An automated sample preparation workflow for the analysis of nitrosamines in metformin

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Goal

The aim of this study is to demonstrate the suitability of an automated sample preparation workflow for the extraction of nitrosamines in drug substance using a Thermo Scientific™ TriPlus™ RSH SMART autosampler.*

Introduction

Sample preparation is one of the most critical steps in analytical workflows as it affects the overall accuracy and reliability of results. It has been estimated that more than sixty percent of the time spent in a chromatographic analysis is dedicated to sample clean-up and extraction to isolate and concentrate the target analytes, providing

a representative and homogenous solution that can be injected into the chromatographic system.

Sample preparation usually requires multiple steps resulting in time-consuming and expensive procedures. Additionally, manual sample handling is prone to errors and cross-contamination and is strongly dependent on the analyst's skills.

Automated sample preparation represents a viable solution to overcome these challenges. The TriPlus RSH autosampler platform provides advanced, built-in robotics that deliver exceptional precision and reproducibility, combined with unprecedented flexibility to fully automate daily sample handling operations or customize more

* Applicable on a TriPlus RSH autosampler with equivalent configuration

complex sample preparation workflows. To offer one level higher in the scale of automation, the new Thermo Scientific™ TriPlus™ RSH SMART autosampler provides an additional layer of reliability and confidence in analytical results, thanks to the automatic SMART syringe identification and usage tracking capabilities.[1]

Nitrosamines are ubiquitous compounds classified as probable carcinogens by the International Agency for Cancer Research (IARC).[2] They, therefore, represent a matter of concern, especially since June 2018 when NDMA was reported to be found in the angiotensin II receptor blocker valsartan and several batches were recalled. The challenges of nitrosamine analysis in pharmaceuticals relate to sensitivity that must be achieved to fulfill the regulation requirements, and selectivity that is critical to avoid false positive non-compliant results. Volatile nitrosamines can be extracted with headspace techniques,[3-9] which are very convenient because almost no sample preparation is required. However, for less volatile nitrosamines and/or thermolabile matrices, liquid-liquid extraction is typically performed before GC-MS analysis. Contamination of the samples can easily occur as small amounts of nitrosamines can be found in the everyday environment, reagents, and laboratory equipment such as plastic and rubber materials, pipettes and even nitrile gloves. The adoption of an automated sample preparation workflow becomes, therefore, beneficial to control and contain possible sources of contamination that cause false positive results, deliver more accurate data for greater confidence, increase sample throughput, and improve the analyst's safety by minimizing the user interactions with samples and potentially harmful chemicals.

In this study, the reliability of an automated workflow for solvent extraction of nitrosamines from metformin drug substance was tested by assessing the long-term extraction repeatability and the recovery.

Experimental

The United States Food and Drug Administration (U.S. FDA) has published several analytical methods that may be considered when determining nitrosamine content in active product ingredient (API) or final pharmaceutical product (FPP),[3-9] providing instructions related to both the sample preparation and the analytical conditions. To automate the sample preparation procedure, a pre-compiled sequence of operations (prep cycle) was developed and used to operate the TriPlus RSH SMART autosampler, equipped as shown in Figure 1. A detailed description of the required autosampler configuration, including a complete list of suggested consumables, is reported in Appendix 1. The use of amber vials and protection of the reagent containing the internal standard (ISTD) with tin foil is strongly suggested as nitrosamines degrade under UV light.

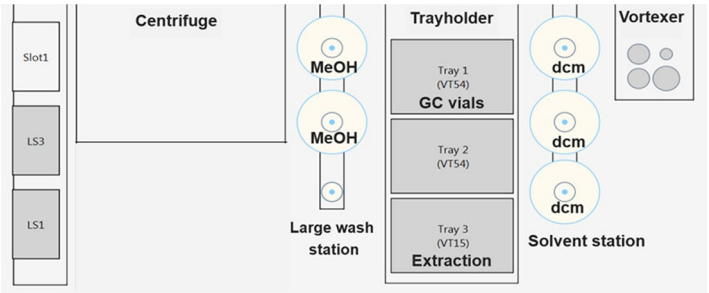

Figure 1. TriPlus RSH SMART autosampler configuration for automated extraction of nitrosamines from drug substance

The sample preparation was performed using a TriPlus RSH SMART bench station for off-line workflow that allowed preparation of samples in batches before GC-MS analysis. The implemented script includes additional steps that can be enabled when the preparation of extracts for LC analysis is required. In this case, a Multiple Headspace Extraction (MHE) module and a headspace (HS) tool are required to evaporate the solvent (DCM) prior to injection into the LC system. A schematic of the developed workflow is reported in Figure 2.

