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Short communication

A practical derivatization LC/MS approach for determination of trace level alkyl sulfonates and dialkyl sulfates genotoxic impurities in drug substances

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ABSTRACT

Derivatization LC/MS methodology has been developed for the determination of a group of commonly encountered alkyl esters of sulfonates or sulfates in drug substances at low ppm levels. This general method uses trimethylamine as the derivatizing reagent for ethyl/propyl/isopropyl esters and triethylamine for methyl esters. The resulting quaternary ammonium derivatization products are highly polar (ionic) and can be retained by a hydrophilic interaction liquid chromatography (HILIC) column and readily separated from the main interfering active pharmaceutical ingredient (API) peak that is usually present at very high concentration. The method gives excellent sensitivity for all the alkyl esters at typical target analyte level of 1–2 ppm when the API samples were prepared at 5 mg/mL. The recoveries at 1–2 ppm were generally above 85% for all the alkyl esters in the various APIs tested. The injection precisions of the lowest concentration standards were excellent with R.S.D. = 0.4–4%. A linear range for concentrations from 0.2 to 20 ppm has been established with $R^2 \geq 0.99$. This general method has been tested in a number of API matrices and used successfully for determination of alkyl sulfonates or dialkyl sulfates in support of API batch releases at GlaxoSmithKline.

1. Introduction

Methanesulfonic, benzenesulfonic, *p*-toluenesulfonic and sulfuric acids are commonly used acids for salt formation of active pharmaceutical ingredients (API) or employed as reagents in synthesis. Methanol, ethanol, propanol or isopropanol, on the other hand, are frequently used as solvents for crystallization or purification of drug substances. Interactions between the sulfonic acids (or sulfonyl chlorides) and the alcohols could lead to the formation of their corresponding alkyl esters. The presence of trace level of the alkyl esters of these acids in drug substance or drug product is of genotoxicity concern and has been closely scrutinized by regulatory agencies and pharmaceutical industries [1]. The 'threshold of toxicological concern' (TTC) of 1.5 μg/day (exposure of genotoxic impurity in drugs that will be tested or dosed for longer than 12 months) has been suggested by the European Medicines Agency's (EMEA) "Guideline on the limits of genotoxic impurities" [2] and the PhRMA's white paper [3]. The 'staged' TTC in the later is believed to be the likely basis for the FDA's guideline which will appear soon. Based on the TTC, the concentration limits of genotoxic impurity in drug substances or drug products can then be derived based on the maximum daily dose: concentration limit (ppm) = [1.5 μg/day]/[dose (g/day)]. For a drug dosed at 1 g/day, for example, 1.5 ppm would be the limit of a specific genotoxic impurity which would also be the 'target analyte level' (TAL) from an analytical perspective. Given such a low ppm concentration limit, besides the control challenges in process chemistry, developing sensitive and robust methodology for their detection poses a tremendous analytical challenge for the pharmaceutical industry [4].

A comprehensive summary of the analytical challenges on the determination of various alkyl esters of alkyl and aryl sulfonates has been presented recently [5]. Direct injection GC/FID [6] or direct injection GC/MS [7,8] methods, including derivatization followed by direct injection GC/MS [9], have been reported for the determination of volatile molecules of this structural class including methyl methanesulfonate (MMS), ethyl methanesulfonate (EMS), etc. For ppm-level detection, however, injection of very high concentrations of drug substance onto a GC column is unavoidable; thus direct injection GC methods suffer from severe contamination issues. In addition, because of the high reactivity of these molecules (alkylation) and the presence of high concentration of API, the alkyl sulfonates often react with API in the GC liner which is generally heated. Therefore, method recoveries are often a major issue for such trace analyses. To overcome various limitations of the reported methods (poor sensitivity, poor specificity, or low recovery due

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to contamination or decomposition), Alzaga et al. recently developed a derivatization headspace (HS) GC/MS method [10]. This general method stabilizes the analytes by using pentafluorothiophenol as the derivatizing reagent causing the analytes to be more amenable to HS sample introduction in order to minimize liner or column contamination. However, technical issues such as trace level interference of pentafluorothiophenol methyl derivative in the blanks and variable recoveries depending on the sample matrices were experienced. Isotopically labeled internal standards appear to be required to compensate the low recovery for some alkyl esters.

