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Determination of methyl and ethyl esters of methanesulfonic, benzenesulfonic and *p*-toluenesulfonic acids in active pharmaceutical ingredients by solid-phase microextraction (SPME) coupled to GC/SIM-MS

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Abstract

The development, optimization and validation of an extraction method for methyl and ethyl esters of various sulfonic acids is presented. The extraction and determination of these esters in active pharmaceutical ingredients (APIs) was accomplished using solid-phase microextraction coupled to GC/MS in the SIM mode. The factors affecting the extraction efficiency are discussed. This method was validated as a limits test and allows the determination of the sulfonic esters at the 5 ppm level in APIs. The method proved to be reproducible (%R.S.D.s less than 6%) and suitable for use with external standard quantitation, and also met basic validation requirements. This method offers numerous advantages over liquid–liquid extraction methods and was also compared to other extraction techniques such as solid-phase extraction (SPE) and liquid-phase microextraction (LPME) also being developed in our laboratories.

Keywords: Solid-phase microextraction; SPME; Sulfonate esters; Impurity analysis; Active pharmaceutical ingredients; SPE; LPME; Sulfonic acid esters

1. Introduction

The development and validation of appropriate analytical methodology for the determination of impurities in pharmaceutical products is required to understand synthetic processes and degradation pathways and is one key aspect in establishing appropriate regulatory controls. This methodology is particularly critical for the determination of process-related impurities of potential adverse toxicity such as alkyl halides and sulfonic esters.

Sulfonic acids, such as methane sulfonic acid (MSA), benzene sulfonic acid (BSA) or *p*-toluene sulfonic acid (*p*-TSA), are routinely employed to produce conjugates with active pharmaceutical ingredients (APIs) to improve its pharmacological properties. The formation of these conjugate salts usually improves the solubility, absorption and physical properties of APIs, increasing the feasibility of a successful formulation. However, the use of these acids potentially could result in the presence of trace levels of the sulfonic acid esters in the final API. The two main routes into the API include as process-related impurity during the manufacture of the acid, and carried through the process or formed in situ by the reaction of these acids with residual solvents (e.g. alcohols) used throughout the synthesis.

The potential toxicity of sulfonic acid esters, particularly methyl methanesulfonate (MMS) and ethyl methanesulfonate (EMS), has been documented [1,2]. MMS and EMS can alkylate cellular DNA [3,4] and analytical methods have been developed to quantitate the degree of alkylation by measuring ethylated and methylated DNA adducts [5], mostly employing liquid chromatography (HPLC). Gas chromatography has been used for the determination of *S*-methylcysteine and other alkylated amino acids as a marker for exposure to MMS and other alkylating agents [6].

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The reactivity of sulfonic acid esters decreases with increasing side-chain length with methanesulfonates being the most reactive. It is of interest to develop general sensitive methodology to determine the sulfonic esters content in the final API. Raw material or purchase controls for the parts per million content of these esters in the bulk sulfonic acid are typically established. Control of the sulfonic acid esters content in the bulk acid is sufficient when there is no chance of forming these esters in the salt formation step (e.g. no alcohols used as solvents during salt formation).

Liquid–liquid extraction (LLE) followed by GC/MS has been employed to extract methyl, ethyl and propyl sulfonate esters in methane sulfonic acid (MSA) [7]. LLE methods, although reliable, can be tedious and prone to interferences due to the use of organic solvents and concentration steps. Using LLE can also lead to the formation of difficult to break emulsions during the extraction process. Although there is a growing need for sensitive and reliable methods for these esters in the final API, very few methods developed for this purpose are reported in the literature. In one study [8], direct injection into a GC/MS system was used to quantitate MMS and EMS in a bismesylate salt API. This study was focused mostly on the ester detection by selected ion monitoring (SIM) and does not offer alternatives to extract the esters from more complex API solutions. Direct injection of complex API solutions can raise contamination issues in the GC injection port due to the presence of non-volatile components. This contamination can result in irreproducibility problems that will prevent successful validation of methods. The lack of fast, reproducible and sensitive methodology for the analysis of these esters in APIs can be revisited now, especially with the recent advances in sample preparation techniques such as solid-phase extraction (SPE), solid-phase microextraction (SPME) and more recently liquid-phase microextraction (LPME). These techniques have made possible the facile and rapid determination of low-level analytes in complex matrices.

Liquid phase microextraction (or LPME) was first introduced in the late 1990s primarily as a way of sample preparation prior to analysis by capillary electrophoresis [9]. Rapidly thereafter, the scope of this technique has been expanded to drug analysis [10,11] and environmental applications [12,13]. In this technique, a capillary hollow membrane filled with μL volumes of an extracting solvent is introduced into a liquid sample prepared in an immiscible solvent. The analytes of interest in the sample will partition with the extracting solvent inside the porous membrane. After equilibrium is reached, the solvent inside the membrane can be removed using a syringe and injected into a chromatographic system. This technique preserves all the advantages of a liquid–liquid extraction with a much improved concentration power due to the micro-volumes of extracting solvent used.

