Direct Quantitation of Formaldehyde in Pressurised Metered Dose Inhaler (pMDI) Devices.

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Summary

A direct sampling procedure has been successfully developed for the derivatisation of residual levels of formaldehyde in pressurised metered dose inhaler (pMDI) devices, enabling total quantitation using standard high performance liquid chromatography coupled with ultra-violet detection (HPLC-UV).

The potential presence of formaldehyde, which can be derived from a range of commonly utilised polymeric components, is a particular area of concern for high exposure risk drug delivery systems such as pMDI devices[1,2].

Formaldehyde is toxic, allergenic and is a known human carcinogen. The compound is also highly reactive and has the potential to adversely interact with the active pharmaceutical ingredient (API) or other excipient components. This can cause issues in terms of product safety and have a negative impact on the overall efficacy of the drug product.

The short term exposure limit for formaldehyde is under review and is likely to be reduced from the current level of 2 parts per million (ppm)[3]. In order to assess the viability of current sampling methodologies a study was performed where placebo pMDI devices were spiked with known amounts of formaldehyde around the level of concern. The results from this study showed that existing procedures would not be suitable.

The developed method has been fully validated in accordance with ICH guidelines and is capable of sub µg / canister detection levels, in a range of generic drug product formulations, with a limit of detection (LOD) of 0.55 µg / canister and reproducibility typically <2% RSD.

Introduction

A method was required to determine the total quantity of residual formaldehyde present in metered dose inhalers (pMDIs). These devices are typically sampled either by actuation of the device, or by removal of the total contents of the canister following freezing. Due to the low levels of detection required and the volatile nature of formaldehyde neither of these methods was found to be suitable and so an alternate sampling procedure was developed.

Initial work, using both HPLC-UV and HPLC-MS/MS detection, analysed formaldehyde as its dinitrophenyl hydrazone (DNPH) derivative. During development it was identified that the available DNPH derivatising reagent contained low levels of both formaldehyde-DNPH and acetaldehyde-DNPH.

Low levels of formaldehyde and acetaldehyde also appeared to be present in various solvents used to process the samples. This required a significantly greater proportion of the total content of the pMDI to be sampled in order for the desired assay sensitivity of 1 to 20 µg total formaldehyde in pMDI to be attained.

A method for carrying out this sampling based upon allowing the liquefied pMDI contents to evaporate and bubble through DNPH reagent was developed. The purpose of this validation study was to determine the performance of the developed methodology used to quantify the total formaldehyde content present in pMDI samples using HPLC-UV. The validation used calibration curves constructed from pre-derivatised formaldehyde-DNPH standards. Propionaldehyde-DNPH was used as an internal standard and calibration lines and subsequent quantification was based upon area response.

Experimental Details – Sample Preparation

pMDI samples are prepared as follows:

- The pMDI can, a labelled 20 mL headspace vial and cap is placed on dry ice.
- 4 mL of receptor solution is added to 2 x 10 mL headspace vials.
- Lengths of deactivated fused silica capillary column (i.d. 0.32 mm) are introduced into the capped receptor vials.
- The valve of the chilled pMDI can is removed with pipe cutters to allow the contents to be transferred to the chilled 20 mL headspace vial. The vial is then was capped as illustrated in figure 1.
- The gas evolved from the sample propellant is allowed to bubble through the vials containing a receptor solution.
- The sample is purged with nitrogen.

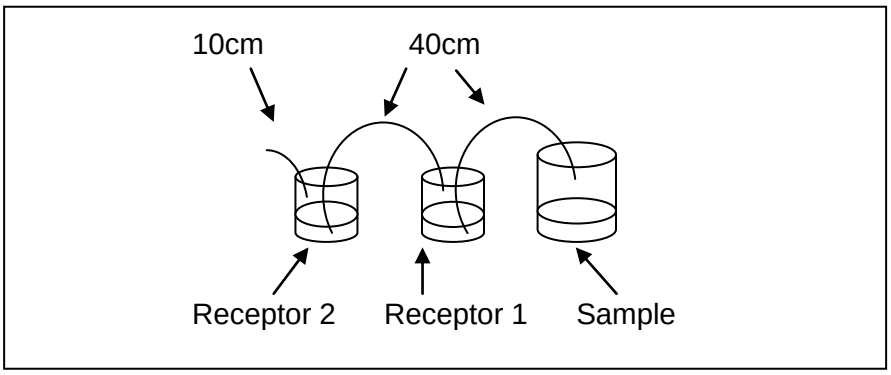

Figure 1 - pMDI Sampling Configuration

- Solvent is added to the receptor vials which are recapped, mixed and sonicated.
- A portion of the contents is finally transferred to LC auto-sampler vials for analysis using the standard HPLC-UV method[4] described below.

A 37 % weight in weight (% w/w) solution of formaldehyde in water stabilised with methanol (formalin) was used to spike placebo samples and pMDI cans at the 1, 10, 20 µg level. The latter were spiked with formalin prior to the addition of propellant.

Assessments of recovery, accuracy and precision were performed using both recovered material from spiked placebo and spiked pMDI cans. Direct derivatisation of the formalin solution used to prepare the spiked pMDIs was used to validate the actual concentration of formaldehyde in solution.

The initial results suggested that the formaldehyde spiked into the pMDI cans was either absorbed onto surfaces in the device, absorbed onto the drug active, or polymerised to paraformaldehyde. Amendments to the extraction procedure were made to include the addition of acetonitrile which significantly improved the level of recovery.