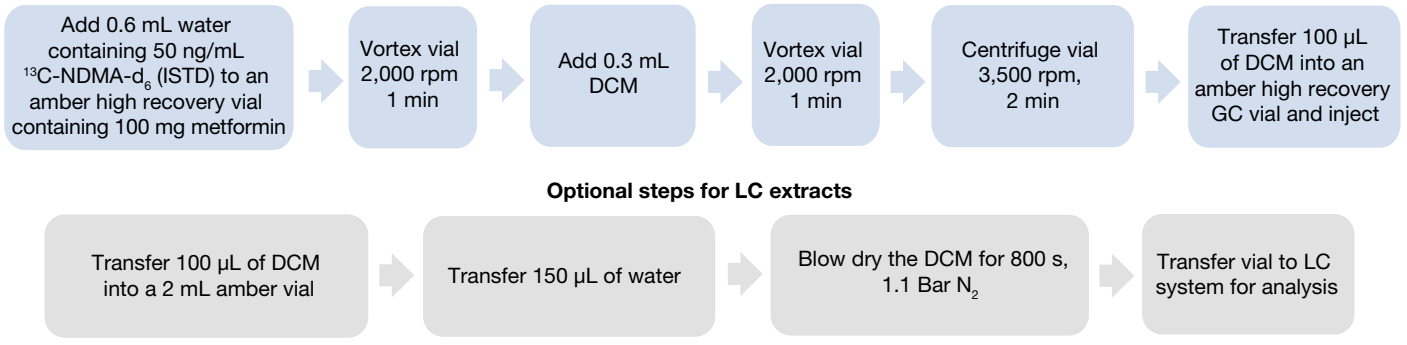

Figure 2. Workflow showing the sample extraction procedure for GC and LC (optional)

The TriPlus RSH SMART autosampler, when installed on top of the GC-MS, can execute the same sample preparation workflow with on-line GC-MS injection, where each sample is injected just after the extraction. The analyst is only required to weigh the sample, while the entire extraction procedure is performed by the autosampler. The automatic tool change (ATC) station available on the autosampler is key to automatically select up to three dedicated syringes of different volumes for reagents dispensing and for transferring the supernatant to a clean vial before injection into the analytical system. The solvent station, with up to three 100 mL bottles, provides a reservoir for the addition of different reagents. The vortex mixer delivers efficient extraction of nitrosamines from the matrix, and the centrifuge ensures that the analytes of interest are separated from the aqueous phase containing the unwanted matrix components, before transferring the organic phase to a clean vial for analysis.[10] Moreover, multiple bottles can be used for syringe washing, thus minimizing the risk of carryover and cross-contamination.

After the sample extraction was completed for a batch of six samples, vials were transferred to the analytical system. A Thermo Scientific™ Orbitrap™ Exploris™ GC mass spectrometer coupled to another TriPlus RSH SMART autosampler was used to validate both the sample preparation workflow and the GC-MS method in accordance with the ICH guidelines[11] used in U.S. FDA and worldwide GMP regulatory validation procedures. The results regarding the analytical method validation, such as linearity, sensitivity, precision, and quantitative performance, as well as the operating conditions and the list of target analytes, are detailed in Application Note 10753.[12]

Data acquisition, processing, and reporting

The TriPlus RSH SMART autosampler is integrated with Thermo Scientific™ Chromeleon™ 7.3 Chromatography Data System (CDS) software to ensure a streamlined automated workflow—from off-line sample preparation, to sequence setup, data acquisition, and reporting. The SMART technology provides a traceable usage-based approach to gas chromatography (GC) syringe management directly from the CDS, resulting in increased reliability, instrument up-time, confidence in the results, and full traceability. Moreover, with the ever-evolving compliance requirements for data integrity and data security, Chromeleon CDS provides a secure platform for analytical laboratories to comply with modern regulatory guidelines, including FDA 21 CFR Part 11 and European Commission (EU) Annex 11.