SPME is a solvent-free extraction technique introduced in 1990 by Pawliszyn's group. In this technique, a 1 cm fused-silica fiber with an immobilized polymeric coating is exposed to a gas (headspace) or liquid sample (direct immersion). Analytes are then extracted by means of a partitioning mechanism between the sample matrix and the fiber coating. Analytes are then desorbed by the use of high temperature in a gas chromatograph (GC) injection port or by re-dissolving them using a different solvent system in a liquid chromatograph (LC) injector. SPME has been successful in many areas including environmental (water, pesticides, solids, air), food analysis, forensic science and toxicology [14–17]. However, few applications have been published discussing applications of SPME in the pharmaceutical industry. The majority of the publications present significant improvements to the conventional headspace-GC determination of residual solvents [18–20]. The use of isotope dilution to improve the reproducibility and other validation parameters of the SPME has also been reported for headspace analyses [21]. Other applications coupling SPME with GC include the determination of flavors [22,23] or odors [24] in drug formulations. More recently, SPME coupled to HPLC/MS for the analysis of APIs and their metabolites in biological matrices such as blood and urine, for drug metabolism and pharmacokinetics studies [25]. For these applications, in-tube SPME has allowed automation of the sample preparation, separation and quantitation, increasing sample throughput with high degree of sensitivity and selectivity.

In this study, an analytical method using SPME coupled to GC/MS in the selected-ion monitoring (SIM) mode for the determination of methyl and ethyl esters of methanesulfonic, benzenesulfonic and *p*-toluenesulfonic acids in APIs. The study includes the optimization of the SPME extraction procedure, which is expected to decrease the analysis time and offer high degree of sensitivity. This method was validated as a limit test for the determination of these esters at the 5 ppm level in mesylate, besylate or tosylate salts of APIs and compared briefly to other techniques available in our laboratory, particularly solid-phase extraction (SPE) and liquid-phase microextraction (LPME).

2. Experimental

2.1. Chemicals and materials

Analytical standards of purity greater than 98% of methyl methanesulfonate (MMS), ethyl methanesulfonate (EMS), isopropyl methanesulfonate (IMS), methyl benzenesulfonate (MBS), ethyl benzenesulfonate (EBS), methyl *p*-toluenesulfonate (Mp-TS) and ethyl *p*-toluenesulfonate (Ep-TS) were obtained from Sigma–Aldrich (St. Louis, MO). J.T. Baker HPLC grade solvents (methanol, THF, 1-octanol, toluene, dichloroethane, acetonitrile and acetone) were purchased through VWR (Bridgeport, NJ). A Milli Q water purifying system was used to prepare buffers and aqueous solutions. Phosphate buffers were mostly used in this study. Potassium hydrogen or dihydrogen phosphate was purchased from Sigma–Aldrich and the pH was adjusted

as needed using J.T. Baker phosphoric acid or sodium hydroxide 1 M volumetric solution.

SPME devices were purchased from Supelco Inc. (Bellefonte, PA), as well as the 15 mL glass vials used for all extractions. For SPE experiments, cartridges were obtained from Phenomenex (Torrance, CA) or Waters (Milford, MA). The Q 3/2 Accurel hollow fibers used for LPME were obtained from Akzo Nobel (Wupertal, Germany).

2.2. GC/MS analysis

All samples were analyzed by gas chromatography using an Agilent 6890N Network GC System (Palo Alto, CA) equipped with an Agilent 5973N Network Mass Selective (MS) Detector and a split/splitless injector with a SPME injection insert (0.75 mm i.d.). This injector was held at 230 °C and operated in the pulsed splitless mode at 40 psi for 1.00 min, when the purge valve was opened. A DB-1701 column (Agilent) with 30 m length × 250 μm and 1.0 μm film thickness was used for all analyses. Ultra high purity helium was used as carrier gas and the column flow was kept constant throughout the run at 2.0 mL/min. The oven was set at 70 °C with a 1.0 min hold and then ramped to 250 °C at 20 °C/min. The column was held at 250 °C for 5.0 min for a total run time of 15.0 min. The MS was operated in the selected ion monitoring (SIM) mode scanning 15 ions that included the base and two qualifier ions for each analyte. These are *m/z*: 77, 79, 80, 91, 94, 95, 109, 110, 123, 124, 141, 155, 172, 186 and 200. The transfer line was set at 280 °C and the source temperature was 230 °C.

