

Two Options for Analyzing Potential Genotoxic Impurities in Active Pharmaceutical Ingredients

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 both sulfonate esters and alkyl halides on the **Rtx[®]-200 column**.

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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 in API. Some of these compounds are potentially genotoxic impurities (PGIs) and may raise concern about cancer and/or birth defects. Because of the toxicity of these compounds, it is essential that they be controlled to low levels in API after synthesis. In January of 2007, the European Medicines Agency (EMA) released guidance on acceptable limits of PGIs in APIs (Guideline on the Limits of Genotoxic Impurities (EMA/CHMP/QWP/251344/2006)). Developing new methods for sensitive detection of impurities is an increasingly active area of research across the pharmaceutical industry.

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

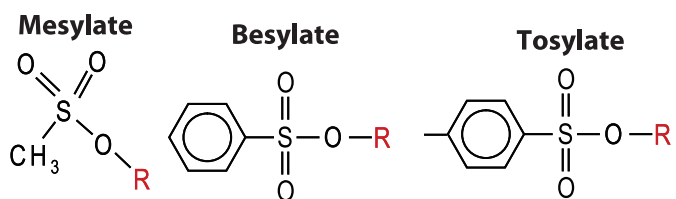
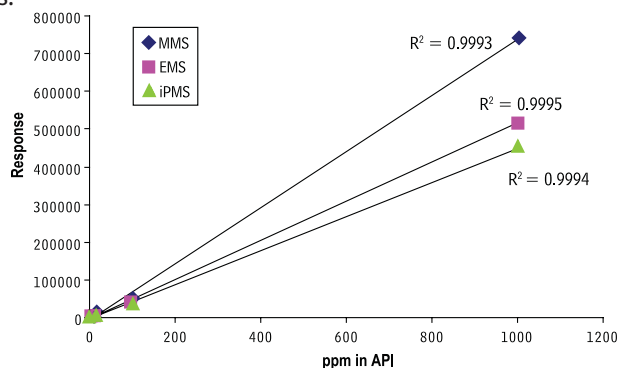


Figure 2 Linearity of fast GC/MS analysis for selected sulfonate esters.



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.

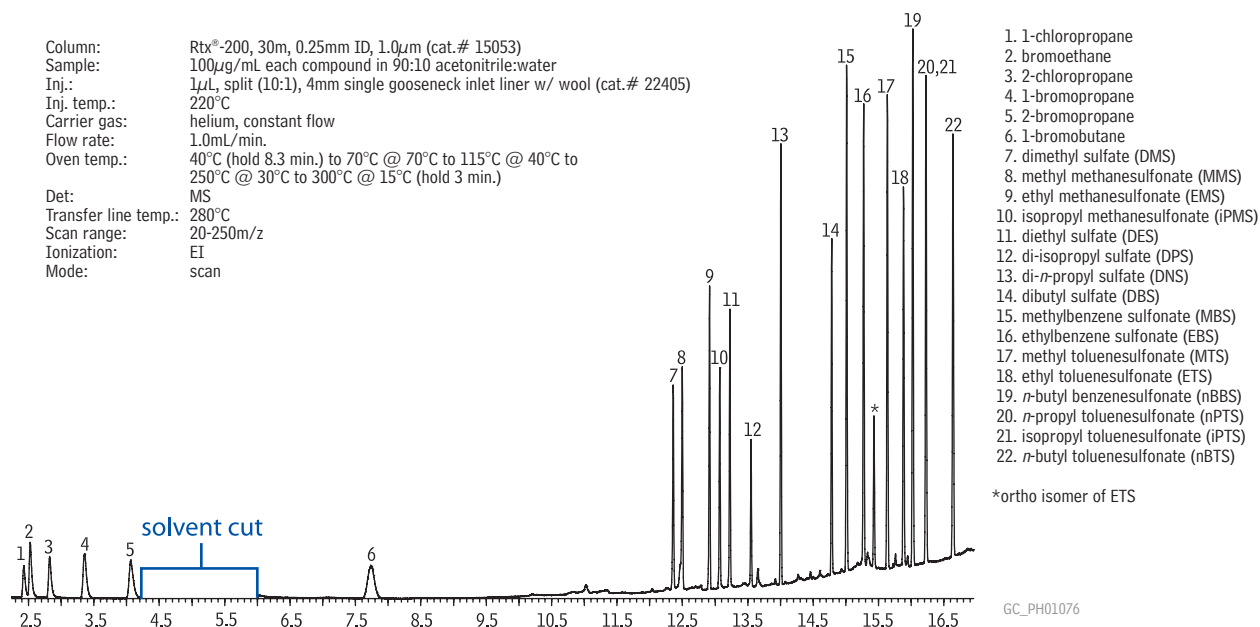
Two options for the analysis of PGIs in API have been developed to meet different laboratory needs. The first option is a fast method for the analysis of sulfonate esters on the Rxi[®]-5Sil MS column. The second option is a comprehensive method for the analysis of both sulfonate esters and alkyl halides 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. The use of a thin film Rxi[®]-5Sil MS column allows for speedy analysis of these active 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. A linearity study performed by Merck shows that this method is linear for sample concentrations from 1ppm to 1,000ppm in API (Figure 2). Depending on the dose of API to the patient, it may be necessary to detect levels of impurities as low as 1 ppm in order to meet EMA requirements. The 1ppm spike represents the threshold for toxicological concern (TTC) as set by the EMA for PGIs.

Figure 3 Small, polar alkyl halides are well-retained on the fluorinated Rtx®-200 column, as are less volatile sulfonate esters.

Column: Rtx®-200, 30m, 0.25mm ID, 1.0µm (cat.# 15053)
 Sample: 100µg/mL each compound in 90:10 acetonitrile:water
 Inj.: 1µL, split (10:1), 4mm single gooseneck inlet liner w/ wool (cat.# 22405)
 Inj. temp.: 220°C
 Carrier gas: helium, constant flow
 Flow rate: 1.0mL/min.
 Oven temp.: 40°C (hold 8.3 min.) to 70°C @ 70°C to 115°C @ 40°C to 250°C @ 30°C to 300°C @ 15°C (hold 3 min.)
 Det: MS
 Transfer line temp.: 280°C
 Scan range: 20-250m/z
 Ionization: EI
 Mode: scan



Option 2: Comprehensive PGI Method

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 a 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 3). 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 (Figure 4). 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.

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.

Product Listing

Rtx®-200 Columns (fused silica)

(Crossbond® trifluoropropylmethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #	price
0.18mm	0.20	-20 to 310/330°C	20-Meter	45002	
0.25mm	1.00	-20 to 290/310°C	30-Meter	15053	

Rxi®-5Sil MS Columns (fused silica)

(Crossbond®, selectivity close to 5% diphenyl/95% dimethyl polysiloxane)

ID	df (µm)	temp. limits	length	cat. #	price
0.18mm	0.18	-60 to 330/350°C	20-Meter	43602	

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