Journal of Pharmaceutical and Biomedical Analysis 106 (2015) 204–209

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Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Analytical protocol for the sensitive determination of mannitol, sorbitol and glucose containing powders in pharmaceutical workplaces by ion chromatography using a pulsed amperometric detector

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ARTICLE INFO

Article history:
Received 29 July 2014
Received in revised form 2 October 2014
Accepted 4 October 2014
Available online 12 October 2014

Keywords:
Workplace
Exposure
Containment
Ion chromatography
Surrogates

ABSTRACT

Workers in the pharmaceutical industry can potentially be exposed to airborne dusts and powders that can contain potent active pharmaceutical ingredients (API). Occupational hygienists and health and safety professionals need to assess and ultimately minimise such inhalation and dermal exposure risks. Containment of dusts at source is the first line of defence but the performance of such technologies needs to be verified, for which purpose the good practice guide: *assessing the particulate containment performance of pharmaceutical equipment*, produced by the International Society for Pharmaceutical Engineering (ISPE), is a widely used reference document. This guide recommends the use of surrogate powders that can be used to challenge the performance of such containment systems. Materials such as lactose and mannitol are recommended as their physical properties (adhesion, compactability, dustiness, flow characteristics and particle sizes) mimic those of API-containing materials typically handled. Furthermore they are safe materials to use, are available in high purity and can be procured at a reasonable cost. The aim of this work was to develop and validate a sensitive ion-chromatography based analytical procedure for the determination of surrogate powders collected on filter samples so as to meet analytical requirements set out in this ISPE guide.

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1. Introduction

Workers in the pharmaceutical industry can be exposed to airborne dusts and powders that may contain potent active pharmaceutical ingredients (API). Occupational hygienists and health and safety professionals need to assess and ultimately minimise such inhalation and dermal exposure risks. Containment of dusts at source is the first line of defence but the performance of such technologies needs to be verified, for which purpose the ISPE good practice guide: *assessing the particulate containment performance of pharmaceutical equipment*, and now in its second edition is a widely used reference [1]. This document provides guidance and consistent methodologies for evaluating the particle containment (particulate emissions) of pharmaceutical equipment and systems.

In this guide the use of surrogate powders, e.g. mannitol, to challenge the performance of containment systems is recommended as their physical properties such as: adhesion, compactability, dustiness, flow characteristics and particle sizes often mimic those of API containing powders typically handled. They are safe materials to use, are available in high purity and can be procured at a reasonable cost. From an analytical viewpoint, being water soluble, they are relatively easy to handle in the laboratory.

This challenge function involves introducing a known quantity of a surrogate powder into a containment system and verifying its performance by ascertaining the extent of loss of material into the wider working environment, i.e. the fraction of surrogate powder that escapes from the containment system. By measuring the levels of surrogate material in air, through filter sampling and subsequent laboratory analysis, the extent of loss can be quantified and hence an estimation of the containment efficiency made. Similarly, assessment can be made on dusts sedimented on work surfaces through swabbing and subsequent analysis. A useful overview of containment testing for pharmaceutical equipment performance has been described by Petroka [2].

The aim of this paper is to describe our approach to the development and validation of a sensitive analytical procedure for