2.3. SPME procedures

A target concentration of 5 ppm of the esters in a 100 mg API sample results in a 50 ppb solution of the esters when the API sample is dissolved in 10 mL of aqueous buffer. For the optimization of the extraction procedure, a 50 ppm stock solution of the seven esters was prepared using methanol as solvent. Extraction vials (15 mL) were then spiked with 100 μL of this solution followed by addition of 10.0 mL of 20 mM phosphate buffer of the appropriate pH. For validation experiments, the concentration of the spiking solution was 5 ppm to yield samples in the 40–60 ppb range. Samples were then extracted using direct immersion SPME under constant agitation. For API samples, 100 mg of the active were accurately weighed into a 15 mL extraction vial and spiked (when desired) with 100 μL of the 5 ppm spiking solution. The spiked API samples were dissolved by the addition of 10.0 mL of 20 mM phosphate buffer (sonicated for 1 min when necessary) and extracted as described for the buffer solutions. The extractions were performed at ambient temperature. Unless otherwise specified, samples were prepared in triplicate, analyzed and the average result reported with its correspondent %R.S.D. The SPME fiber was left in the injector for approximately 10 min for proper conditioning before the next extraction.

2.4. SPE procedures

For comparison purposes, some data were obtained on the capabilities of SPE for the sensitive and reproducible extraction of methyl and ethyl methanesulfonates. The Strata brand cartridges evaluated (Strata-X, Strata-Phenyl, Strata-SDB) were purchased from Phenomenex (Torrance, CA) and the Oasis-HLB from Waters (Milford, MA). Buffer samples containing 500 ppb of MMS and EMS were prepared and 10.0 mL were loaded into a SPE cartridge previously conditioned with 2 mL of methanol and 2 mL of the buffered dissolving solvent. After sample loading, the cartridges were dried under vacuum for 30 s and eluted with 5.0 mL of organic solvent. Various organic solvents were evaluated for elution, including acetone, methanol, acetonitrile and THF (J.T. Baker). At this concentration (∼1 ppm), no solvent evaporation was needed prior to GC analysis. An aliquot of the eluent was injected directly into the chromatographic system.

2.5. LPME procedures

The LPME device consisted of two conventional 0.8 mm o.d. medical syringe needles (Becton Dickinson, Sparks, MD) inserted through the silicon septum in the screw cap of a 15 mL headspace sampling vial (Supelco, Bellefonte, PA) as previously described [10]. A small piece of polyethylene tubing (0.965 mm i.d. Becton Dickinson) was connected to the top of each syringe guide to facilitate the introduction of the extraction solvent into the capillary membrane. The opposite ends of the syringe needles were then connected by a 10 cm piece of Q 3/2 Accurel KM propylene hollow fiber (Akzo Nobel, Wupertal, Germany). The i.d. of this fiber is 600 μm and the pore size is 0.2 μm. The 10 cm pieces were discarded after a single use.

The sample to be extracted (15 mL) was placed into the glass vial. Samples consisted of phosphate buffer at pH 4.7 spiked with 50 ppb of the sulfonic esters. The fiber was exposed to the organic solvent to be used for a few seconds to immobilize solvent in the fiber pores. This fiber assembly was capped to the sample vial and the fiber loop filled with 40–50 μL of extracting solvent using a conventional Hamilton syringe. Samples were then extracted for 1 h under constant agitation and ambient temperature. After extraction, the solvent inside the capillary membrane was removed using a Hamilton syringe and either injected neat into the chromatographic system or was further diluted prior to analysis. These extraction devices are disposable.

3. Results and discussion

3.1. Chromatographic conditions

A 50 ppm standard solution of the seven sulfonate esters in methanol was used to optimize the chromatographic conditions. Due to their high degree of polarity, a DB-1701 column

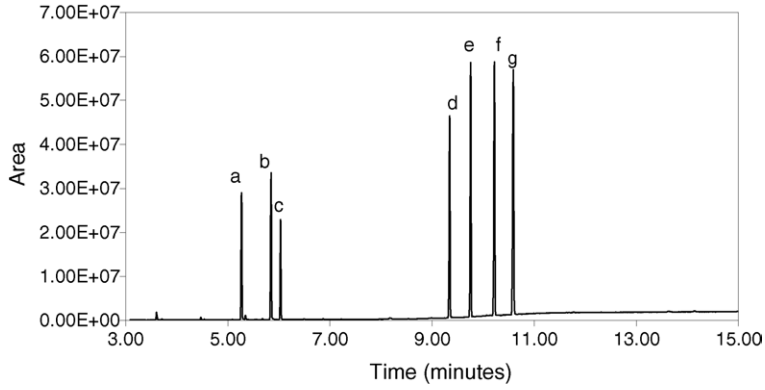

Fig. 1. GC/SIM-MS chromatogram for a 50 ppm standard solution of MMS (a), EMS (b), IMS (c), MBS (d), EBS (e), Mp-TS (f) and Ep-TS (g).

with a 1.0 μm film thickness was selected to ensure appropriate retention of the analytes. A DB-WAX column has been employed previously for their determination by GC [8]. The selected conditions described in Section 2 provided baseline separation for all seven components in 15 min. Since the limit test was designed to analyze solutions in the lower ppb range (ppm relative to the API), selected ion monitoring (SIM) was needed to increase the sensitivity over the MS SCAN mode or the FID detector. The analysis of the standard solution in the SCAN mode provided reliable spectra for each compound, which were used to select approximately three ions for the quantitative analysis of each ester although some of the ions are common to more than one ester. These ions are: MMS (80, 95, 110), EMS (79, 109, 124), IMS (79, 123), MBS (79, 94, 172), EMS (77, 141, 186), Mp-TS (91, 155, 186) and Ep-TS (91, 155, 200).

