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Quantification of residual solvents in antibody drug conjugates using gas chromatography

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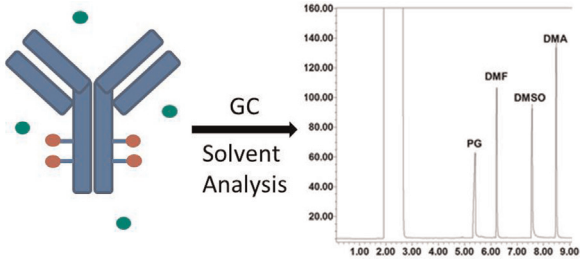
HIGHLIGHTS

- Sensitive residual solvents detection in ADCs.
- 125 ppm QL for common conjugation solvents.
- Generic and validatable method.

GRAPHICAL ABSTRACT



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ABSTRACT

The detection and quantification of residual solvents present in clinical and commercial pharmaceutical products is necessary from both patient safety and regulatory perspectives. Head-space gas chromatography is routinely used for quantitation of residual solvents for small molecule APIs produced through synthetic processes; however residual solvent analysis is generally not needed for protein based pharmaceuticals produced through cultured cell lines where solvents are not introduced. In contrast, antibody drug conjugates and other protein conjugates where a drug or other molecule is covalently bound to a protein typically use solvents such as *N*,*N*-dimethylacetamide (DMA), *N*,*N*-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), or propylene glycol (PG) to dissolve the hydrophobic small molecule drug for conjugation to the protein. The levels of the solvent remaining following the conjugation step are therefore important to patient safety as these parental drug products are introduced directly into the patients bloodstream. We have developed a rapid sample preparation followed by a gas chromatography separation for the detection and quantification of several solvents typically used in these conjugation reactions. This generic method has been validated and can be easily implemented for use in quality control testing for clinical or commercial bioconjugated products.

1. Introduction

Conjugated proteins can encompass a wide range of pharmaceutical products from conjugated vaccines to toxin linked antibody drug conjugates. As more and more therapeutics utilize conjugated proteins including commercial products like ADCs Kadcyla [1], Adcetris [2], or conjugated vaccines [3] like Prevnar, there is increasing interest in developing analytical methods and strategies to address their unique complexities. While the molecules conjugated to proteins include peptides [4,5], polysaccharides [3], and small molecule drugs and toxins [1,2], the one common link between all of these species is that they undergo a conjugation step to be attached to the protein. While the protein itself is contained in an aqueous buffer, the species to be conjugated are typically more hydrophobic and require dissolving in an inert, water-miscible organic solvent, such as *N*,*N*-dimethylacetamide (DMA), dimethyl

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sulfoxide (DMSO), *N*,*N*-dimethylformamide (DMF), and propylene glycol (PG). In addition, the conjugation chemistries generally employed such as succinimidyl esters, TFP esters, or maleimide functional groups are sensitive to hydrolysis, hence using water as the diluent can lead to reduced conjugation efficiencies and degradation of the drug itself.

Residual solvents require strict testing and control strategies in order to protect patient safety. Due to toxicity concerns, the USP, EP, and ICH have guidelines specifying the allowable amounts of specific residual solvents that are allowed in pharmaceutical products. Although TGA/loss on drying [6], FT-IR [7], and NMR [8] have been used for the analysis of residual solvents, specificity and sensitivity limitations persist with these techniques. Gas chromatography (GC) is the most commonly used analytical technique for routine detection and quantification of residual solvents in pharmaceutical active pharmaceutical ingredients (APIs). Several approaches have been developed for the GC analysis of residual solvents in pharmaceutical products including head space injection of samples dissolved in a solvent [9], dynamic injection of the headspace [10], pre-concentration of headspace solvents prior to analysis [11], or direct injection of the sample onto the GC [12]. The effectiveness of GC for residual solvent analysis has been demonstrated repeatedly through separating dozens of different residual solvents on a variety of columns [13–16]. GC based approaches also possess the reproducibility, sensitivity, and specificity to be an effective tool for routine residual solvent analysis in quality control laboratories [17]. However, as noted above, GC residual solvent methods have not been applied to the complicated matrixes of protein formulations.

