Genotoxische Sulfonat Ester in Pharmazeutika – eine schnelle Analytik auf Rxi-5Silms und Rtx-200 Säulen

Sulfonat Ester (Mesylate, Besylate & Tosylate) können als Syntheserückstände in Pharmazeutika vorhanden sein. Innerhalb von 4.5 min ist es möglich, diese Komponenten mit einer Splitinjektion auf der Rxi-5Silms zu analysieren. Wenn auch die Analyse von Alkyhaliden ansteht – hier ist es möglich auf der mittelpolaren und dennoch temperaturstabilen Dickfilm Rtx-200 beide Substanzklassen zu trennen. Sie finden hier auch Hinweise zu einer einfachen Probenvorbereitung und Tipps zur besseren Löslichkeit.

Two Options for Analyzing Potential Genotoxic Impurities in Active Pharmaceutical Ingredients

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Laboratory needs for analyzing PGIs in API vary. Here we developed both a fast analysis of sulfonate esters on the Rxi®-5Sil MS column, and a comprehensive method for sulfonate esters and alkyl halides on the Rtx®-200 column.

Compounds that are used in the synthesis of active pharmaceutical ingredients (API), or reaction byproducts that form during synthesis, have the potential to remain as impurities. Some of these compounds are potentially genotoxic impurities (PGI) and may raise concern about cancer and/or birth defects.

Scientists from Merck, in collaboration with Restek, have developed a fast method for the analysis of sulfonate esters on the RXI® - 5 Sil MS column.

Four structural classes of PGIs are discussed in this article. The first three classes, known collectively as sulfonate esters, include mesylates, besylates, and tosylates (Figure 1). These alkylating sulfonic acid esters may form when sulfonic acid reacts with an alcohol solvent. The first three classes are differentiated by the group that forms an ester with the sulfur: mesylates contain a methyl group, besylates contain a phenyl (benzyl) group, and tosylates contain a toluene group. The fourth class of PGIs tested here, alkyl halides, consists of short alkyl chains with halogen constituents. Since alkyl halides are polar and very volatile, they are not retained well on thin film stationary phases. This can make analysis of a mixture of sulfonate esters and alkyl halides quite problematic.

Two options for the analysis of PGIs in API have been developed to meet different laboratory needs. The first option is a fast method for the analysis of sulfonate esters on the Rxi®-5Sil MS column. The second option is a comprehensive method for the analysis of both sulfonate esters and alkyl halides on the Rtx®-200 column. Both methods require very little sample preparation, which helps increase laboratory productivity.

Option 1: Fast Analysis of Sulfonate Esters

Scientists from Merck, in collaboration with Restek, have developed a fast method for the analysis of sulfonate esters on the Rxi®-5Sil MS column. Since PGIs can differ dramatically from one another, several types of API were spiked and analyzed to ensure the robustness of the method. Figure 2 shows an example of API spiked at 1ppm, which is the threshold for toxological concern (TTC) for PGIs in API as set by the EMEA. Depending on the dose of API to the patient, it may be necessary to detect levels of impurities as low as 1ppm in order to meet requirements. A linearity study was also performed and shows that this method is linear for sample concentrations from 1ppm to 1,000ppm in API (Figure 3).

The use of a thin film Rxi®-5Sil MS column allows for fast analysis of active PGI compounds. Since the Rxi®-5Sil MS column is very selective toward sulfonate esters, a fast oven program can be used to speed analysis. This method allows for the analysis of selected sulfonate esters in less than 4.5 minutes. However, note that since the Rxi®-5Sil MS column is a mid-polarity stationary phase, the use of polar sample solvents, such as methanol, is not recommended. Due to the polarity of this phase, splitless injections of sample solvents with water may cause peaks to split. The method shown here utilizes a 10:1 split, which allows for the injection of relatively polar sample solvents, such as 90:10 acetonitrile:water.

Figure 1 Sulfonate ester PGIs. Differences between sulfonate esters and alkyl halides make analysis of mixtures challenging.

