FULLY AUTOMATED METHOD FOR DRUG PRODUCT LEACHABLE STUDIES

APPLICATION NOTE AS-252

Authors

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Abstract

This work describes the application of a fully automated sample preparation applied to an extractables and leachables (E+L) study. Laboratory analysts are often faced with laborious sample preparation which is time consuming and prone to errors and therefore look for ways the processes can be automated. In addition, some sample types encountered in +EL studies can be challenging both manually and for automated preparation.

With relative standard deviations (RSDs) of 13% or less, much lower than the limit of 25%, it is demonstrated that automation can meet the precision targets required. With sensitivity sometimes an issue for analysts, automated solvent exchange with the GERSTEL mVap has been shown to improve method sensitivities without reduction in performance of recovery or repeatability.

Fully Automated Method for Drug Product Leachables studies

Introduction

Extractables and leachables analyses are performed on pharmaceuticals drug products and the materials that come in contact with the drug product, to investigate what compounds have the potential to leach (extractables analysis) or do leach (targeted leachables analysis) into that product [1].

As these methods frequently contain many extraction steps, data quality can vary due to errors linked with the complexity of the extraction or the number of test articles extracted. Improvements in data quality and reproducibility are the primary reasons analytical scientists look to automation of sample extraction.

Automation has been shown to be a valuable tool to reduce the number of manual steps required in sample extraction[2] with an accompanying improvement in data quality due to the high reproducible nature of automated platforms[3]. This application note describes an automated liquid-liquid extraction for the application in a targeted leachables analysis. This work also shows how use of the ^{m}VAP, GERSTEL's evaporation station, improves sensitivity by providing sample enrichment, a factor which is vital, as regulatory requirements drives Analytical Evaluation Thresholds (AET), and therefore analytical detection and quantitation limits requirements ever lower.

Instrumentation

- GERSTEL MultiPurposeSampler 2 metre dual head Robotic/Robotic^{Pro}
- Anatune CF200 centrifuge
- GERSTEL QuickMix
- GERSTEL ^{m}VAP
- Split – Splitless inlet
- Agilent 7890B GC
- 5977A Inert^{Plus} MS

Figure 1. GERSTEL MultiPurposeSampler which can be used for Extractables and Leachables studies.

Method

An ethyl acetate/acetone solvent extraction method was developed to extract 1 mL of sterile injectable drug product. The sample was manually pipetted to a 4 mL vial. The remainder of the process was then fully automated as shown in figure 2.

Two spiking solutions of 120 and 12 ppm were prepared containing 1-tridecene, 1-bromotridecane, eicosane, 1-bromoeicosane and butylated hydroxytoluene (BHT) and were used to spike blank drug matrix which was performed using the GERSTEL MPS.

A validation protocol was followed to ensure the suitability of the method to meet specified criteria with a calibration range of 0.6 to 6 ppm with levels at 0.6, 1.6, 3.0, 4.5 and 6 ppm. Samples were spiked in triplicate at 0.6 and 6 ppm with six replicates of the mid-level spike at 3 ppm.

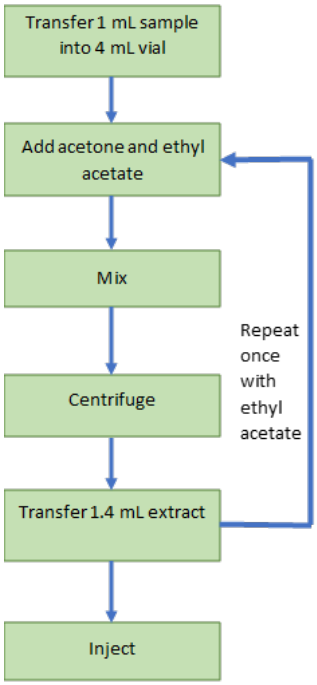

Figure 2. GERSTEL MultiPurposeSampler which can be used for Extractables and Leachables studies.