We have developed a generic method for the detection and quantification of residual solvents commonly used in conjugation reactions from ADC containing samples. The method involves using an organic solvent-based protein precipitation followed by a GC separation for quantitation of each solvent. The method was successfully able to detect and assay the amount of DMA, DMF, DMSO, and PG present in an ADC containing sample. The suitability and validation potential of the method was demonstrated on three different ADCs, each with a different drug and antibody combination demonstrating the potential of the method to be adopted as a generic method for the quantitation of residual solvents in formulated ADC samples.

2. Methods and materials

2.1. Sample preparation

ADC samples ADC1, ADC2, and ADC3 were prepared by aliquoting 1 mL of the formulated drug product into 2.0 mL acetonitrile and incubating for 60 min at −10 °C. Following incubation, the samples were vortexed briefly, centrifuged at 13,000 rpm for 10 min, and then the supernatant was transferred to a vial for analysis. Standards were prepared by diluting the solvents of interest (PG, DMF, DMSO, and DMA) in Milli-Q purified water. All solvents were HPLC grade or ≥99%.

2.2. GC conditions

An Agilent 7890A gas chromatography system with a 7683B injector and G2614A autosampler tray was used to analyze all of the samples except for the GC–MS samples that were analyzed on an Agilent 6890N gas chromatography system with an Agilent 5975C MSD. The GC conditions for all analysis are as follows: inlet temperature 225 °C; split ratio 50:1; column flow 1.5 mL min^{-1} He; run time 22 min; FID detector temperature: 300 °C; detector gas flow H_2 at 40 mL min^{-1}, air at 400 mL min^{-1}, He (makeup) at 30 mL min^{-1}. Peak integration and signal to noise calculations were performed in Empower software (Waters) while subsequent data analysis was performed in Microsoft Excel (Table 1).

3. Results and discussion

3.1. Sample preparation development

The initial method development activities focused on identifying an ADC sample preparation technique compatible with the GC. Due to the possibility of clogging and column fouling by the protein from direct injection, the ADC drug product efforts were focused on sample introduction techniques that would remove the protein. The three major sample preparations evaluated were headspace injection, solvent precipitation of the protein, and removal of the protein using a centrifuge filter. The use of a headspace injector was attempted first because it allows simple sample handling and preparation. For the headspace autosampler injection, an oven temperature of 100 °C was used with a 10 min vial equilibration time with a 0.1 min injection time. The headspace injection technique was suitable for DMA, DMF and DMSO, however a PG peak was not observed in any of the headspace injection samples. As propylene glycol is a standard conjugation solvent used for very hydrophobic linker drugs, this approach was not further evaluated. The next two approaches focused on removal of the protein from the sample prior to direct injection of the sample. Initially, centrifuge filters with a 20 kDa cutoff were used to remove the ADC from the sample. For these experiments, 1 mL of the ADC was added to the centrifuge filter and then centrifuged at 4000 rpm for 45 min. After centrifuging, the flow through was collected and analyzed using direct injection GC. While initially promising, sample sets utilizing this approach suffered from repeated injector needle clogging that would stop an injection sequence after two–six injections indicating less than 100% efficient removal of the protein. Finally, a solvent precipitation was evaluated using 2 parts acetonitrile to 1 part sample (ADC drug product) followed by incubating at −10 °C for 30 min. Samples were vortexed and then centrifuged for 20 min at 13,000 rpm. The supernatant was then analyzed on the GC using direct injection. The protein precipitation preparation did not exhibit the injector needle clogging issue even after thirty injections; and all four solvents were detected using a direct injection.

3.2. Chromatography optimization

With the proper sample preparation procedure defined, the focus turned to developing an optimized GC separation that was capable of detecting and resolving all four solvents. Using a 1000 ppm standard of the PG, DMA, DMF, and DMSO with direct injection, four different 30 m GC capillary columns with diverse stationary phases were initially screened: Restek RTX-5 Amine, an Agilent DB-624, an Agilent DB-FFAP, and a Zebron ZB-Wax. Sample chromatograms for the four columns with the solvent mixture are shown in Fig. 1.

Based on the column screening, the DB-624 column, the stationary phase from the USP compendial residual solvents method proved to be unsuitable for use based on the poor shape and poor resolution between two of the solvents. The ZB-Wax

Table 1
Oven temperature program.

Ramp (°C min^{-1})	Hold time (min)	Final temp (°C)
N/A	4	40
8	0	60
5	2	85
30	2	280