Fig. 1 shows a chromatogram obtained by SIM MS using the ions and conditions specified in Section 2 showing the baseline separation of all components.

3.2. Optimization of the SPME fiber coating

Preliminary studies performed in our laboratory using the more polar esters MMS and EMS, showed that the polydimethyl siloxane (PDMS) and the polyacrylate fibers did not have significant affinity for the esters at the lower pH (4.7) used for the extraction. Therefore, the main focus of the optimization using all seven sulfonate esters was geared towards the more polar SPME fibers such as carboxen (CAR)/PDMS, PDMS/divinyl benzene (DVB) and carbowax (CW)/DVB.

Samples were prepared by spiking 500 ppb of the esters in methanol to a 10.0 mL sample of 20 mM KH_2PO_4 (pH ~4.7). The samples were extracted by direct immersion SPME for 30 min at ambient temperature. The lower pH used in the extraction allows for a large number of sulfonate API salts to be in the ionized state, thus minimizing interferences in the extraction. The fiber optimization experiments were run in triplicate and the average area with its correspondent standard deviation is presented in Fig. 2. As expected, the less polar esters were extracted with more efficiency by all fibers. The CW/DVB fiber did not extract measurable amounts of the three more polar esters MMS, EMS and IMS. The CAR/PDMS fiber showed greater affinity for these three esters compared to the other two fibers although the PDMS/DVB fiber was the best one for extracting MBS, EBS, Mp-TS and Ep-TS. It is important to notice that the standard deviation for the responses obtained with the CAR/PDMS fiber were significantly higher than the ones obtained using the PDMS/DVB fiber. For the CAR/PDMS, the %R.S.D.s obtained were relatively high, within 7% (Ep-

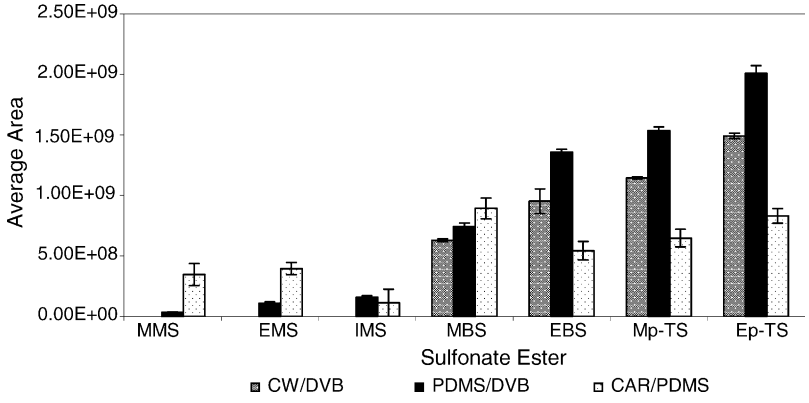

Fig. 2. Effect of fiber coating on the extraction efficiency for 20 mM phosphate buffer (pH 4.7) samples spiked with 500 ppb of seven sulfonate esters. Samples were prepared in triplicate and extracted for 30 min under constant agitation at ambient temperature.

TS) and 65% (IMS). The PDMS/DVB fiber showed acceptable reproducibility, with %R.S.D.s within 2% (EBS and Mp-TS) and 12% (MMS). It was common to both fibers that the higher R.S.D. values were obtained for MMS, EMS and IMS, whose extraction and MS detection is not as efficient as for MBS, EBS, Mp-TS and Ep-TS. Based on the overall performance and reproducibility, the PDMS/DVB fiber was selected for this method although the potential of the CAR/PDMS fiber for this application has also been demonstrated.

3.3. PDMS/DVB fiber extraction time profiles

It is important to evaluate the saturation and equilibration behavior of the seven esters into the SPME fiber during the optimization of the extraction procedure. Again, 10.0 mL samples of 20 mM KH_2PO_4 (pH ~4.7) were spiked with 500 ppb of the sulfonate esters and extracted at different time intervals, ranging from 5 to 60 min. As shown in Figs. 3a and b, saturation of the SPME fiber was basically achieved at 40 min. After 40 min, an interesting phenomenon can be observed, where the higher molecular weight esters (particularly Mp-TS and Ep-TS) show an increase in signal at 60 min. In contrast, the lower molecular weight esters (particularly MMS and IMS) showed a decrease in signal at 60 min. As concluded from other experiments, the less polar esters have more affinity to the SPME fiber and after 40 min there appears to be a displacement of the more polar mesylate esters by the toluene and benzene sulfonic esters, a result of the competition for adsorption sites on the fiber as judged from the extraction time profiles.