High Performance Liquid Chromatography Method Details

Analysis of samples and standards was performed with a Dionex Ultimate 3000 HPLC-DAD system using the following method parameters;

Parameter	**Setting**				
Instrument	Dionex Ultimate 3000 or equivalent				
Column Temperature	45 °C				
Column	Waters Atlantis T3 100 mm x 2.1 mm x 3 µm				
Solvent Programme	Time (min):	Flow (mL/min)	0.1% Acetic acid in water (%)	Acetonitrile (%)	
	0.0	0.4	65	35	
	4.0	0.4	65	35	
	11.0	0.4	0	100	
	13.0	0.4	0	100	
	13.1	0.4	65	35	
	17.0	0.4	65	35	
Injector Volume	5 µL				

Table 1 – HPLC Instrument Parameters

Injection Mode	'Normal'
UV Detector Wavelength	380 nm
Band Width	2 nm
Data Collection Rate	5 Hz

Table 2 – UV Detector Parameters

A typical HPLC-UV chromatogram for a derivatised product formulation sample is shown below;

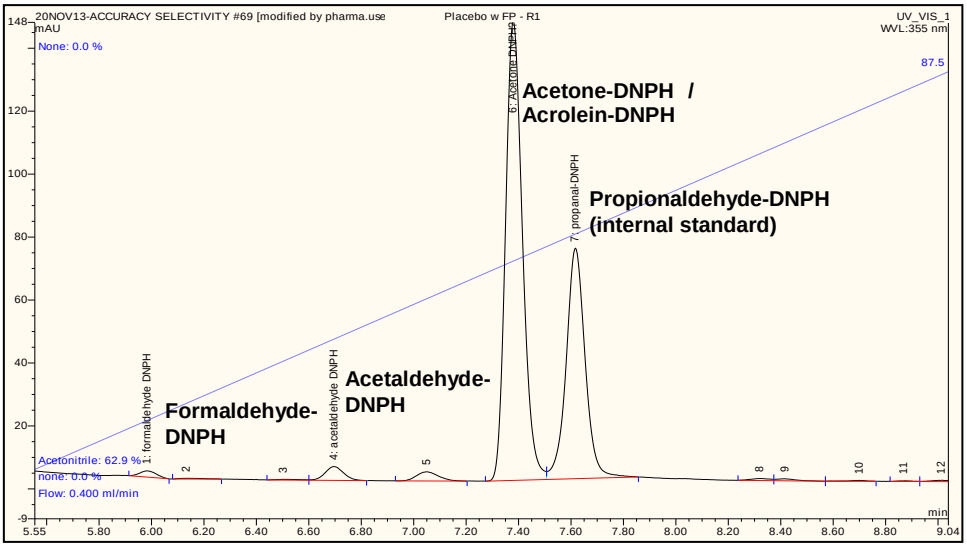

Figure 2 – LC-UV Chromatogram for Derivatised Placebo Sample

Method Validation

The method was fully validated in accordance with current ICH guidelines[5] and a summary of results is shown in the following tables:

Area Assessed	Acceptance Criteria	Result	Pass/Fail
Accuracy and Precision	%RSD <2% for initial precision	0.29-1.14%	Pass
	%RSD <5% for further precision	0.32-1.20%	Pass
	% agreement is 95-105%	99.3-102.5%	Pass
Selectivity	No significant overlap with peaks of interest	There was no significant overlap with the peaks of interest.	Pass

Table 2 – Accuracy, Precision and Selectivity

Area Assessed	Acceptance Criteria	Result	Pass/Fail
Limit of Detection (LOD)	%RSD <30%	<3%% LOD = 0.55µg / can	Pass
Limit of Quantification (LOQ)	%RSD <15%	<1.5%	Pass
	Mean recovery 70-130%	95.2% LOQ = 1µg / can	
Linearity	Linear correlation >0.99	0.9999	Pass
Range	No set criteria	0.55µg - 20µg equivalent formaldehyde pMDI content	N/A

Table 3 – LOD, LOQ, Linearity and Range

Area Assessed	Acceptance Criteria	Result	Pass/Fail
Intermediate Precision – Formalin	Mean recovery 90-110%	105%	Pass
	%RSD <15%	<5%	Pass
	%RSD <20%	<10%	Pass
Stability	The difference for Day 1 & 4 should be <15%	<15%	Pass
Robustness	The difference from default should be 90-110%	All results 90 – 110%	Pass

Table 4 – Intermediate Precision, Stability and Robustness

Conclusion

In order to both achieve required sensitivity and to prevent the loss of analyte associated with conventional sampling methods a novel procedure has been successfully developed to directly determine the total quantity of formaldehyde in metered dose inhalers (pMDIs) as its dinitrophenyl hydrazone (DNPH) derivative using a standard HPLC-UV based assay. The method is fully validated for routine sample analysis in accordance with current ICH guidelines, with an LOD of 0.55 µg / canister.

References

[1] Madan A K, Kushwaha P: *Extractables and Leachables: An Overview of Emerging Challenges, Pharmaceutical Technology 2008.* 32:8 (online only)

[2] Ball D J, Norwood D L, Stults C L M, Nagao L M (eds): *Leachable and Extractables Handbook – Safety Evaluation, Qualification, and Best Practices Applied to Inhalation Drug Products:* pp, , 2012.

[3] http://www.hse.gov.uk/pubns/iacl88.htm

[4] Dionex Application Note 97: Determination of Carbonyl Compounds by Reversed Phase High-Performance Liquid Chromatography

[5] ICH Harmonised Tripartite Guideline – Validation of Analytical Procedures: Text and Methodology Q2 (R1) November 2005