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Short Communication

Chromatoprobe as a sample-sparing technique for residual solvent analysis of drug discovery candidates by gas chromatography

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ABSTRACT

In drug discovery research, residual solvent measurement is an integral part of purity analysis for synthesis of a drug candidate before it is used for toxicity testing. This is usually carried out using gas chromatography (GC) with direct injection sample introduction. This method requires testing compounds to be soluble at high concentrations (> 50 mg/mL, usually in DMSO) to achieve acceptable sensitivity, a hurdle which is not always achievable for some samples such as cyclic peptides and oligonucleotides. To overcome the limitation associated with the direct injection approach, a new method using the Chromatoprobe thermal extraction device was developed for quantifying residual solvents of drug discovery compounds. This method not only consumes significantly less material (less than 1 mg), but also shows higher sensitivity than the direct injection approach. In addition, because no diluent is required with the Chromatoprobe thermal extraction, all residual solvents can be detected and measured without further method optimization. In our study, we compared data from GC residual solvent analysis using the Chromatoprobe solid sample introduction to those of the direct injection method for seven in-house samples. Our results showed a good agreement between the data from these two sample introduction methods. Thus, the Chromatoprobe sample introduction method provided a sample-sparing alternative to the direct injection method for the measurement of residual solvents in drug discovery. This method can be particularly useful for residual solvent analysis in samples that are available only in limited amounts, poorly soluble, and/or unstable in the diluents used for the direct injection method.

1. Introduction

A lead compound for a drug discovery target usually undergoes vigorous in vitro and in vivo toxicity testing before it is advanced as a drug development candidate. To minimize the potential adverse effects caused by impurities, the compound is subjected to an in-depth analytical characterization for purity determination and structure verification. As an integral part of purity determination, residual solvent analysis quantifies the amount of residual solvent present in a synthesis lot of a lead compound. Measuring residual solvents of an active pharmaceutical ingredient (API) is not only necessary to avoid potential toxicity but also to accurately calculate the API dose for toxicology studies. Residual solvents are divided into three classes in the USP < 467 > [1]. Class I solvents, such as benzene and carbon tetrachloride, are to be avoided if possible, and have very low allowable limits (2–8 ppm). Class II solvents are more commonly used in chemical synthesis and have limits ranging from 50 ppm to several thousand ppm. Class III solvents are not restricted but need to be quantified. USP < 467 > recommends measuring residual solvents in pharmaceutical compounds by gas chromatography (GC) using headspace sampling for class I and II solvents, and the loss on drying method for class III solvents. In the experimental procedure described in USP < 467 > for class I and II solvents, around 250 mg of drug is needed for the GC analysis with headspace sampling. For class III solvents, over 1 g is needed for the loss on drying procedure. In addition to requiring a substantial amount of material, the headspace procedure requires between 45 and 60 min for sample equilibration prior to injection. Others have investigated a variety of alternative sampling techniques including purge and trap [2], direct thermal extraction [3,4], solid-phase microextraction (SPME) [5–7], and single-drop microextraction (SDME) techniques [8,9] for measuring residual solvents in pharmaceutical samples. The purge and trap and direct thermal extraction techniques offer good sensitivity and only need a few milligrams of material, but they require customized apparatus and method optimization for each sample lot. The micro-extraction methods also offer good sensitivity, but require ~100 mg of drug material in addition to long equilibration time and extensive method optimization, which are more suitable for a development or