The %R.S.D.s for all time points between 10 and 30 min was within the acceptable range of 1–9%. As expected, the %R.S.D. decreased as the extraction time increased for most esters. For the methane sulfonates, the %R.S.D. increased significantly (although <10%) after 30 min, possibly due to their displacement by the less polar esters. Therefore, a 30-min extraction was chosen, since it offers a balance between sensitivity, speed of analysis and reproducibility. Extraction times longer than 30 min did not offer any significant advantages even for individual families of compounds (e.g. mesylates, benzenesulfonates or *p*-toluenesulfonates).

3.4. Effect of pH on extraction efficiency

Altering the pH of the extraction media could not only affect the extraction efficiency for analytes but could also play a key role in minimizing interferences from the extraction. Choosing a pH at which the main component (API) and structurally similar interferences are ionized, could drastically

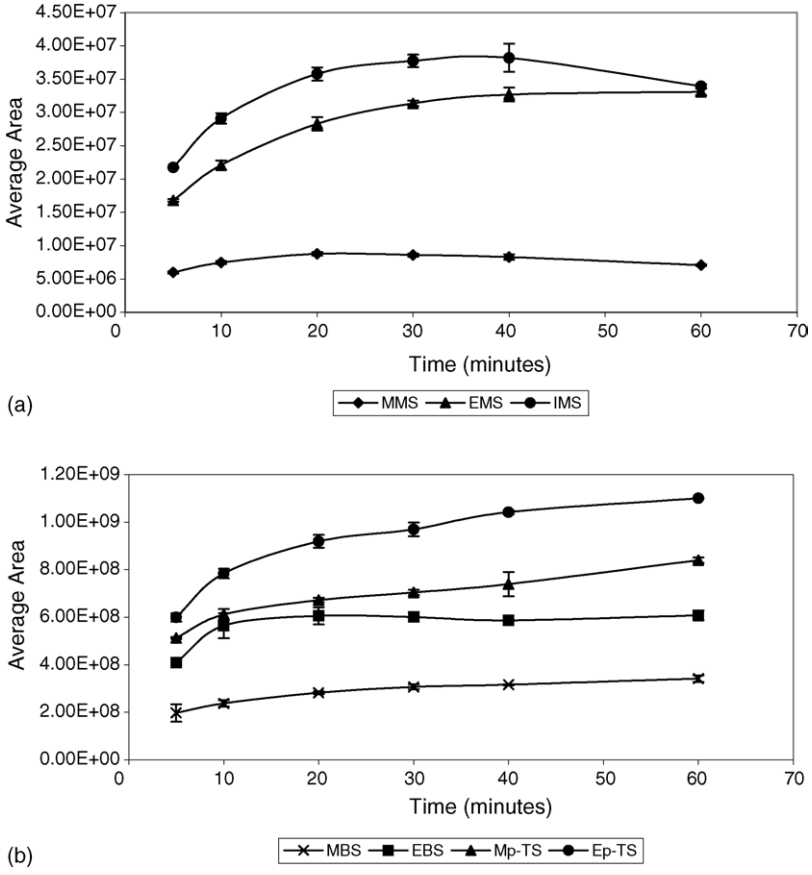

Fig. 3. Extraction time profiles for the PDMS/DVB fiber for 20 mM phosphate buffer (pH 4.7) samples spiked with 500 ppb of MMS, EMS, IMS (a) and MBS, EBS, Mp-TS and Ep-TS (b). Samples were prepared in triplicate and results are shown in two graphs due to significant differences in response factors.

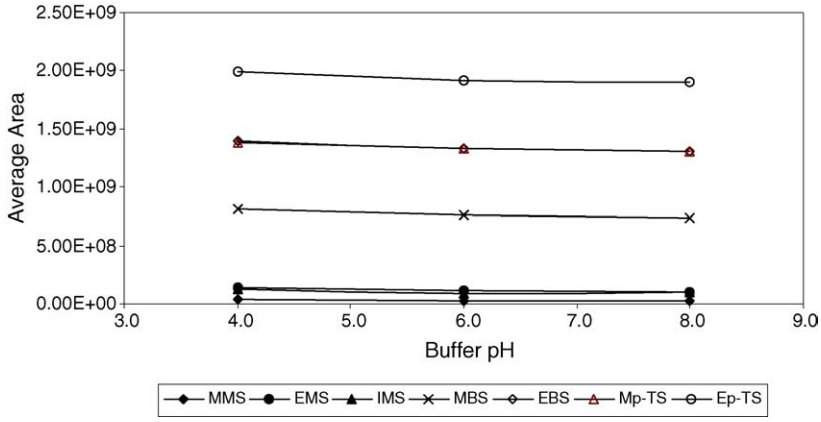

Fig. 4. Effect of pH on the extraction efficiency for 20 mM phosphate buffer (pH 4.7) samples spiked with 500 ppb of the seven sulfonic esters.