Results and discussion

When using an automated sample preparation workflow, it is vital that the instrument works unattended, producing repeatable and reliable extracts over time. Long-term extraction repeatability, recovery, and precision were assessed to test the performance and compliance of the developed workflow for everyday use in regulated environments.

Long-term extraction repeatability

Calibration standards and blanks, as well as metformin samples, were extracted during both the validation and method development phases and injected into the chromatographic system over a period of several weeks to monitor the extraction performance. The consistency of the results was evaluated by monitoring the peak area repeatability of the ISTD (^{13}C-NDMA-d_6) across the analyzed samples. The exceptional precision of the TriPlus RSH SMART autosampler ensured reproducible addition of the ISTD, combined with effective analyte extraction with peak area % RSD of 7.7, confirming an outstanding extraction stability over a long-term period, as reported in Figure 3.

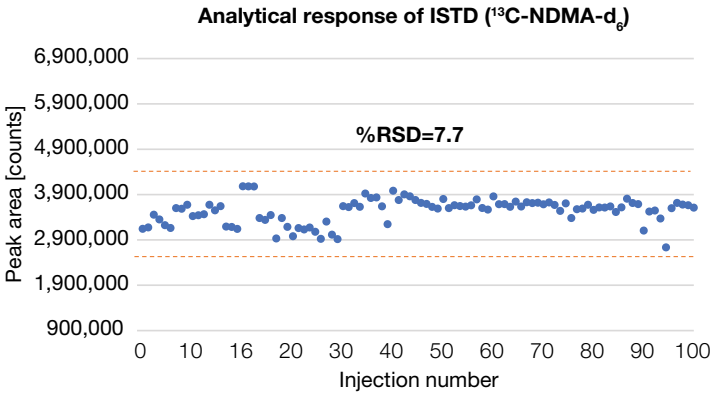

Figure 3. ISTD (^{13}C-NDMA-d_6) peak area % RSD obtained analyzing extracts (n=100) over a period of several weeks

Accuracy (recovery) and precision

Linearity was assessed by spiking DCM with nitrosamine standard mix in a range of 0.6–6,000 ng/g in metformin drug substance. Metformin samples (100 mg) were spiked prior to extraction at 1.0, 2.0, 2.5, and 10 ng/g (w/w) and used to assess recovery and precision. Each level was prepared and injected in triplicate. According to the ICH Guidelines Q2 (R1), recovery should be between 70 and 130% and the calculated amount should fall within 20% of the spiked concentration. The vortexer module ensured effective extraction of the target analytes, meeting the ICH performance criteria with calculated recovery ranging from 77 to 105% and overall precision lower than 12% as reported in Table 1.

Table 1. Accuracy and precision table showing the spiked concentration (ng/g), the calculated amount (ng/g), the calculated recovery (%), and the recovery % RSD for metformin drug substance spiked with 1.0, 2.0, 2.5, and 10 ng/g (w/w) nitrosamine standards. Each level was prepared and injected in triplicate.

Compound	Spiked concentration (ng/g)	Average calculated amount (n=3, ng/g)	Average calculated recovery (n=3, %)	Amount % RSD
NDMA	2.0	2.1	104	7.8
NMEA	1.0	1.0	98	9.4
NDEA	2.0	2.1	105	4.6
NIPEA	1.0	1.0	103	7.4
NDIPA	1.0	0.9	88	10.6
NMPA	2.5	2.3	91	11.1
NDPA	2.0	2.2	111	7.8
NEBA	2.0	1.7	86	5.2
NEPhA	10.0	7.7	77	12
NMOR	2.5	2.1	83	13.2
NDBEA	10.0	9.8	98	10.9
NPYR	1.0	0.9	86	4.1
NPIP	2.0	2.1	105	3.7
NDBA	2.0	2.1	105	8.5
NDPhA	2.5	2.5	99	8.2

Conclusions

The results of these experiments demonstrate that the automated sample preparation capability of the TriPlus RSH SMART autosampler provides an ideal solution for pharma QC analytical science laboratories looking to improve productivity and deliver confident results.