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manufacturing environment. In a fast-paced drug discovery environment, where there are often multiple lead compounds that are usually synthesized in limited amounts, and where multiple lots of a given lead compound are often obtained using different synthetic routes, optimizing an analytical method for each sample lot is not feasible. The residual solvent analysis in the drug discovery stage is often carried out using direct injection GC analysis of solubilized sample [10,11]. This method requires 5–10 mg of material, and can be used for a wide range of sample types without further optimization, but it has several limitations. The method requires the material to be soluble at high concentrations (typically 50–100 mg/mL in DMSO) in order to achieve acceptable sensitivity. With this method, the sample solution is injected using a split injection, which is necessary to avoid diluent overloading of the GC column. Typically, the residual solvent from only 1/500th of sample material is introduced to the column (1 μL injection of a 100 mg/mL solution, split 50:1). The majority of the sample is wasted as a result of necessary pre-injection syringe rinsing and pumping. The nearly universal solvent DMSO is often used as a diluent because it can dissolve a variety of chemotypes and usually elutes after the peaks of interest on the GC columns used for solvent analysis. However, not all samples dissolve easily in DMSO. When samples have only limited solubility in DMSO, the lower sample concentration can impact the sensitivity of the analysis, which can be an issue for class I and some of the class II solvents. In addition, DMSO reacts with HCl or TFA salts in the hot injector, producing artifacts that can interfere with the interpretation of the resulting chromatogram. Furthermore, when the API is not volatile, it can foul the GC inlet or column resulting in higher maintenance costs and downtime for the instrument. Moreover, it is impossible to quantify the residual solvent that is used as the diluent for the sample analyzed with this method. As cyclic peptides and antisense oligonucleotides (ASOs) entered the drug discovery portfolio, these issues were exacerbated. Many of these more complex molecules were difficult to synthesize, the quantity of material was limited, and many showed poor solubility and stability in DMSO. To address the limitations of the direct injection method and the large amount of sample required for the headspace method of residual solvent analysis, the use of the Chromatoprobe sample introduction device was explored. The Chromatoprobe, developed by Aviv Amarov (Tel Aviv University), is a direct thermal extraction device that allows GC analysis of small quantities of solid sample or dilute solutions. This sample introduction device has been used for forensic and environmental analysis, but it has not been reported for residual solvent analysis of pharmaceutical compounds [12–14]. In this report, we describe the use of the Chromatoprobe sample introduction device and compare the analytical results of seven in-house compounds by both the Chromatoprobe and direct injection methods to determine if the Chromatoprobe sample introduction method can serve as a sample-sparing alternative for drug discovery support.

2. Experimental

2.1. Chemicals and materials

All solvents and chemicals were of analytical grade or better. Analytical samples were provided by Bristol-Myers Squibb drug discovery chemists in Wallingford, CT, USA.

2.2. GC residual solvent analysis using Chromatoprobe

All GC residual solvent analyses using Chromatoprobe were performed on a Varian 3800GC with CO_2 cooling coupled with a 4000MS detector using a Restek Rtx 502.2 GC column (30 m×0.25 mm ID, 1.4 μm df) with a press-fit "Y"-connector to separate inlets (Chromatoprobe and direct injection) via a Restek polar deactivated guard column (5 m×0.25 mm). The unused inlet was maintained at 0.5 psi throughout the experiment to prevent backflow. The sample vial was placed into the Chromatoprobe holder and then assembly inserted into the inlet to initiate the analysis. A constant flow of helium at 2.0 mL/min with an initial pressure pulse of 30 psi for 2.7 min was used. The inlet was cooled to 20 °C prior to sample introduction. Starting at 0.10 min after the Chromatoprobe with sample was inserted into the inlet, the inlet temperature was increased to 250 °C at 200 °C/min and held there for 2.25 min before cooling back to 20 °C at 200 °C/min. The inlet split was initially set at 100:1 when the Chromatoprobe was inserted into the inlet. A trial run was conducted using a small aliquot of sample under splitless conditions. Data from this sample were used to determine if the split needed to be adjusted to avoid overloading the detector. Based on the trial run, at 0.01 min the split was adjusted to splitless, 25:1, 50:1 or 100:1. The oven temperature program was 0 °C for 4 min followed by a 20 °C/min gradient to 220 °C and held there until the end of the run at 18.0 min. Three small aliquots of sample were placed in Chromatoprobe sample vials, weighed and analyzed by GC–MS. The appropriate ions for the residual solvents present were extracted and the resulting chromatograms were integrated to obtain the peak area/ng drug.

Calibration standards were prepared by diluting the residual solvents to 1.0%, 0.1% and 0.01% (v/v) in a solvent that was not present in the drug sample. Calibrants were analyzed in triplicate on the same GC column as used for samples. The inlet was maintained at 250 °C and the split was the same as that used for the sample analysis. The oven program was 35 °C for 4.0 min followed by a 6 °C/min gradient up to 90 °C and then a 15 °C/min gradient to 220 °C and held there until the end of the run at 19.0 min. The appropriate ions for the residual solvents present were extracted and the resulting chromatograms were integrated to obtain the peak area/ng of solvent. The results were plotted to generate a standard curve for the calculation of the amount of residual solvent in each drug sample.