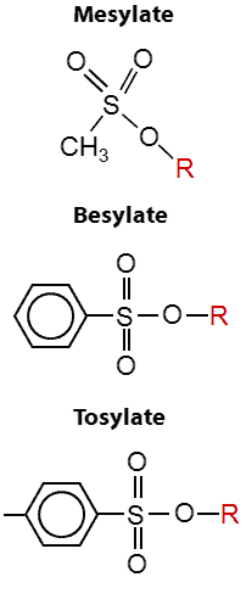
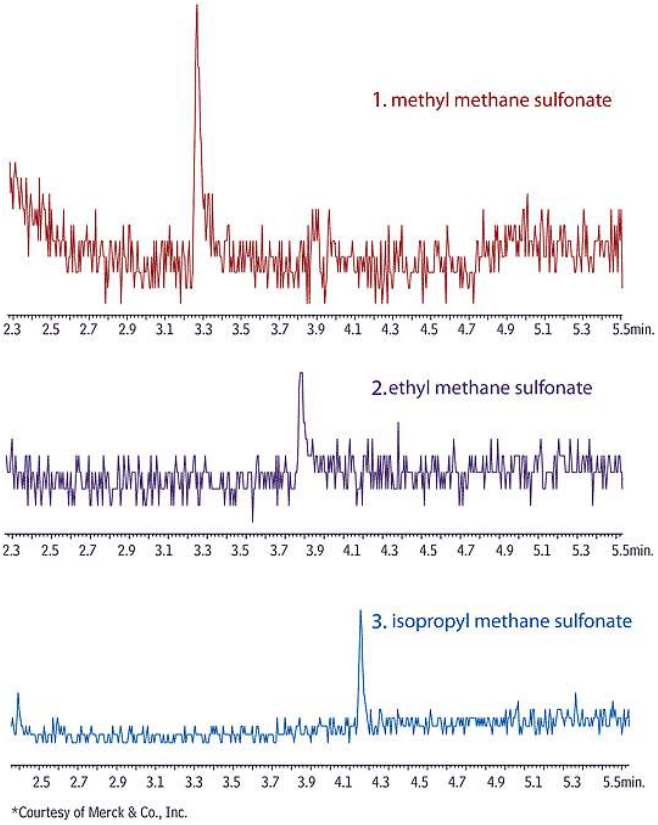


Figure 2 Fast separation of sulfonate ester PGIs in API (1ppm).



*Courtesy of Merck & Co., Inc.

GC_PH01072A

Column	Rxi®-5Sil MS, 20 m, 0.18 mm ID, 0.18 µm (cat.# 43602)
Sample	API
Diluent:	90:10 acetonitrile:water
Conc.:	10 mg/mL
Injection	
Inj. Vol.:	1.0 µL split (split ratio 10:1)
Liner:	Gooseneck Splitless (4mm) w/Wool (cat.# 22405)
Inj. Temp.:	250 °C
Oven	
Oven Temp.:	50 °C (hold 1 min) to 150 °C at 20 °C/min to 250 °C at 50 °C/min
Carrier Gas	He, constant pressure (10.19 psi, 70.3 kPa)
Detector	MS
Mode:	SIM
SIM Program:	80, 109, 123 m/z
Transfer Line Temp.:	280 °C
Ionization Mode:	EI
Acknowledgement	Merck & Co., Inc

Figure 3 Linearity of fast GC/MS analysis for selected sulfonate esters.

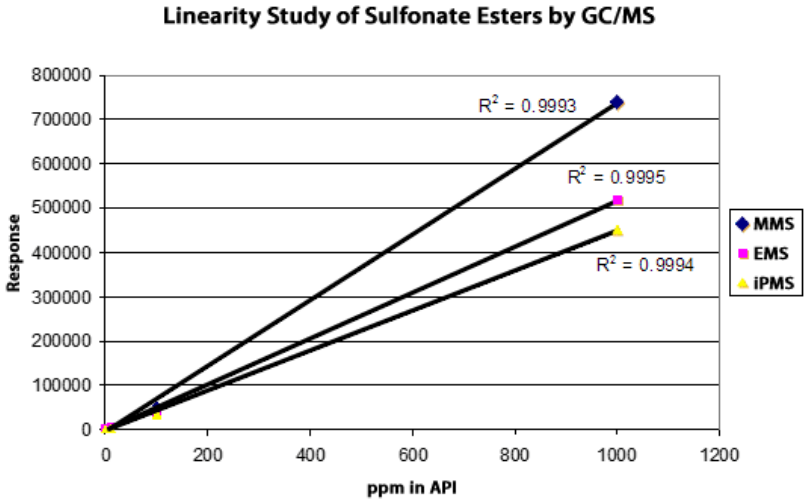


Table I Excellent linearity is also obtained using the comprehensive PGI methodology.