minimize interferences in the SPME extraction. Therefore, the extraction efficiency of all seven sulfonate esters was evaluated as a function of pH and the results are summarized in Fig. 4. Buffered samples were prepared utilizing 20 mM KH_2PO_4 or K_2HPO_4 and adjusting to the desired pH with KOH or H_3PO_4. These buffered samples were spiked with 500 ppb of the seven sulfonate esters.

A significant number of mesylate, besylate and tosylate salts could be ionized at lower pH (e.g. ~4). As shown in the graph, there appears to be a slight decrease of the extraction efficiency with increasing pH. However, this difference is not considered significant. As with previous optimization experiments, the less polar besylate and tosylate esters were affected the least by changes in pH with %R.S.D.s within 2% (Ep-TS) and 5% (MBS). For the mesylate esters (with the lower response and extraction efficiencies), the %R.S.D.s ranged between 12% (EMS) and 20% (IMS). It has been demonstrated by this experiment that since the extraction efficiency of the esters is not significantly affected by pH, the analyst performing the analysis has the freedom to use pH as a tool to minimize interferences and to maximize the solubility of the API in the extraction media to obtain a sample suitable for SPME extraction without the use of organic solvents.

3.5. Validation of limit test

As stated, the main objective of this development work is to optimize and validate a limit test for the presence of these esters in API solutions at a 5 ppm level (relative to API). For this purpose, 100 mg of API were accurately weighed and dissolved in 10.0 mL of 20 mM phosphate buffer at a selected pH. Based on our model compounds, pH 4.7 was appropriate to dissolve and ionize the drug substance. Table 1 summarizes some of the physical properties of model APIs used in the performance testing and validation of this method. When 100 mg API are spiked with a relative level of 5 ppm of the esters, the concentration of these esters in the resultant solution for extraction is 50 ppb. At these low concentrations, a 10% R.S.D. in the reproducibility of a method is commonly accepted.

Even though this method is intended to be used as a limit test, its linearity within the 80–120% of the intended concentration was evaluated to evaluate potential saturation problems and the likelihood of false positives/negatives to the test. Although the linearity factor is not crucial for this validation, it is expected that the R^2 values for all esters are close to 0.9. Furthermore, the response of the analytes was evaluated with and without the presence of API to assess method bias and the possibility of reliable external quantitation. As suggested for other types of validations, it would be ideal if the responses at the target concentration of 5 ppm, with and without the API, were within 10%.

The extensive validation work was done with compound 1. For the other compounds, only a response check with and without API was performed prior to the analysis of samples.

(a) Method reproducibility

Five replicate samples of 100 mg compound 1 were spiked with 5 ppm of the esters. After dissolution and extraction, the resulting responses for each analysis were averaged. At this target concentration, all %R.S.D. were <6% (meeting validation requirement) for all sulfonate esters: MMS (5.5%), EMS (3.0%), IMS (3.9%), MBS (3.5%), EBS (1.5%), Mp-TS (3.7%) and Ep-TS (1.7%).

(b) Linearity in the 80–120% range

The method showed to be linear in the 4–6 ppm range with R^2 values ≥0.9 with and without the presence of the API. For the linearity experiment without the API the R^2 values were: MMS (0.99%), EMS (0.99%), IMS

Table 1
Selected physical properties of the active pharmaceutical ingredients (APIs) used in this study

Name	Salt form	pK_a Value	Solubility 20 mM KH_2PO_4 pH 4 (mg/mL)
Compound 1	Mesylate	7.1	12.3
Compound 2	Tosylate	9.4	13.3
Compound 3	Besylate	9.4	>10
Compound 4	Besylate	10.9	>10

(0.90%), MBS (0.99%), EBS (0.98%), Mp-TS (0.99%) and Ep-TS (0.99%). For the linearity in the presence of API: MMS (0.99%), EMS (0.99%), IMS (0.97%), MBS (0.89%), EBS (0.94%), Mp-TS (0.91%) and Ep-TS (0.99%). Although all values were close to 0.9, it was noted that the R^2 values of the linearity with API were, in general, lower with respect to the values obtained without the API present. This result is not surprising, since the presence of API could make integration of the peaks at these low concentrations more challenging. However, these results are remarkable considering the low concentration levels and acceptable for the validation of this limit test. Based on the results, saturation problems are not expected at these concentrations and this method is able to distinguish between a "negative" result (esters concentrations <5 ppm) and a "positive" result for samples with esters present at levels ≥5 ppm.