- Unattended operations and improved sample throughput can be achieved through the automated sample preparation workflow, requiring only 6 minutes per sample. Automation minimizes solvent consumption (0.3 mL of DCM were used compared to the 2 mL suggested in the U.S. FDA method[13]) and reduces the risk of errors and cross-contamination. Additionally, it improves the analyst's safety by limiting exposure to toxic chemicals.
- Reproducible ISTD addition and preparation of extracts of metformin samples were demonstrated over a long-term period with peak area % RSD for ^{13}C-NDMA-d_6 in the injected extracts (n=100) of 7.7.
- High extraction efficiency in compliance with the ICH guidelines was demonstrated with recovery between 70 and 130% and calculated amount within 20% the spiked concentration for extracted standards ranging from 1 to 10 ng/g.

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Appendix

TriPlus RSH SMART autosampler configuration for automated extraction of nitrosamines from drug substance and list of suggested consumables

TriPlus RSH SMART minimum configuration	Part number	Qty
TriPlus RSH SMART Advanced For Liquid Injections, regular rail*	1R77010-2003	1
Automatic Tool Change (ATC) station Stores and changes automatically up to three syringe tools. Up to two ATC stations can be configured on each TriPlus RSH SMART Advanced autosampler.	1R77010-1019	1
Solvent station Solvent station for 3× 100 mL solvent bottles. Bottles with seal and caps are included.	1R77010-1031	1
Large wash station Large wash station for 2× 100 mL solvent bottles and one waste position. Bottles with seal and caps are included.	1R77010-1030	1
Vortexer module For the intensive mixing of one vial at a time. Suitable for 2, 10, or 20 mL vial.	1R77010-1033	1
Tray holder for VT15, VT54, VT70, and R60 Vial Trays Tray holder able to house up to 3 different sample trays among VT15, VT54, VT70. Alternatively, the tray holder can accommodate one aluminum sample tray R60. Sample trays are not included.	1R77010-1021	1
VT54 vial tray Sample tray for fifty-four 2 mL vials	1R77010-1023	1
Centrifuge combi 2× 10 mL vials, 2× 20 mL vials or up to 4× 2 mL vials. Maximum speed of 4,800 rpm (2,000 × g RCF)	1R77010-1193	1
Liquid syringe tool For syringes of 0.5, 1.0, 5, 10, 25, 50, or 100 µL with a 57 mm needle length	1R77010-1007	1
10 µL Thermo Scientific™ GC SMART Syringes 57 mm needle length, 26S gauge, Cone needle type	365D0291-SM	1
1 mL Gas-Tight HT SMART Headspace syringe Up to 150 °C	365K2871-SM	1
100 µL Fixed Needle Gas-Tight SMART Syringe 57 mm needle length, 26S Gauge	365H2161-SM	1
Optional modules required for LC sample prep	**Part number**	**Qty**
Headspace tool For a 1,000 µL syringe with a needle length of 65 mm	1R77010-1012	1
Multiple headspace module Comes with MHE station module, MHE tool and MHE needle gauge 22/5	1R77010-1036	1
Suggested consumables	**Part Number**	
9 mm Target DP Micro-V tapered MicroVial With 150 µL reservoir, total volume 1.4 mL, usable volume 1.0 mL, screw cap, amber	C4000-V2	
Magnetic 9 mm open top cap 6 mm hole, soft septum, PTFE/blue silicone	C5000-46M	
9 mm Target DP MacroVial 350 µL, fused insert, total volume 475 µL, usable volume 350 µL, screw cap, amber	C4000-LV2W	
9 mm Open top short screw AVCS cap (not magnetic) 6 mm hole, white silicone/red PTFE	9-SCK(B)-ST1	
Thermo Scientific™ TraceGOLD™ TG-1701 MS column 30 m, 0.25 mm, 0.50 µm	26090-2230	
Thermo Scientific™ LinerGOLD™ Splitless/Split GC liner With single taper with quartz wool	453A1925-UI	

*Or equivalent TriPlus RSH base liquid configuration

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