Results

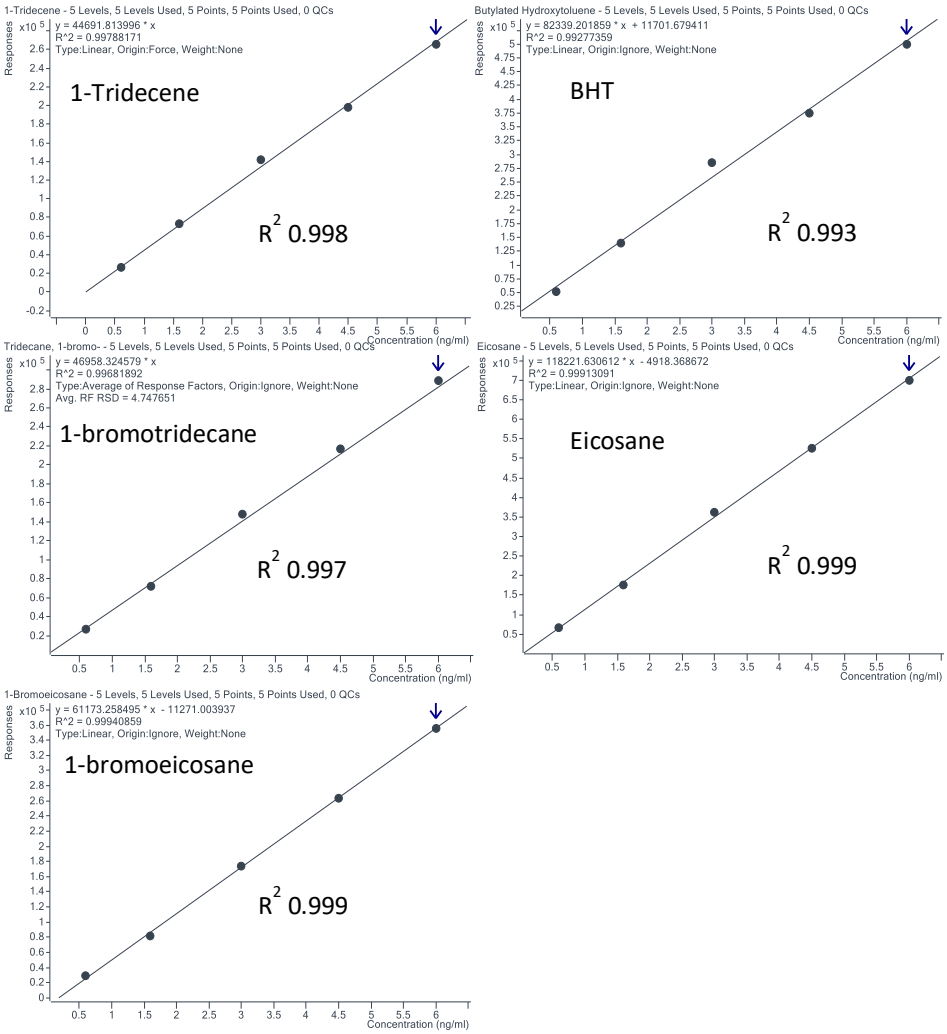

Figure 3. Calibration curves for each analyte with all R^2 above 0.992.

The method showed good performance during validation with all recoveries falling between a target of 70-130% and relative standard deviations of less than 25% with exception to BHT, which showed over recoveries at 3 and 6 ppm. However, precision for this analyte was well within tolerance at all levels. Table 1 shows the results from the validation. Good precision was observed for all analytes.

Table 1. Validation Results

Compound	0.6 ppm		3 ppm		6 ppm	
	RSD %	Recovery %	RSD %	Recovery %	RSD %	Recovery %
1-Tridecene	7.45	113	3.45	123	0.02	126
Butylated hydroxytoluene	13.0	106	1.74	146	0.97	147
1-Bromotridecane	9.28	114	1.92	126	1.41	124
Eicosane	10.2	108	1.88	115	3.55	119
1-Bromoeicosane	12.4	138	5.25	117	3.87	102

Signal to noise was above 10:1 for all compounds at 0.6 ppm, with the lowest at 30 for 1-bromotridecane (figure 4), however use of the GERSTEL ^{m}VAP was evaluated to further improve sensitivity of the method.

Once extraction was complete, the extract vial was transported to the ^{m}VAP and the extract was reduced and solvent exchanged to heptane. Typically, ^{m}VAP samples are evaporated to dryness, however in this application this would risk evaporation of analytes of interest. Therefore, half of the extract volume was evaporated for 9 minutes followed by addition of heptane with further evaporation for 3 minutes. This was performed in triplicate and precision and recovery measured, comparing to a solvent standard spiked at the expected concentration, Table 2.