(c) Bias of the response at the target concentration

The average response of the method (samples were prepared in triplicate) with and without the presence of the API was calculated at the target concentration. These data were obtained from the linearity experiment. All esters gave similar responses with and without the presence of API (within 10%). Individual agreement values were as follows: MMS (99.7%), EMS (104.0%), IMS (96.3%), MBS (97.2%), EBS (93.1%), Mp-TS (92.0%) and Ep-TS (93.1%). Results show that this method could be suitable for external quantitation with spiked control samples run at the time of analysis. This was demonstrated with authentic samples for other compounds as presented in the next section.

3.6. Analysis of API samples

Two samples were accurately weighed (ca. 100 mg) for each API listed in Table 1 and dissolved in 10.0 mL of phosphate buffer (pH 4.7). One of the samples was spiked with 5 ppm of the sulfonic esters (100 μL of 5 ppm stock solution in MeOH) while the second sample was only spiked with 100 μL of MeOH prior to extraction to simulate the exact spiking conditions. Quality control samples were also prepared in triplicate without API to check the reproducibility of the method. For these samples (analyzed prior to API samples), the %R.S.D.s were <2.8%, meeting the 10% R.S.D. requirement of the method. Fig. 5a and b shows representative chromatograms for spiked and unspiked samples of compound 4 (besylate salt). As shown, the sample-related interferences were minimal, allowing accurate determination of the esters in the sample. For all API samples analyzed the corresponding esters were not detected and reported as <5 ppm, not detected.

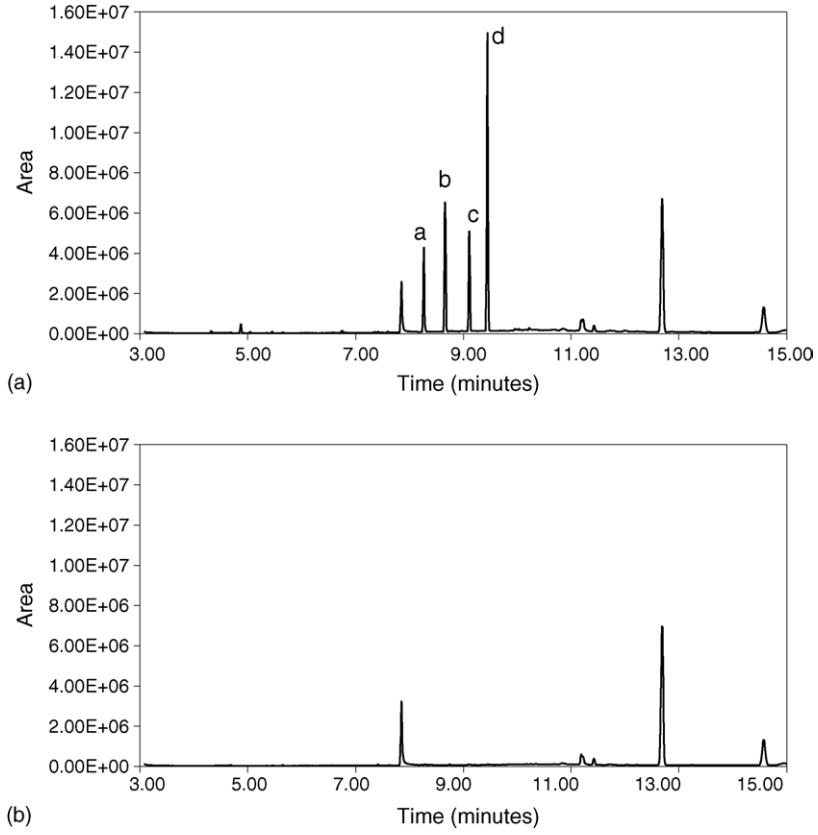

Fig. 5. Sample chromatograms for compound 4 spiked with 5 ppm (relative to API) of (a) MBS, (b) EBS, (c) Mp-TS and (d) Ep-TS (5a) and compound 4 not spiked (5b) extracted with the optimized SPME procedure.

3.7. Applicability of the SPME extraction method

The feasibility of a SPME extraction method followed by GC/SIM-MS analysis for the determination of sulfonic esters in APIs has been demonstrated by the results presented in this study. However, there are some limitations to this method that should be taken into consideration, mostly related to the efficient removal of interferences and the dissolving solvents used. Adjustment of pH is crucial for the ionization of potential interferences. In cases where the API or similar process-related impurities cannot be ionized in the pH range from 4 to 9 (working range of current commercial SPME fibers for direct immersion), more development effort must be spent in eliminating potential interferences. Another factor to consider is the aqueous solubility of the API to be analyzed. In preliminary feasibility studies, it has been demonstrated that this SPME extraction procedure is not compatible with the use of organic solvents when the organic percentage exceeds 5%. Therefore, the API needs to be soluble in aqueous solutions of pH ~4 at a concentration of approximately 10 mg/mL for a 5 ppm limit test. A large number of mesylate, besylate or tosylate salts meet this requirement. The present studies are being conducted adding 1% (of a 10.0 mL total sample volume) of organic solvent (methanol, acetone, etc.) for the spiking experiments and to help in the dissolution of the sample. A significant increase in baseline noise and artifact peaks has been observed for samples that are not completely dissolved when extracted.