We report herein a derivatization LC/MS general approach that had been developed at GlaxoSmithKline (GSK) during a similar time frame to Alzaga et al. developing the derivatization HSGC/MS method [10]. The derivatization LC/MS method has been used at GSK successfully for several years. Compared to GC capillary columns, LC columns typically can tolerate much higher sample loading; thus system contamination is less of issue. Direct analysis of sulfonate alkyl esters by LC/MS seemed to be achievable for some arylsulfonates and good recoveries have been demonstrated [11]. However, a major drawback of the direct analysis method is the poor stability of some analytes in an aqueous environment, i.e., sample degradation occurs during the course of analysis. It is expected that direct analysis of alkyl esters of alkylsulfonates would be more problematic. In order to develop an LC/MS amenable method that is general to common alkyl esters of both aryl and alkylsulfonates, employing a derivatization strategy to stabilize the analytes before LC/MS analysis seems necessary.

2. Experimental

2.1. Reagents

Methyl methanesulfonate (MMS), ethyl methanesulfonate (EMS), methyl *p*-toluenesulfonate (MTS), dimethyl sulfate (DMS), ethyl *p*-toluenesulfonate (ETS), diethyl sulfate (DES) were purchased from Sigma–Aldrich (Milwaukee, WI, USA). Propyl methanesulfonate (PMS), propyl benzenesulfonate (PBS) and isopropyl benzenesulfonate (IPBS) were obtained from Wilmington PharmaTech (Wilmington, DE, USA). Methyl benzenesulfonate (MBS), propyl benzenesulfonate (PBS) and di-*n*-propyl sulfate (DPS) were supplied by TCI American (Portland, OR, USA). Ethyl benzenesulfonate (EBS) and isopropyl methanesulfonate (IPMS) were purchased from PFALTZ & BAUER, Inc. (Waterbury, CT, USA). Isopropyl *p*-toluenesulfonate (IPTS) was purchased from Wako Chemicals USA (Richmond, VA, USA). Diisopropyl sulfate (DIPS) was obtained from TCI-EP (Tokyo Kasei, Japan). Trimethylamine (25% in water), triethylamine and ammonium formate were obtained from Aldrich (Milwaukee, WI, USA). Formic acid was purchased from Fluka (Milwaukee, WI, USA). Acetonitrile (HPLC grade) was purchased from Burdick & Jackson (Morristown, New Jersey, USA). All water used in the experiment was purified by an in-house Milli-Q system (Millipore, Billerica, MA, USA). All drug substances used for validation and testing were obtained from current projects at GlaxoSmithKline and prepared in house.

2.2. LC/MS conditions

An Agilent 1100 HPLC/MSD system was used. Chromatographic separations were achieved on a Waters Atlantis HILIC silica (3 μm, 50 mm x 2.1 mm, part no. 186002011) column (Waters, Milford, MA, USA). The column temperature was set to 35 °C with an isocratic elution using a combination of mobile phases of 85% A (acetonitrile, weak solvent) and 15% B (water with 50 mM ammonium formate and 0.1% (v/v) formic acid, strong solvent) at a flow rate of 0.3 mL/min. At 6.8 min after each injection, the flow rate was ramped to 1 mL/min to flush the column for 5 min. With this elution profile, the main interfering API peaks typically elute near the void; therefore, MSD data collection can start 3 min after injection. The MSD was operated in the electrospray ionization (ESI) positive ion mode with the capillary voltage set to 3 kV. The fragmentor was set to 70 V. The drying gas flow was 10 L/min with a temperature of 350 °C. The derivatization products of the alkyl sulfonates were detected by single ion monitoring (SIM) at *m*/*z* 88, 102 or 116 for ethyl trimethyl ammonium, (iso)propyl trimethyl ammonium, and methyl triethyl ammonium, respectively.

2.3. Preparation of standard and sample solutions

The stock solutions of all alkyl sulfonates or dialkyl sulfates were prepared at approximately 1 mg/mL in pure acetonitrile. For linearity validation, the stock solutions of alkyl sulfonates were diluted using HPLC-grade acetonitrile to give standards at 1, 5, 10, 50, and 100 ng/mL, respectively. The testing API samples were typically prepared at approximately 5 mg/mL in HPLC-grade acetonitrile. The solutions used for method recovery tests were prepared by dissolving 5 mg drug substance in 1 mL of 5 or 10 ng/mL alkyl sulfonates standard solutions. These correspond to 1 or 2 ppm concentration of analytes, respectively, which is the typical 'target analyte level' (TAL).