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http://dx.doi.org/10.1016/j.jpba.2014.10.006
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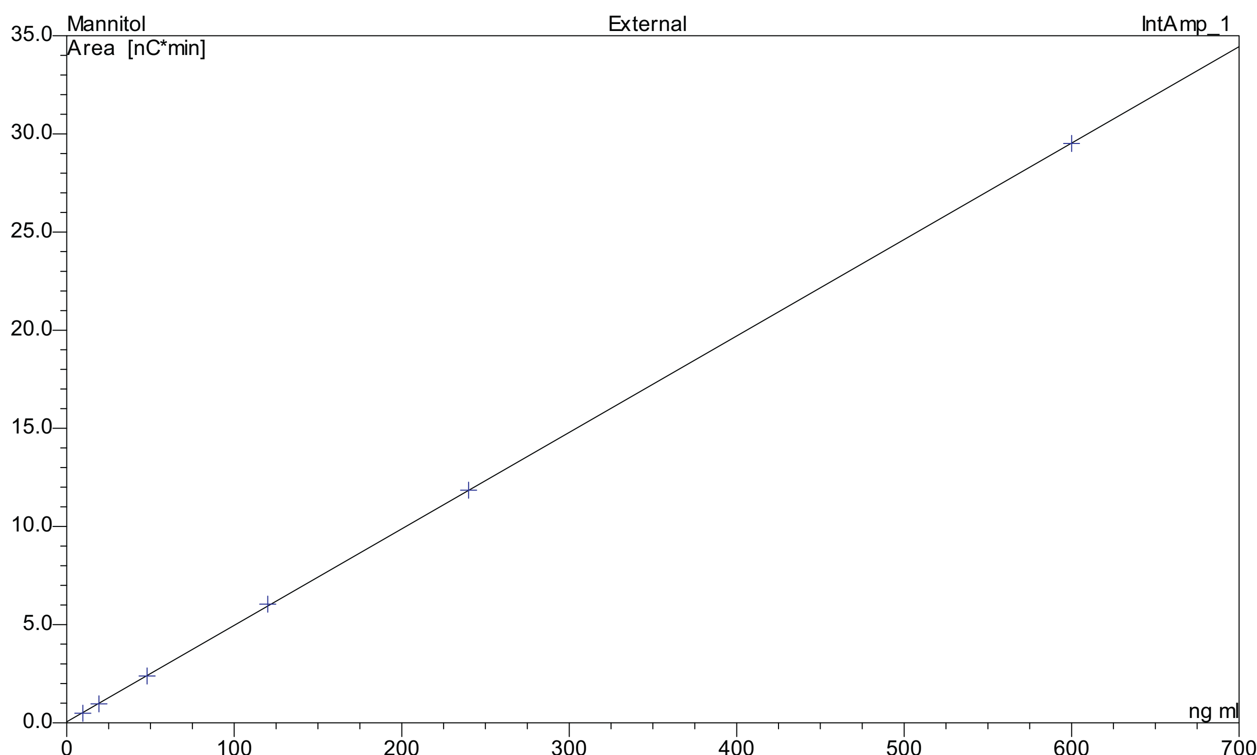

Fig. 1. Calibration plot for Mannitol over 0–600 ng mL^{-1} range.

the determination of mannitol, sorbitol and glucose surrogates collected on air filter samples. The approach described is also amenable to swab samples although this not reported here. This methodology involves leaching sampling media in water, filtration of the resultant solution and analysis using an ion chromatography (IC) system equipped with a pulsed amperometric detector (PAD). This work builds upon earlier HSL work in which an IC-PAD method for the determination of lactose [3] was developed in support of recommendations in the first edition of the ISPE guide [1].

2. Materials and methods

2.1. Instrumental considerations

Initially it was assumed that the method developed for lactose could be extended to encompass a number of additional surrogate materials recommended now for inclusion in this revised ISPE guide [1]. This original method used a Dionex PA20 5 μm column (3 mm × 150 mm with a 3 mm × 50 mm guard) and a 150 mM NaOH eluent [3]. Modifying this method, by varying the eluent concentration, did not produce the desired result of resolving the selected glucose, fructose, lactose, mannitol, sorbitol and sucrose surrogates from each other. An alternative approach using a Dionex MA1 4 mm × 250 mm column and 480 mM NaOH eluent was then examined [4]. Here, mannitol and sorbitol separated with good resolution but glucose and fructose peaks were found to co-elute. Furthermore the retention times for lactose and sucrose were too long, with a total sample run-time of ca. 45 min.

As gradient elution capability was not available at the Health and Safety Laboratory, it was decided that the existing method for lactose would be retained (sample run time of ∼8 min) and the new method validated now only for mannitol, sorbitol and glucose (sample run time of ∼25 min). It is anticipated that future measurement of surrogates would focus on the use of such substances. In summary the instrumental conditions presented in Table 1 were those subsequently used for method validation purposes.

2.2. Preparation of calibration standards

Stock standard solutions, at a nominal 1000 mg L^{-1}, were prepared by dissolving known masses of high purity surrogate materials, procured from a number of different vendors, in deionised water. From these single compound standards, a composite stock calibration standard solution was prepared at a nominal 24 mg L^{-1} from which working calibration standards and a limit of detection (LOD) test standard were prepared (Table 2).

Calibration verification standards were prepared in the same manner but using starting materials from a different vendor or from a different lot number to those used for calibration purposes.

2.3. Validation strategy

The ISPE guide [1] does not provide detail on how to validate an analytical method for the determination of surrogate analytes. For

Table 1
IC-PAD instrument conditions.