2.3. GC residual solvent analysis using direct injection

All GC residual solvent analyses using the Direct Injection Method were performed on Agilent 6890 with a 5795 MS and FID, using Agilent DB-Wax column (30 m×0.32 mm ID, 0.5 μm df). The inlet temperature was kept at 250 °C and a split of 20:1 was used throughout the analysis. The oven temperature program was 35 °C for 4.0 min followed by a gradient of 15 °C/min to 200 °C and held there until the end of the run at 20.0 min. Samples were prepared as 50 mg/mL solutions in DMSO. 1 μL of each sample solution was injected using a constant flow of helium at 1.0 mL/min and a 10:1 split and analyzed with a GC–MS method to determine which solvents were present.

For quantitation, samples were dissolved in DMSO with 0.05% (v/v) of an internal standard (IS) to obtain a 50 mg/mL or 100 mg/mL solution. Calibration standards were made to be 0.1% and 0.01% (v/v) residual solvent with 0.05% (v/v) IS in DMSO. After the sample solution and standards were analyzed using the same conditions as above with a 50:1 injection split, a "response factor" was calculated by dividing the solvent's peak area by the IS peak area. The values obtained from the standard solutions were plotted to obtain the linear equation used to calculate the amount of solvent in each drug sample.

3. Results and discussion

3.1. Chromatoprobe sample introduction device

The Chromatoprobe device (Fig. 1), developed by Aviv Amirav consists of a stainless steel holder protected by a Silcosteel coating. It holds a small vial (1.8 mm ID×15 mm) that can be used with a liquid (volume < 40 μL) or solid sample. The sample vial is placed in the holder and then inserted into the GC inlet at a relatively cool temperature (below the analytes' boiling points). As the inlet temperature is increased, the sample volatilizes and is swept onto the column. The oven is kept at a temperature which allows the analyte to condense

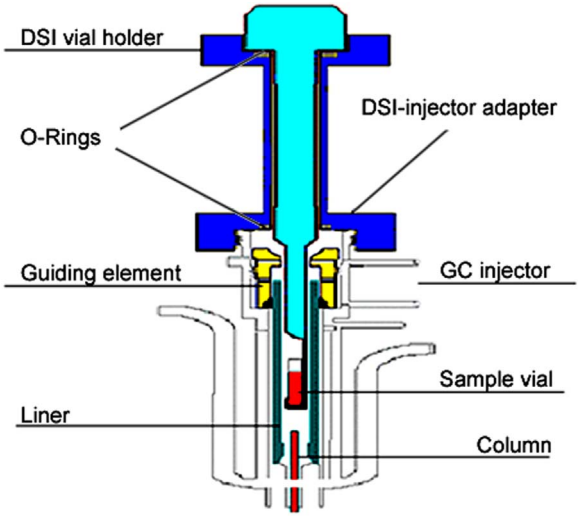

Fig. 1. Chromatoprobe diagram [15].

on the column. After all of the analytes are deposited onto the column, the oven temperature gradient is started to resolve the components and elute them to the detector.

3.2. Residual solvent quantitation comparative study

Seven in-house samples with varying polarity and molecular weights were selected for this comparative study and their characteristics are shown in Table 1. For each of these seven in-house samples, the residual solvent analysis was performed by using both direct injection and Chromatoprobe sample introduction methods. The residual solvent analysis results obtained by both methods are summarized in Table 2. For the Chromatoprobe method, each sample was analyzed in triplicate to evaluate its analysis reproducibility, and for each residual solvent, an average value of the amount (% m/m) and standard deviation from the triplicate analyses were reported. The results showed that both methods yielded overall similar data on the residual solvent analysis with no significant disagreement. In addition, the triplicate analyses with the Chromatoprobe method were very consistent as shown by the standard deviation in Table 2. While the direct injection method usually required more than 2–4 mg per injection, the Chromatoprobe method typically consumed a 100–300 μg of material per injection.