2.4. Derivatization procedures

An aqueous solution of 10% (v/v) triethylamine was used for derivatizing methyl sulfonates or dimethyl sulfates, whereas an aqueous solution of 10% (v/v) trimethylamine was used for all others including ethyl, propyl or isopropyl esters of sulfonates or sulfates. The derivatization reactions were carried out by adding 100 μL of derivatizing agent into 2-mL HPLC vials that contain standards or samples in 1 mL ACN (sometimes up to 20% water can been used as solvents to improve the API solubility). All vials were capped tightly, vortexed, and then heated at 50–60 °C for 60 min. Upon completion of the reaction, the vials containing the corresponding quaternary ammonium derivatization products were subject to LC/MS analysis directly. A typical injection volume of 5 μL was used, which can be increased to improve the method sensitivity if desired.

3. Results and discussion

Two factors impairing the accurate determination of trace level alkyl sulfonates and sulfates in pharmaceutical products by LC/MS are their high reactivity (poor stability) and poor ionization potential (low sensitivity). For the former, any nucleophiles present in the sample matrix, such as high concentration of API or unknown impurities can potentially decompose the alkyl esters. For these reasons, developing a highly selective derivatization procedure to transform the alkyl esters into stable products with enhanced ionization efficiency is an attractive approach. In order for the method to be 'general', several important criteria should be considered. Firstly, for the method to have true applications it should offer sustainable sensitivity and specificity for all 16 possible alkyl esters (Fig. 1) in the presence of various APIs. Secondly, generic chromatographic conditions must provide adequate separation: all the analyte peaks (derivatization products) should be sufficiently resolved from the main interfering API peaks as well as unknown impurities. The later is more easily said than done because the impurities are often unknown. Nonetheless, achieving this selectivity seems very important for trace level method since the LOD

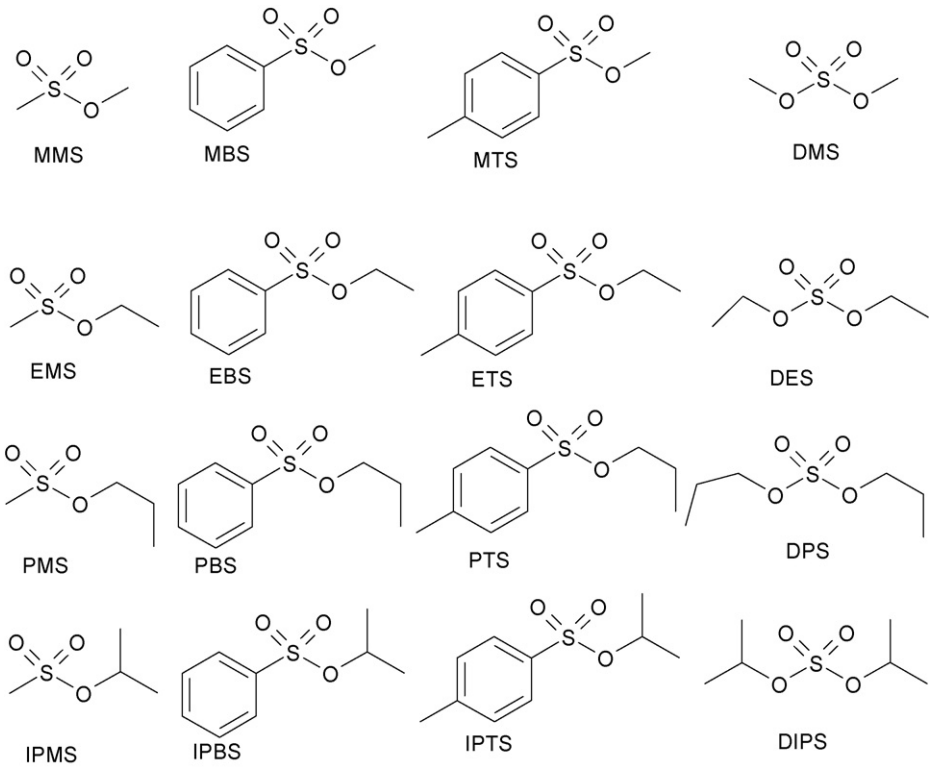
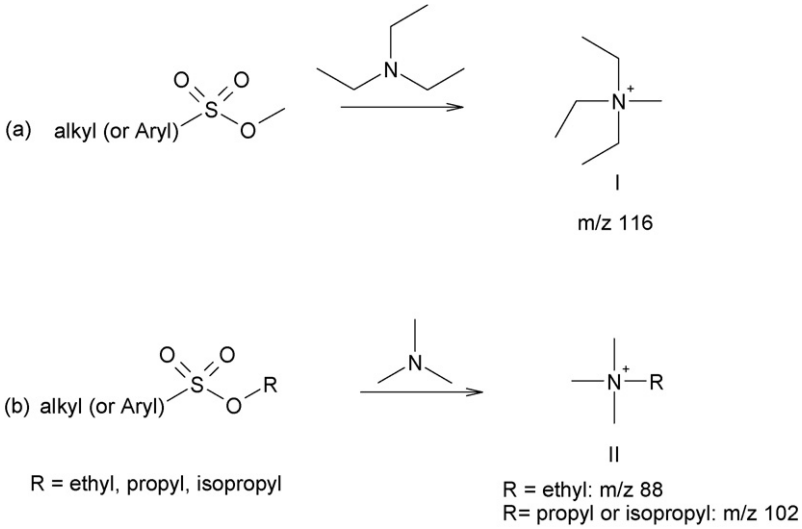