Compound Name	R-squared Value
dimethyl sulfate (DMS)	0.9984
methyl methanesulfonate (MMS)	0.9988
ethyl methanesulfonte (EMS)	0.9988
isopropyl methanesulfonate (iPMS)	0.999
diethyl sulfate (DES)	0.999
diisopropyl sulfate (DPS)	0.9993
di-*n*-butyl sulfate (DBS)	0.9996
methylbenzene sulfonate (MBS)	0.9991
benzenesulfonic acid ethyl ester (EBS)	0.9995
methyl-*p*-toluenesulfonate (MTS)	0.9996
p-toluenesulfonic acid ethyl ester (ETS)	0.9996
p-toluenesulfonic acid *n*-propyl ester (nPTS)	0.9994
benzenesulfonic acid *n*-butyl ester (nBBS)	0.9995
isopropyl *p*-toluenesulfonate (iPTS)	0.9996
p-toluenesulfonic acid *n*-butyl ester (nBTS)	0.9994

8-point standard curves, 1-1,000ng/mL (equivalent to 1-1,000ppm in API matrix)

Option 2: Comprehensive Method for Analysis of PGIs

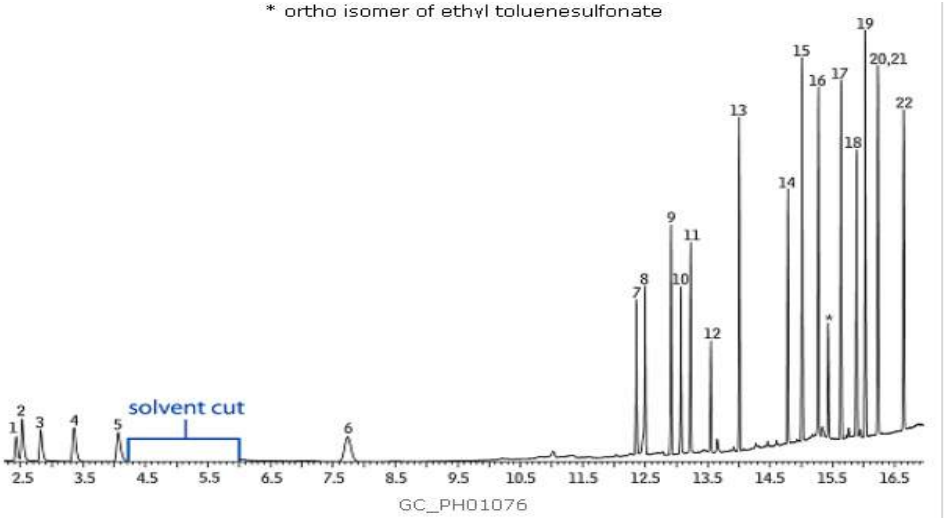
Although the thin film Rxi®-5Sil MS column allows for fast analysis of sulfonate esters, the smaller, more polar alkyl halides are not well retained. To take advantage of the halogen constituents on the alkyl halides, a thick film Rtx®-200 column was used to develop this comprehensive method for both volatile alkyl halides and less volatile sulfonate esters. Since the Rtx®-200 column has a fluorinated stationary phase, the alkyl halides are well-retained (Figure 4). Note that all of the alkyl halides elute at a low temperature and some of the more volatile compounds elute prior to the sample solvent (acetonitrile). Because of this, the solvent cut time must be carefully measured. The Rtx®-200 column is also selective for sulfonate esters, providing baseline resolution for 20 out of 22 of the compounds analyzed. Additionally, the increased polarity of the fluorinated Rtx®-200 phase allows for the use of splitless injection of more polar sample solvents, such as methanol.