Instrument	Dionex DX-500
Autosampler	Dionex AS50
Pump	Dionex IP25
Column oven	Dionex AS50
Detector	Dionex EC50
Analytical column	Dionex MA1 4 mm × 250 mm
Guard column	Dionex MA1 4 mm × 50 mm
Column temperature	40 °C
Eluent	480 mM NaOH, carbonate free
Flowrate	0.5 mL min^{-1}
Detection technique	Pulsed amperometry
Waveform	Waveform A (Dionex, TN 21)
Injection volume	200 μL

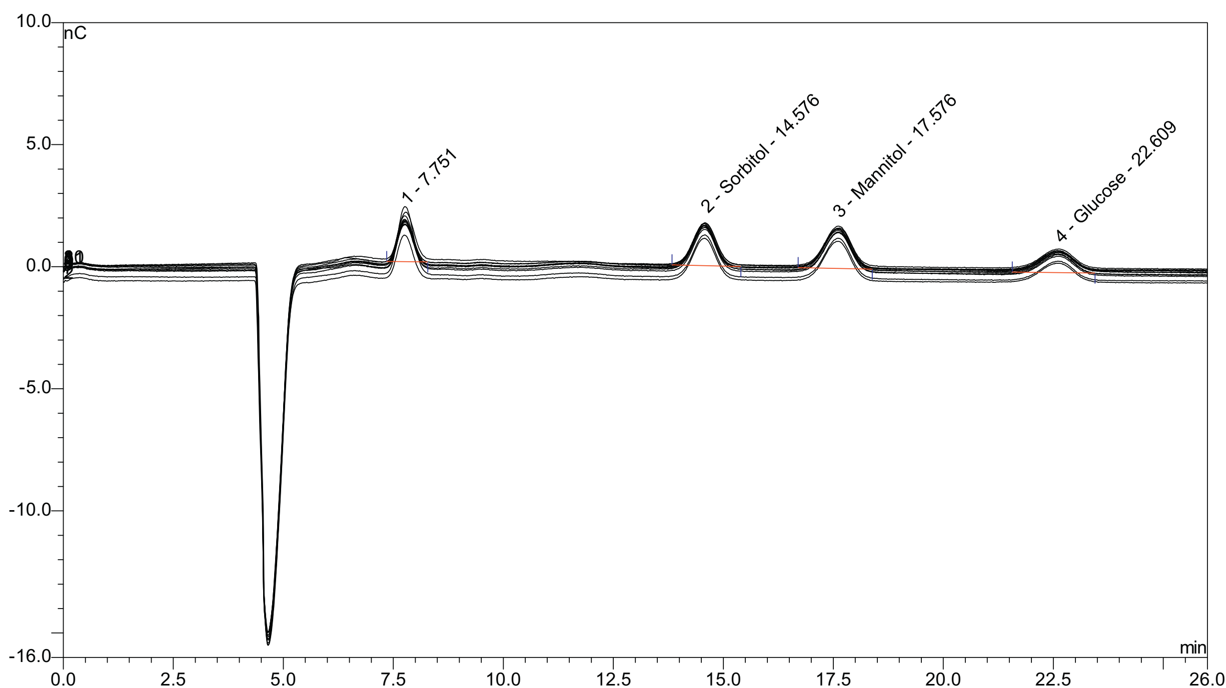

Fig. 2. Chromatogram of a 24 ng mL^{-1} standard.