Other extraction techniques should be considered in cases where API does not meet one or more of the requirements previously discussed. Initial development results have shown the feasibility of solid-phase extraction (SPE) and liquid-phase microextraction (LPME) for the sensitive extraction and detection of these esters.

(a) Solid-phase extraction (SPE)

Based on the polarity of the analytes, polymeric and more "universal" SPE cartridges were included in this evaluation. Spiked phosphate buffer samples (500 ppb) were loaded into Strata-X, Strata-SDB, Strata-Phenyl and Oasis-HLB SPE cartridges. All cartridges gave similar results, with recovery values higher than 85%. These recovery experiments were repeated using 80:20 (%, v/v) KH_2PO_4 (pH 4.7):Acetonitrile as the spiked sample. As expected, the addition of 20% acetonitrile to the dissolving solvent drastically decreased the extraction efficiency. The more universal phases such as Strata-X and Oasis-HLB gave the lowest recovery values (<10%), while the polymeric phases Strata-SDB and Strata-Phenyl, still retained the analytes but with recovery values of approximately 50%. Acetone, methanol, acetonitrile and THF were evaluated as solvents for the elution of the cartridges. For the Strata-SDB and Strata-Phenyl cartridges, acetone and methanol gave similar results and higher recovery values, followed by THF and acetonitrile. Despite the lower recovery values, this method was able to extract the esters reproducibly at the 5 ppm concentration in APIs. This method also proved to be linear for ester concentrations between 2.5 and 50 ppm (relative to API) with R^2 values greater than 0.9. Further improvements in sensitivity could be achieved by adding a solvent concentration step prior to GC analysis. These development results demonstrate that SPE could potentially offer an alternative to the SPME extraction procedure in cases where the API is not highly soluble in 100% phosphate buffer and require some addition of organic solvent. SPE cartridges are commercially available in a wide variety of phases for various elution modes that could also offer advantages in terms of selectivity and pre-concentration. Further validation work will assess the viability of this approach for the routine analysis of pharmaceutical samples.

(b) Liquid-phase microextraction (LPME)

The polar nature of the samples to be extracted require a low polarity extraction solvent inside the hollow fiber to prevent mixing of the two solvents and further leaching of the extraction solvent into the sample dissolving solvent. For this reason, 1-octanol, toluene and dichloroethane are preferred choices of organic solvents to perform the extraction. This may pose a challenge for the determination of the more polar methanesulfonic esters. The high boiling point of 1-octanol caused numerous interferences at the retention time of the methanesulfonic esters. A decrease in S/N ratio was also observed for the benzenesulfonic and *p*-toluenesulfonic esters due to higher baseline noise with 1-octanol as the extraction solvent. With toluene as extraction solvent, the benzenesulfonic and *p*-toluenesulfonic esters were efficiently extracted and as expected, no signal was observed for the methanesulfonic esters. The concentration factors for the extracted analytes were between 81% and 261% and as observed for SPME, the highest efficiencies and reproducibility values were obtained for the less polar tosylate esters. The mesylate esters were extracted with dichloroethane. This solvent also resulted in high extraction efficiency values and concentration factors ranging from 189% to 247%. These preliminary results demonstrate the ability of this technique to achieve the target quantitation limit of 5 ppm relative to the API. This technique could have limitations in terms of the addition of organic solvent to the sample, but its working pH range may be broader than for SPME.

4. Conclusion

A limit test for the determination of methyl and ethyl esters of methanesulfonic (including isopropyl mesylate), benzenesulfonic and *p*-toluenesulfonic acids at the 5 ppm level in active pharmaceutical ingredients was successfully developed and validated. This method used direct immer-

sion SPME for the extraction of the analytes from the sample matrix and SIM-MS detection for improved sensitivity. This extraction procedure is a "universal" method for sulfonic acid esters determination in APIs and potentially has numerous advantages over liquid–liquid extraction in terms of speed and sensitivity. Interferences from the sample matrix were successfully minimized by ionization at lower pH values. This method proved to be reproducible and linear, suitable for routine pharmaceutical analysis.

The capabilities of other extraction techniques such as SPE and LPME have also been demonstrated for the determination of these sulfonic esters, which could provide viable alternatives to the SPME methodology in cases where the API has limited solubility in aqueous matrices.

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