3.3. Quantitation sensitivity

With the direct injection analysis, a diluent, typically DMSO, was used in the direct injection analysis, and a sample solution had to be injected with a split ratio to control the adverse effect of diluent on the chromatography. Lowering the split ratio allowed loading more analytes, but unfortunately also more unwanted diluent on the GC column. Even with only 1 μL injection, a minimum split ratio 1:20 was necessary to avoid overloading of the diluent on the GC column. On the other hand, the split ratio also regulated the amount of analytes injected onto the column, and the higher the split ratio was, the less analytes were loaded onto the column, affecting the sensitivity of the analysis. The final split ratio, which could vary depending on the properties of the samples, was thus selected by balancing these two opposing objectives, increasing sensitivity by injecting more samples on the column and avoiding the diluent overload. In contrast, with the Chromatoprobe method, where solid sample was used, all of the residual solvents present in the material were transferred to the column without adversely affecting the chromatography, leading to a significant increase of the sensitivity. In fact, since it was not feasible to accurately weigh less than 100 μg of sample material, most samples required an inlet split to reduce the sample amount to avoid overloading the detector. The sensitivity difference between the direct injection and Chromatoprobe methods could be further illustrated by the residual solvent analysis of sample #5. As shown in Fig. 2A, chromatogram was obtained using the direct injection method, 1 μL injection of a 50 mg/mL solution in DMSO with 1:100 split ratio. With this experimental condition, only the residual solvent from 0.5 μg of sample material was introduced to the column for the quantitative analysis, and the majority of the sample was discarded for the pre-injection syringe rinsing and pumping. As a result, the peak of acetamide was hardly above the noise level and was difficult to be quantitated. As shown in Fig. 2 B, chromatogram was obtained by using the Chromatoprobe method with 1.6 μg solid sample introduced to the column (160 μg with a 100:1 split). Even with much less sample consumption, the peaks of both acetic acid and acetamide were much higher in the chromatogram with the Chromatoprobe method, leading to more accurate quantitation.

Table 1
Sample description.

Sample	MW	Description
1	390	Small polar molecule
2	303	Small polar molecule
3	441	Small moderately polar molecule
4	853	Small non-polar molecule
5	1889	Synthetic cyclic peptide
6	1859	Synthetic cyclic peptide
7	3452/3480	Mixture of naturally occurring cyclic peptides

Table 2
Comparison of results from direct injection and Chromatoprobe methods.

Sample	Residual solvent	Direct injection amount (% m/m)	Chromatoprobe	
			Amount (% m/m)	Std. Dev. (% m/m, n=3)
1	THF	0.51	0.53	0.05
	1,4-Dioxane	0.30	0.31	0.05
2	IPA	0.08	0.13	< 0.01
3	Chloroform	0.22	0.22	0.04
4	Diacetone-alcohol[a]	0.05	0.03	0.01
5	Acetic acid	2.11	2.93	0.11
	Acetamide[b]	0.39	0.59	0.02
6	Acetic acid	0.23	0.15	< 0.01
	Acetamide[b]	0.07	0.04	< 0.01
	DMSO	0.94	0.56	0.05
7	Acetic acid	3.61	2.84	0.12
	Acetamide[b]	0.48	0.33	0.03

[a] Diacetone alcohol: not listed in the USP < 467 >, a condensation product of acetone with some reported toxicity.
[b] Acetamide: not listed in the USP < 467 >, a suspected carcinogen.

3.4. Diluent interference

The solvent of first choice as diluent for the direct injection method was DMSO due to its dissolving power and its long retention resulting in elution after most of the peaks of interest. However, diluent interference occurred with any sample that contained DMSO as one of the residual solvents to be quantified. When this information was available before the analysis, a different diluent was used, but this was not possible for most of our samples in drug discovery. In fact, the analysts did not know all the solvents that were used during synthesis

