Method selection for trace analysis of potentially genotoxic impurities in active pharmaceutical ingredients

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INTRODUCTION

The analysis of potentially genotoxic impurities (PGIs) in an active pharmaceutical ingredient (API) is a challenging task. The target limit of detection for a “PGI in API” method is about 500 times lower than for classical impurity analysis (1 ppm versus 0.050%), while the target solutes with a structural alert functionality cover a broad range of polarities and volatilities. It is clear that classical methods using direct introduction of the sample (API solution) cannot be used or at least major modifications are needed to allow a robust and accurate determination of the target PGIs.

METHOD SELECTION

A number of generic methods were developed to cover different classes of PGIs in different APIs. Depending on the solute characteristics, either gas chromatography (GC) or liquid chromatography (LC) were used. Different sample preparation and sample introduction methods were developed with special attention to automation, miniaturization, toxic solvent reduction and hyphenation (Fig. 1). These methods were applied to different classes of genotoxic impurities covering a broad range of polarities and volatilities [1].

HALIDES AND HALOALKENES: SHS or SPME-GC-MS

Volatile halides and halogenated solvents were analyzed by static headspace or headspace-SPE coupled to GC-MS. The API was dissolved in a DMSO/water mixture. Both techniques were complementary. SHS provided the highest robustness and sensitivity for the most volatile solutes, such as vinyl chloride and chloromethane. SPME resulted in a higher sensitivity for the less volatile solutes that were enriched on the SPME fiber. The methods were validated for a target solute spiked at 0.5 ppm level and compared to HPLC. The higher sensitivity obtained by SPME for the late eluting solutes is obvious.

SULFONATES: derivatization-SHS-GC-MS

Sulfonate esters are not volatile enough for headspace techniques: they were analyzed by in-situ derivatization-SHS-GC-MS (Fig. 3) with the deuterated equivalents as internal standards [2].

N-MUSTARDS: derivatization-SPME-GC-MS

N-mustards were acylated using ethyl chloroformate (ECF) in a 2:1:1 mixture of water/ethanol/pyridine, and analyzed by HS SPME-GC-MS 100 μm PDMS fiber.

ALDEHYDES: derivatization-RPLC-MS

Some PGIs can be analyzed either using GC or LC. Method selection is based on ease-of-use, robustness and automation of the method. For aldehydes, separation is not critical but detection is difficult, in particular for formaldehyde. Therefore, derivatization is often used. Automated pre-column derivatization using DNPH was applied for carbonyl solutes, allowing the simultaneous analysis of volatile (eg. C1-C5 aldehydes) and less volatile (eg. 4-hydroxybenzaldehyde) aldehydes (Fig. 7).

HALOALCOHOLS: GC-GC-MS

Halocarbons could not be detected using headspace techniques, even after derivatization: direct injection using heart-cutting was the best option to minimize chromatographic interferences and system contamination [3].

A concentrated solution of API was injected on an apolar first dimension column. The fraction containing the PGI was heart-cut using the Deans Switch (capillary flow technology) to a second column installed in a separate oven (LTM), which enables an independent temperature control of both dimensions. The main solutes, derivatization reagents, solvents… were not introduced in the 2nd column, avoiding overloading and contamination of this column and of the MS source (Fig. 4).

An API (carbamazepine) was dissolved in pyridine, spiked with halocarbons at the 1 ppm level. 1μL of the mixture was injected after automated silylation and LLE (Fig. 5).

AZIRIDINES: RPLC/HILIC-MS

Aziridines were analyzed by HS-SPME-GC-MS. A concentrated solution of the API was directly injected. The analysis was performed on a hydrophobic interaction LC (HILIC) column and on a reversed phase (RPLC) column. The most polar solutes were well retained and separated from the API on the HILIC column. More hydrophobic solutes were best analyzed on the RPLC column (Fig. 6). Both columns were installed on a single instrument using a column selection valve and could be operated with the same solvents using dedicated gradient programs.

REFERENCES