Fig. 1. Chemical structures of 16 most commonly encountered alkyl sulfonates and dialkyl sulfates.

is often compromised by method specificity. Based on the above considerations and taking advantages of the electrophilicity that is common to all 16 alkyl esters, converting the analytes into quaternary ammonium by trialkylamine offers an attractive way forward. Besides the benefit of stabilizing the alkyl esters, trialkylamine derivatization generates positively charged quaternary ammonium ions that also enhance mass spectrometric detection in ESI MS in the positive ion mode. By using a HILIC column, ionic quaternary ammonium derivatization products are readily separated from relatively non-polar APIs and impurities using a generic method. This derivatization procedure followed by LC/MS SIM detection affords greater method specificity.

3.1. Derivatization

The derivatization procedure developed here takes advantage of the chemical reactivity of all 16 alkyl esters. The relatively labile alkyl esters are transformed into stable quaternary ammonium cations that are well suited for MS detection. The highly reactive methyl sulfonates and dimethyl sulfates can be readily derivatized by triethylamine to give methyl triethyl ammonium (**I**) as shown in Scheme 1(a). Triethylamine, rather than trimethylamine, was used in this instance because that the later reagent contains tetramethyl ammonium interference. It is worth noting that the triethylamine reagent blank also contains (or produces)



Scheme 1. Illustration of derivatization reactions converting the alkyl sulfonates into corresponding quaternary ammonium ions for LC/MS detection of (a) methyl triethyl ammonium at *m*/*z* 116 and (b) ethyl trimethyl ammonium at *m*/*z* 88 and (iso)propyl trimethyl ammonium at *m*/*z* 102.

trace amounts of methyl triethyl ammonium, the anticipated derivatization products of methyl sulfonates. The level of the interference presented at approximately 10% of TAL of 1–2 ppm typically when 100 μl of the derivatizing regent was used and this appeared to be quite reproducible. Therefore, subtraction of peak area of the interference in the reagent blank from that of the standards or samples can be applied as a correction if desired. This approach was proven to be successful for limit tests of dimethyl sulfonates in house. The less reactive ethyl, propyl or isopropyl esters, on the other hand, can be derivatized by trimethylamine affording ethyl trimethyl ammonium, propyl trimethyl ammonium, or isopropyl trimethyl ammonium cations (**II**), respectively (Scheme 1(b)).

The corresponding quaternary ammonium derivatization products are positively charged and are thus well suited for mass spectrometric detection in ESI positive ion mode. Ethyl and (iso)propyl trimethyl ammonium were monitored at *m*/*z* 88 and 102, respectively, while methyl triethyl ammonium was monitored at *m*/*z* 116. The derivatization procedure is simple to perform and the derivatizing reagents, triethylamine and trimethylamine, are readily available at minimal cost. An additional advantage of converting alkyl sulfonates to the corresponding quaternary ammonium cations is that the later are highly polar and can thus be easily separated from the main interfering API peaks chromatographically.

As for the method specificity with regard to the derivatization reaction, it is expected that other alkylating agents such as alkyl halide, if present, would interfere with the analysis. If risk assessment shows this potential, the interference would need to be evaluated using a different method such as headspace GC/MS (or ECD) methods.