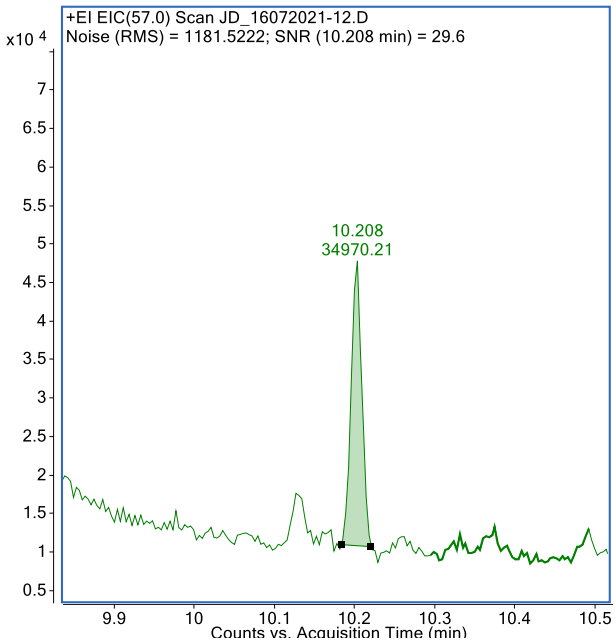

Figure 4. Chromatogram of 1-bromotridecane at 0.6 ppm

Good recoveries were achieved between 90% and below 120% showing that no loss of compounds was observed and that the extract concentration had not been enhanced when compared to the solvent standard, due to over evaporation. RSDs were all below 10% highlighting the reproducibility of this approach, Table 2. Extract volume was reduced to 0.8 mL from 2.4 mL increasing the extract concentration three-fold which provides a significant improvement on peak height, Figure 5. An improvement in root mean squared signal-to-noise (rms S/N) of almost 10x was also observed for 1-bromotridecane, 682 pre-enrichment and greater than 5000 post-enrichment.

Table 2. Precision and recovery values of solvent exchange using GERSTEL^{m}VAP

	Recovery %	RSD %
1-Tridecene	93.7	6.7
Butylated hydroxytoluene	96.1	8.0
1-Bromotridecane	101	7.4
Eicosane	108	6.4
1-Bromoeicosane	116	9.1

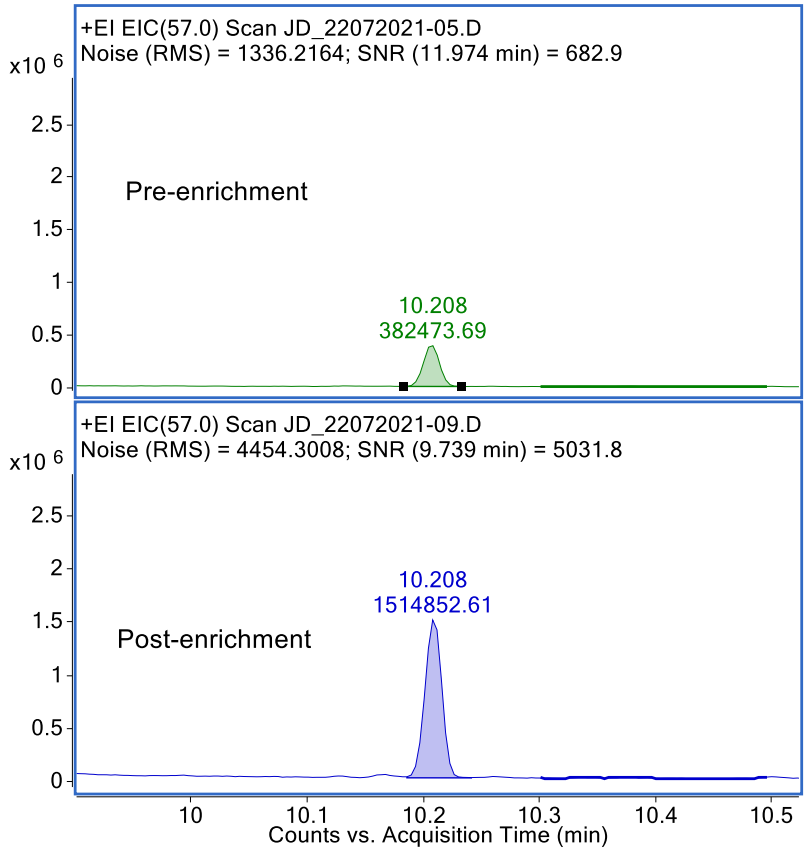

Figure 5. Peak height comparison pre- and post-solvent exchange

Conclusion

The work describes here shows a successful validation of a method for extraction of a drug product in a typical targeted leachables study. With precision and recoveries within the desired targets, the automated method has shown that good data quality can be achieved whilst reducing the number of manual steps needed.

Whilst it can sometimes be challenging to reach low detection limits with methods that require small sample volumes, use of GERSTEL's ^{m}VAP, a fully automated evaporation station, can counteract this and provide an improvement in the concentration factor of a method, enabling analysts to use smaller sample volumes; in this case, 1 mL.

References

1. https://www.smithers.com/en-gb/industries/life-science/medical-device/chemical-analysis/extractables-and-leachables
2. AS182- Fully Automated Method Using DiLLME for Extractables and Leachables Studies, Dan Carrier, Alan Hutchinson, 2017.
3. AS246, Simulant Solution Extraction Using Automated Dispersive Liquid-Liquid Microextraction (DiLLME) in Extractables and Leachables Studies, Jon Dunscombe, 2021.