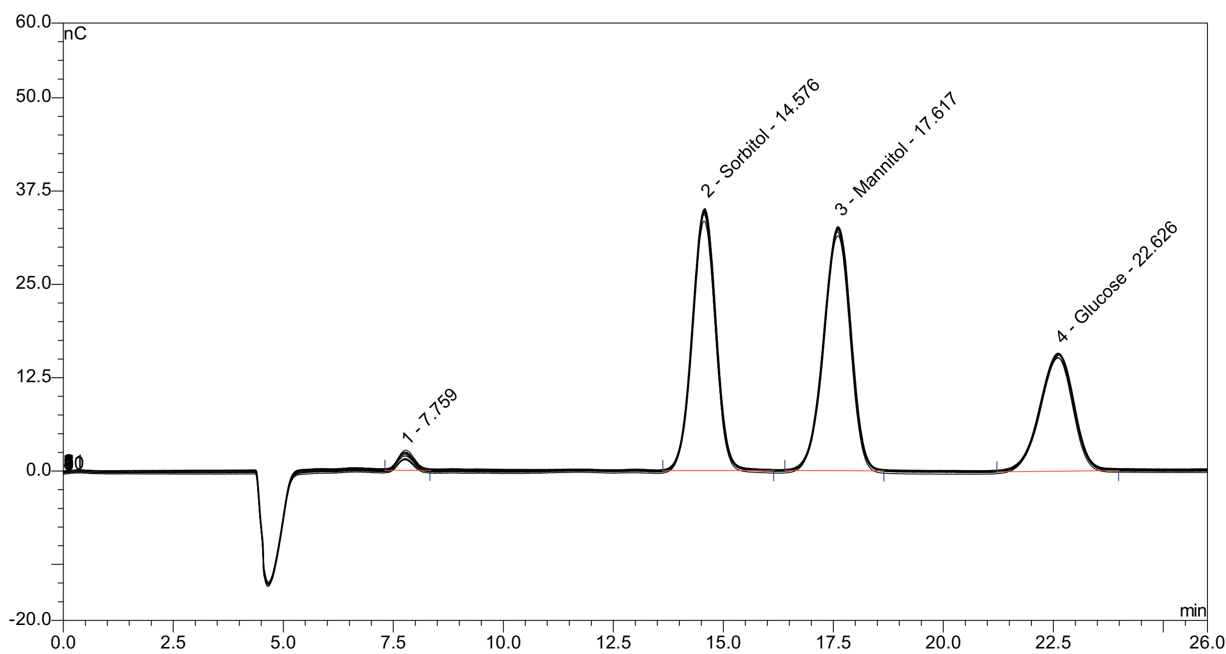

Fig. 3. Chromatogram of a 480 ng mL^{-1} standard.

Table 2
Preparation of working calibration standards.

Calibration solution	Solution to be diluted	Volume to dilute to 50 ml (ml)	Resulting concentration (ng mL^{-1})
A	Mixed calibration standard solution 24 mg L^{-1}	1.25	600
B	Mixed calibration standard solution 24 mg L^{-1}	0.5	240
C	Mixed calibration standard solution 24 mg L^{-1}	0.25	120
D	Calibration solution C	20	48
E	Calibration solution C	8	19.2
F	Calibration solution C	4	9.6
LOD test	Calibration solution A	0.5	6

consistency, a four-step validation protocol, developed previously for the lactose method [3], was used.

2.3.1. Validation step 1: determination of an instrumental LOD

The instrument was initially calibrated (Figs. 1–3) and then 11 replicates of a 6 ng mL^{-1} test standard sample analysed in one analytical run. An instrumental (LOD) was then calculated for each analyte based on 3× standard deviation of measurements made.

2.3.2. Validation step 2: spiked filter recovery test

Spiked filters were prepared and analysed to ascertain the desorption efficiency of the water extraction step. Eleven spiked filters were prepared at two loadings:

- a 25-mm diameter glass fibre filter (Pall-Gelman GF/F) spiked with 90 ng of each surrogate
- a 25-mm diameter glass fibre filter (Pall-Gelman GF/F) spiked with 1800 ng of each surrogate

Filter samples were placed in vials and 3 ml deionised water added for extraction. After 2 h of gentle agitation on an orbital shaker, ~2 ml solution aliquots were removed and filtered into autosampler vials using disposable 0.2 μm polyethylsulfone (PES) syringe filters.

2.3.3. Validation step 3: determination of a method LOD

Method reproducibility was assessed by the analysis of spiked filter (Section 2.3.2) at six time intervals over a period of 3 weeks. This encompasses analytical conditions one would expect if the method were to be in regular use, e.g. eluent replacement, preparation of new calibration standards, changes, instrument start-ups/shutdowns. Subsequently a method LOD was calculated for each analyte based upon 3× standard deviation of measurements made on twelve replicates of the 90 ng spiked filter.

2.3.4. Validation step 4: determination of sample stability

To ascertain the potential for sample loss, once extracted but prior to analysis, in the eventuality of an instrument failure, a truncated sample stability study was undertaken. Here, six replicate pairs (Section 2.3.2) were extracted, stored in capped vials, in the dark at room temperature for 7 days, before filtration and analysis.

3. Results

Validation results obtained are summarised in Tables 3–6.

4. Discussion

As a prerequisite in considering analytical aspects of this work it is useful to briefly summarise the air sampling requirements.

4.1. Sampling considerations

The ISPE guide [1] cites a number of air sampler designs that can be used to collect airborne dust samples. In the US, the 25-mm and 37-mm closed-face cassette samplers are samplers of choice whilst in the UK, the IOM sampler is widely used. Other designs such as the UK 7-hole cassette sampler and the conical design used in Germany are also mentioned. Generally these samplers operate at a nominal 2 L min^{-1} flow rate. Both personal and fixed location air sampling can be performed and sampling strategies can be found in the guide. Personal air samples are normally collected by placing the sampler in the worker's breathing zone so that the air sample drawn into the device is similar to that inhaled by the worker. Fixed location sampling can be used to identify areas within the workplace where dust may be emitted.