3.2. Hydrophilic interaction liquid chromatography LC/MS

The quaternary ammonium derivatization products are so polar that typical reversed-phase LC columns could not retain the compounds well. Hydrophilic interaction liquid chromatography (HILIC) is a special case of normal phase chromatography used primarily for separation of very polar compounds [12–14]. The typical mobile phase for HILIC chromatography includes acetonitrile with minimal water content. It is generally believed that HILIC column retains analytes through the partitioning of the analytes between the water-rich layer of the hydrophilic stationary phase and the hydrophobic mobile phase. Thus the more polar the compound is (e.g., ionic compound) the better it is retained. For the derivatization products in this experiment, all APIs and their impurities are relatively non-polar and are thus not retained but eluted in the solvent front. The derivatization products of the alkyl sulfonates, positively charged quaternary ammonium ions, on the other hand, were selectively retained and separated on the HILIC column. Fig. 2 shows the typical SIM chromatograms of the four quaternary ammonium derivatization products (a) methyl triethyl ammonium of *m*/*z* 116 at 4.6 min for monitoring dimethyl sulfate at 1 ng/mL, (b) ethyl trimethyl ammonium of *m*/*z* 88 at 4.9 min for monitoring ethyl methanesulfonate at 1 ng/mL, (c) propyl trimethyl ammonium of *m*/*z* 102 at 4.4 min for monitoring dipropyl sulfate at 1 ng/mL, and (d) isopropyl trimethyl ammonium of *m*/*z* 102 at 5.6 min for monitoring di-isopropyl sulfate at 10 ng/mL. The superior selectivity achieved by HILIC separation greatly improves method specificity. Although a Waters Atlantis HILIC column was used for the current method, it is believed that equivalent columns by other manufacturers can also be employed as long as similar method specificity can be demonstrated.

3.3. Validation results

The linearity, limit of detection, injection precision, and recovery of the method were evaluated, and the validation results are summarized in Table 1. A linear range from 1 to 100 ng/mL (equivalent to 0.2–20 ppm relative to 5 mg/mL API samples) was demonstrated for all analytes with $R^2 \geq 0.99$. By calculation based on the S/N ratio of the lowest standards, the LOD's for many alkyl esters are one order of magnitude lower. For methyl sulfonates or dimethyl sulfate, however, interference of methyl triethyl ammonium ion in the reagent blank was encountered. It presented around 10% of the typical TAL of 1–2 ppm when 100 μl of the derivatizing regent was used.