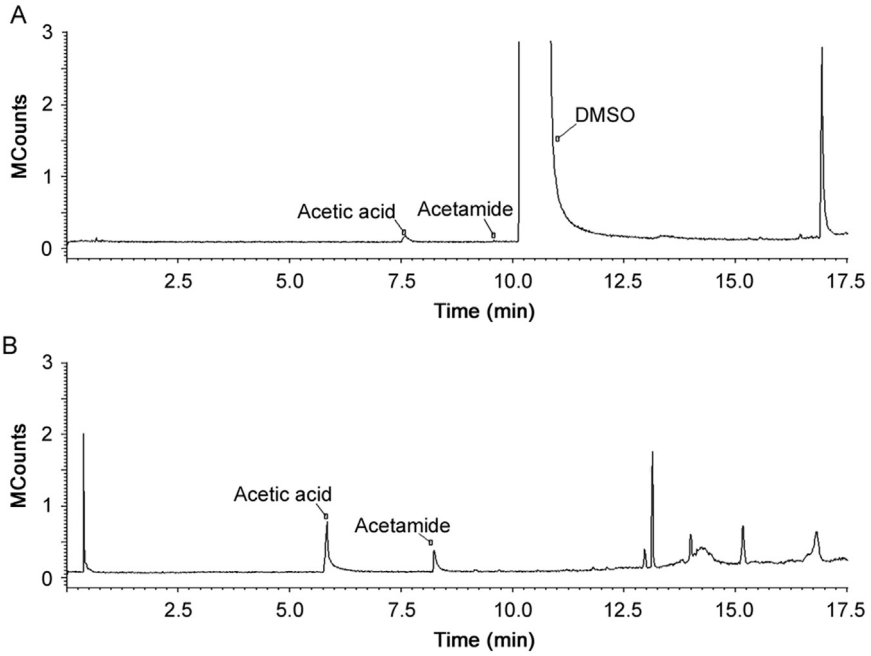

Fig. 2. Sample #5 Analysis. (A) 50 mg/mL solution in DMSO direct injection. (B) 160 μg dry solid sample by Chromatoprobe (Both samples use the Rtx 502.2 column with a 100:1 split. Retention times are different because the oven temperature gradient program starts at 0 °C and is steeper with the Chromatoprobe).

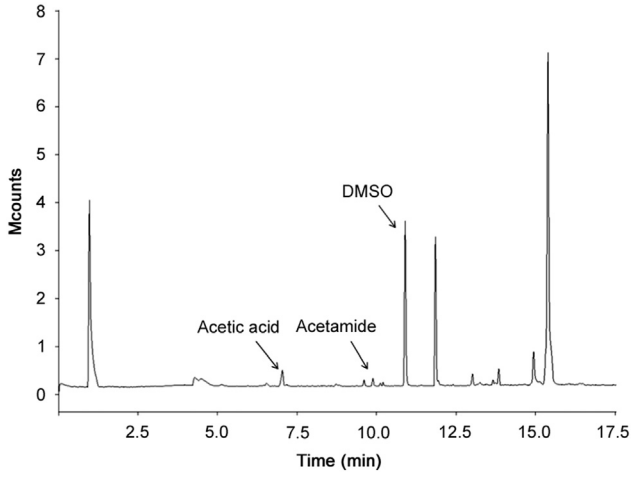

Fig. 3. GC–MS chromatogram of Sample #6 (0.510 mg) using the Chromatoprobe method.

and purification for a given sample. Regardless of the choice of diluents, the direct injection method could not detect and quantify the same residual solvent that was used as the diluent of the sample. With the Chromatoprobe sample introduction as a solid, no diluent was required and therefore diluent interference was avoided. As shown in Fig. 3, the use of the Chromatoprobe sample introduction method led to the detection and quantitation of DMSO as a residual solvent in sample #6, which would have been masked by the use of DMSO as the diluent with the direct injection method. Furthermore, because sample solubility was not an issue for the Chromatoprobe method, the diluent for the calibration standards could be any solvent that was not present as a residual solvent in the sample measured. For sample #6, methanol, which eluted at around 1.0 min, was used to make the acetic acid, acetamide and DMSO standard solutions for quantitation.

4. Conclusions

Our study demonstrates that the results of the residual solvent analysis obtained by Chromatoprobe sample introduction shows a good agreement with results from the direction injection method. Furthermore, the Chromatoprobe sample introduction device offered several advantages for the measurement of residual solvents, compared to direct injection method. In addition to consuming significantly less sample material, it provided increased sensitivity and circumvented the diluent interference issue associated with direct injection method. Furthermore, the Chromatoprobe sample introduction method allowed analysis of those samples that were poorly soluble or unstable in DMSO, such as peptide molecules. Finally, with the Chromatoprobe sample introduction method, only the volatile ingredients of the sample material were introduced to the GC column, and non-volatile components were left in the sample vial. This reduced fouling and instrument maintenance, leading to an extended GC column life.

Thus, the Chromatoprobe sample introduction method can serve as a sample-sparing and sensitive alternative to the direct injection method for the measurement of residual solvents in drug discovery. The limitations of this method are that carbon dioxide is required to cool the inlet and oven, and the sample analysis is not yet automated. We plan to refine the method further and enable automation of this technique for higher sample throughput.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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