately before injection.

Keywords: HPLC; LC HPLC/MS; LC/MS; GC; Sulfonate esters; *p*-Toluenesulfonate esters; Benzenesulfonate esters; Genotoxins; Tosylates; Besylates; Trace analysis; Stability

1. Introduction

Sulfonic acids such as methanesulfonic acid (mesylate), benzenesulfonic acid (besylate) and *p*-toluenesulfonic acids (tosylate) are often used during manufacture of pharmaceuticals, either as counter-ions to form a salt, as acid catalysts or as the result of protecting group removal during the synthesis. However, the presence of any alcohol either in any of the stages of synthesis, or the in the crystallisation stage of the salt may cause the formation of sulfonic acid esters which are considered to be potential alkylating agents. In fact methyl and ethyl methanesulfonate esters are known genotoxins and are known carcinogens in rats and mice [1]. The potential presence of these genotoxins has attracted the attention of regulatory authorities, although no official guidelines have yet been issued. Draft guidelines [2] from the European Agency and feedback from the US Food Drug Administration (FDA) to the pharmaceutical industry via responses to drug applications has enabled the industry to establish interim strategies. Generally it is accepted that genotoxins will be limited to a daily dose of 1.0–1.5 μg/day unless safety studies establish that it is safe to receive a higher dose or that the drug is used for only a short term exposure, e.g. as an antibiotic. As some genotoxicity studies can take up to two years, e.g. carcinogenicity studies, it is preferable for the potential genotoxins to be controlled during the synthesis. In cases where levels cannot be controlled and no safety data yet exists it may be preferable for the pharmaceutical company to change the route of the drug substance or the isolation procedure, though this normally happens only during early development. This is a challenge analytically as well as synthetically as a 1.0 μg/day dose transposes to 1.0 ppm in the drug substance assuming a drug dose of 1 g/day.

Although the methyl and ethyl methanesulfonates are known carcinogens only recently has genotoxicity data been published on tosylates and besylates. Glowienke et al. [3] reported that in a study of methyl, ethyl, propyl, isopropyl, butyl and *n*-butyl tosylates and besylates, only the isobutyl tosylate ester showed inactivity in two in vitro tests (Ames and mouse lymphoma micronucleus). This data would infer that the regulatory authorities may be expected to request levels of besylate and tosylate esters to be controlled to 1 ppm in the drug substance (assuming a 1 g/day daily dose) or safety data to justify

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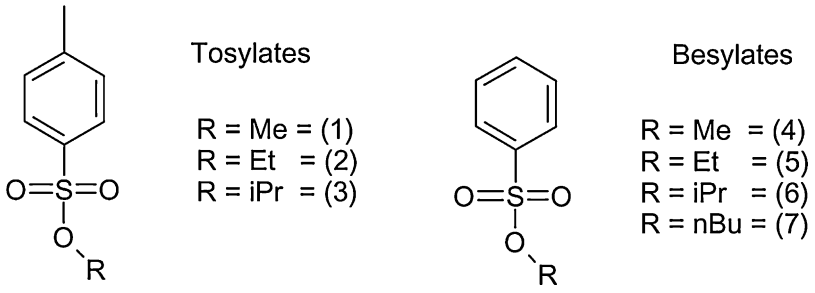

Fig. 1. General structure of the tosylates and besylates esters.

a greater dose. As AstraZeneca had one drug (API 1), existing as a carboxylic acid salt, in late stage development using *p*-toluenesulfonic acid as a catalyst in the synthesis and solvents, methanol, ethanol and isopropanol, it was felt necessary to develop simple, sensitive and validated methods for methyl (1), ethyl (2), isopropyl (3) tosylates (Fig. 1). Additionally a second drug (API 2), in the early stages of development, existed as the besylate salt and various alcohols, methanol, ethanol, isopropanol and *n*-butanol were evaluated in its synthesis, this necessitated a method for methyl (4), ethyl (5), isopropyl (6) and *n*-butyl (7) besylates (Fig. 1). At this stage in development it was important to demonstrate that the besylate esters were <1 ppm as the final dose is not always known. It was also important that the methodology could easily be adapted to other active pharmaceutical ingredients (APIs) or intermediates, ideally with direct injection onto a column avoiding tedious sample work up.

Only one reference could be found on the trace analysis of besylates and tosylates [4], this used solid-phase microextraction (SPME) and GC/MS with selected ion monitoring (SIM), this allowed the determination of besylates, tosylates and mesylates, at the 5 ppm level. Solid-phase extraction (SPE) and liquid-phase microextraction (LPME) were also investigated. All these had limitations in terms of the addition of organic solvents which may be required to solubilise non polar APIs or intermediates, this is especially important when lower levels such as 1 ppm or less are needed as higher sample concentrations may be required. Other references for mesylates included direct GC/MS injection [5], by derivatization with thiocyanate and reaction headspace GC [6] and GC with flame ionisation detection (FID) [7]. As the predicted boiling points of the tosylates and besylates were around 270–315 °C [8] it was thought feasible to attempt direct GC/FID or GC/MS. Additionally as the tosylates and besylates have the phenyl moiety which is UV absorbing HPLC/UV and HPLC/MS were thought to be an alternative option.

This short paper describes a very brief evaluation of GC and a sensitive direct injection HPLC/MS approach which has been used for the two drug substances and has been found applicable to other pharmaceuticals under development. As part of the approach, brief stability data were generated for the tosylates and besylates in various solvents as not all drug substances or intermediates are soluble in aqueous solutions.

2. Experimental

2.1. Chemicals

Methyl and ethyl tosylates were purchased from Aldrich Chemicals (Poole, Dorset, UK). Isopropyl tosylate and besylate were synthesised by AstraZeneca from the corresponding sulphonyl chloride and the alcohol. Methyl, ethyl and *n*-butyl besylate were all purchased from TCI Europe (Zwijndrecht, Belgium). HPLC grade acetonitrile and methanol, ammonium acetate, orthophosphoric acid, DMSO, DMF, and dry pyridine were all obtained from Fisher Chemicals (Loughborough, UK). HPLC grade water was obtained from a Millipore Milli-Q-Gradient ultrapure water system (Millipore, Billerica, MA, USA). The study also used two proprietary AstraZeneca development compounds.

2.2. Preparation of solutions

0.5 mg/mL concentrated stock solutions of the besylate or tosylate esters were prepared by dissolving the compounds in acetonitrile. The diluted stock solution, (0.01 mg/mL), was prepared by diluting 1 mL of the 0.5 mg/mL solutions to 50 mL with acetonitrile, DMSO, DMF, or pyridine (for stability studies with different solvents). The working standard solutions, 0.05 μg/mL, were prepared just before injection by diluting 1 mL of the 0.01 mg/mL solution in acetonitrile to 200 mL with sample diluents (35:65, acetonitrile/water, v/v) or (90:10, acetonitrile/DMSO, v/v). The working standard solutions were always prepared and injected immediately. The sample solution was prepared by accurately weighing about 50 mg of the drug substance into a HPLC vial and adding 1.0 mL of sample solvent, shaking to dissolve and immediately injecting.

Mixed tosylate/besylate standards at 0.09 mg/mL in acetonitrile or aqueous acetonitrile for the GC experiments were prepared by diluting 1.8 mL of each of the mixed besylate, tosylate standards to 10 mL with the required solvent.

2.2.1. Instrumentation GC/FID

An Agilent 6890 GC (Palo Alto, CA, USA) equipped with an autosampler and FID was used for the experiments. Data acquisition and processing was carried out on Atlas software (Thermo Electron Corp.).