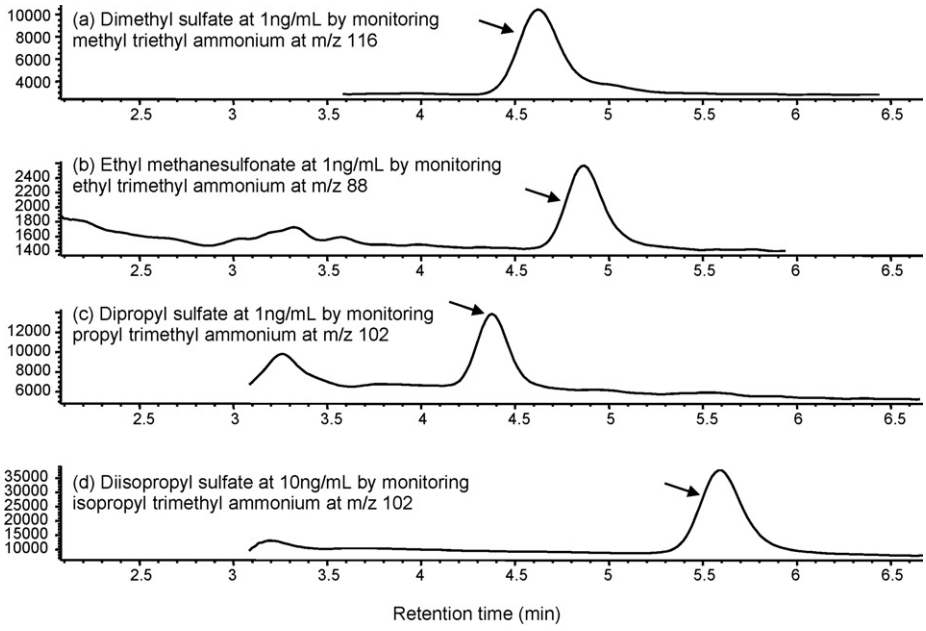


Fig. 2. Typical LC/MS chromatograms showing the peaks of the four quaternary ammonium derivatization products: (a) methyl triethyl ammonium of *m*/*z* 116 at 4.6 min for monitoring dimethyl sulfate at 1 ng/mL, (b) ethyl trimethyl ammonium of *m*/*z* 88 at 4.9 min for monitoring ethyl methanesulfonate at 1 ng/mL, (c) propyl trimethyl ammonium of *m*/*z* 102 at 4.4 min for monitoring dipropyl sulfate at 1 ng/mL, and (d) isopropyl trimethyl ammonium of *m*/*z* 102 at 5.6 min for monitoring di-isopropyl sulfate at 10 ng/mL.

Table 1
Summary for the validation results of 16 alkyl sulfonates and dialkyl sulfates in various APIs

Alkyl sulfonates	Linearity (R^2)	Injection precision[a] R.S.D. (%)	Recovery[b] (%)	API[c]
DMS	0.9985	±0.5	102	GW786XXX
MMS	0.9949	±0.6	85	SB751XXX
MBS	0.9992	±0.6	88	SB751XXX
MTS	0.9999	±2.3	113	SB751XXX
DES	0.9999	±0.4	103	SB751XXX
EMS	1	±2.7	110	GW813XXX
EBS	1	±2.6	137	SB751XXX
ETS	0.9995	±2.0	94	SB751XXX
DPS	0.9916	±0.8	92	SB462XXX
PMS	0.9902	±0.8	94	SB751XXX
PBS	0.9942	±0.7	74	SB751XXX
PTS	0.9988	±0.4	86	GSK424XXX
DIPS	0.9997	±0.8	99	SB462XXX
IPMS	0.9978	±3.4	105	SB462XXX
IPBS	0.9982	±0.9	112	SB462XXX
IPTS	0.9885	±1.7	106	GSK424XXX

[a] The injection precisions were determined using standards at 1–2 ppm (typical TAL).
[b] The recoveries were evaluated by spiking 1–2 ppm of alkyl esters into various APIs.
[c] The compound numbers were masked partially due to proprietary reasons.

To improve the assay accuracy, subtraction of the level of the interference from the standards or samples can be considered since the peak size was reproducible.

Typical recoveries at TAL of 1–2 ppm were all above 85% except for PBS which is 74% (Table 1). The relatively lower recovery of PBS is possibly due to the stronger competitive reaction from the amino and hydroxyl groups of the specific API used in the validation. Increasing the amount of trimethylamine derivatizing reagent is expected to improve the recovery. Nonetheless, for trace level detection of reactive analytes, 75% recovery is generally accepted as reasonable. Relatively high recovery (137%) was noticed for EBS when spiked into the SB751XXX API. The underlying reason remains to be understood. The injection precisions evaluated using the standards at TAL of 1–2 ppm were excellent (R.S.D. = 0.4–4%). This general derivatization LC/MS method has been used successfully for determination of dimethyl sulfate, diethyl sulfate, ethyl methanesulfonate and propyl *p*-toluenesulfonate in support of batch releases for four different GlaxoSmithKline APIs, respectively. It is worth mentioning that these alkyl sulfonates or sulfates were not detected in any of the APIs tested using the current method. The current debate on whether some of these are hypothetical impurities (i.e., what's the likelihood of some of the alkyl sulfonates or sulfates surviving the chemical process) is indeed warranted.

4. Conclusions

A general derivatization LC/MS methodology has been developed for determination of a class of commonly encountered alkyl esters of sulfonates or sulfates. The method uses trialkylamines as the derivatizating agents and converts alkyl esters into the stable quaternary ammonium ions for detection. The polar ionic nature of the derivatization products offers an excellent attribute for their chromatographic separation and detection. By employing an Atlantis HILIC column, major interfering API or unknown impurity peaks are readily washed away in the solvent front. Removing these major interferences that are typically present in extremely high concentrations prior to the MSD help maintain the cleanness of the MS source, thus minimizing contamination. Furthermore, the positively charged quaternary ammonium derivatization products are ideal for ESI MS detection in the positive ion mode. This method offers a general strategy for detecting various alkyl sulfonates or dialkyl sulfates in drug substances or drug products in support of drug development.

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