If the analysis of alkyl halides is not a laboratory concern, a thin film Rtx®-200 column may be used for faster analysis of sulfonate esters. A linearity study was conducted to ensure that this method is linear over the expected range of sulfonate esters in API. Samples were prepared at 8 concentrations ranging from 10ng/mL to 10,000ng/mL in 90:10 ACN:H2O. This is equivalent to 1-1,000ppm in API for samples prepared at 10mg of API/mL of sample solvent. This method shows acceptable linearity for all 15 sulfonate esters analyzed (Table I).

Figure 4 Small, polar alkyl halides are well-retained on the fluorinated Rtx®-200 column, as are less volatile sulfonate esters.

Peaks	Peaks
1. 1-Chloropropane	12. di-Isopropyl sulfate (DPS)
2. Bromoethane	13. di-*n*-Propyl sulfate (DNS)
3. 2-Chloropropane	14. Dibutyl sulfate (DBS)
4. 1-Bromopropane	15. Methylbenzene sulfonate (MBS)
5. 2-Bromopropane	16. Ethylbenzene sulfonate (EBS)
6. 1-Bromobutane	17. Methyl toluenesulfonate (MTS)
7. Dimethyl sulfate (DMS)	18. Ethyl toluenesulfonate (ETS)
8. Methyl methanesulfonate (MMS)	19. *n*-Butyl benzenesulfonate (nBBS)
9. Ethyl methanesulfonate (EMS)	20. *n*-Propyl toluenesulfonate (nPTS)
10. Isopropyl methanesulfonate (iPMS)	21. Isopropyl toluenesulfonate (iPTS)
11. Diethyl sulfate (DES)	22. *n*-Butyl toluenesulfonate (nBTS)

* ortho isomer of ethyl toluenesulfonate



Column	Rtx®-200, 30 m, 0.25 mm ID, 1.0 µm (cat.# 15053)
Sample	
Diluent:	90:10 acetonitrile:water
Conc.:	100 µg/mL
Injection	
Inj. Vol.:	1 µL split (split ratio 10:1)
Liner:	4mm Gooseneck Splitless w/Wool (cat.# 22405)
Inj. Temp.:	220 °C
Oven	
Oven Temp.:	40 °C (hold 8.3 min) to 70 °C at 70 °C/min to 115 °C at 40 °C/min to 250 °C at 30 °C/min to 300 °C at 15 °C/min (hold 3 min)
Carrier Gas	He, constant flow
Flow Rate:	1 mL/min
Detector	MS
Mode:	Scan
Transfer Line Temp.:	280 °C
Ionization Mode:	EI
Scan Range:	20-250 amu

Special Considerations:

Sample Preparation

Minimal sample preparation is needed to successfully run these methods. Samples of neat API were simply diluted to a concentration of 10mg/mL and injected into the GC. However, care must be taken with sample solvent selection, since many APIs are in salt forms and cannot be dissolved in 100% organic solvent. To aid dissolution, up to 10% water may be mixed with a water-miscible organic solvent. The sample solvent used in this application was 90:10 acetonitrile:water.

System Maintenance

Since samples are prepared at a relatively high concentration, nonvolatile API may build up in the liner and/or on the head of the column after repeated injections. Care should be taken to regularly change liners and seals to avoid problems with chromatography stemming from contaminated parts.

Some PGIs, such as isopropyl benzene sulfonate, break down in both the inlet and column. These breakdown products may persist in the analytical column, causing an elevated baseline that resembles a bleed profile. If isopropyl benzene sulfonate is present in the sample, a longer bake-out time (10-15 min. @ 300°C) is recommended at the end of each analytical run in order to remove any degradation products present on the column.

SIM Groups

Care must be taken when choosing SIM groups for PGI compounds during method development. Because these analytes are very similar to one another, many of them share common ions. It is good practice to pick two or three ions to monitor for each compound and to use relatively unique ions for each PGI to aid in peak identification.

Conclusion

Since potential genotoxic impurities are of increasing concern for both regulatory bodies and consumers, the importance of effective methods for detection and quantitation of these compounds is growing. As a result of collaboration between Merck and Restek, two easy, sensitive options are now available for the analysis of PGIs in API using inert, selective columns from Restek.

RELATED SEARCHES

PGI , sulfonate ester , potential genotoxic impurities , rtx-200 , API , Rxi-5Sil MS

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