Developments in sampler technologies since the publication of this guide in 2012 are worth noting. Firstly, the extent of particle losses to internal walls of samplers is currently of much research interest, i.e. the fraction of dust particles which enter the sampler device but does not end up on the filter. Here the ISPE guide [1] correctly states that recovery of all potential material from the sampling device should be achieved either by rinsing or by swabbing. In the case of 25-mm and 37-mm closed-face cassette samplers it is possible to undertake in situ extraction, i.e. to add the extracting solution directly to the sampler to leach both the filter and the internal walls of the sampler as outlined in other methods [5]. For IOM samplers this is not possible and the interior walls either need to be swabbed, typically using a moistened filter of the same type used for air sampling, or to be rinsed. It is therefore important to recognise the potential for wall losses. In our experience, for example, we have recovered substantial quantities of analyte material from sampler walls, particularly if a non-conducting plastic sampler has been used, i.e. due to buildup of static charging. Such findings confirm earlier reported losses of pharmaceutical dusts to walls of samplers [6]. Disposable inserts to prevent losses to walls for use with the 25-mm and 37-mm closed-face cassette samplers are now available [7,8] ensuring that material can be recovered for analysis.

Secondly, there is a growing requirement to increase of sensitivity of air sampling methods. This can be achieved either by using a more sensitive analytical system or by collecting more material on the filter through the use of high volume air samplers. New sampler designs, operating at flow rate of up to 10 l min^{-1}, are now commercially available such as the GSP10 sampler [9] and a high-flow rate derivate of 37-mm closed-face cassette samplers described in the ISPE guide [1] is also being developed [10].

4.2. Analytical considerations

The ISPE guide [1] provides guidance and consistent methodologies for evaluating the particle containment (particulate emissions) of pharmaceutical equipment and systems and thus helps ensure that airborne levels are controlled to levels considered acceptable for workers handling API of high potency. In this context it can be useful to establish an airborne threshold or target concentration for the control of these agents. This is defined as the containment performance target (CPT). Where possible, CPT values can be linked to an occupational exposure limit (OEL) – an upper limit on the acceptable concentration of a hazardous substance in workplace air set and enforced by regulation – or some

Table 3
Validation step 1 results: determination of an instrumental LOD.

		Sorbitol	Mannitol	Glucose
LOD test sample (6 ng mL^{-1})	% mean recovery	86	77	77
	% relative standard deviation	4.4	5.5	5.9
Calculated instrumental LOD (ng mL^{-1})		0.7	0.8	0.8

Table 4
Validation step 2 results: spiked filter recovery tests.

		Sorbitol	Mannitol	Glucose
Low concentration (90 ng)	% mean recovery	93	95	94
	% relative standard deviation	2.0	1.5	2.6
High concentration (1800 ng)	% mean recovery	98	98	98
	% relative standard deviation	0.7	0.3	0.3

Table 5
Validation step 3 results: determination of a method LOD.

		Sorbitol	Mannitol	Glucose
Low concentration (90 ng)	% mean recovery	98	99	95
	% relative standard deviation	3.8	3.5	7.4
Calculated method LOD (ng/filter) using a nominal 3 ml filter extraction volume		7	7	14
Calculated method limit of quantification (LOQ – ng/filter) based upon 3.3× method LOD		23	23	46

Table 6
Results from validation step 4: determination of sample stability.

		Sorbitol	Mannitol	Glucose
Low concentration (90 ng)	% mean recovery after 7 days	94	103	92
	% relative standard deviation	1.5	2.6	2.4
High Concentration (1800 ng)	% mean recovery after 7 days	100	99	93
	% relative standard deviation	0.4	0.6	0.8

fraction of the OEL provided that an OEL value exists for the API in question.

In the absence of compound-specific OELs, occupational exposure bands (OEB) have usually been established. Here banding is a process of assigning a compound to a health hazard banding (HHB) category that corresponds to a range of airborne concentrations, and the associated control mechanisms, needed to ensure safe handling. Such an approach is often used within the pharmaceutical sector given that OELs do not often exist for newly synthesised compounds of a proprietary nature. These can range from HBB1 (low toxicity) to HHB5 (high toxicity) with increasingly stringent requirements for containment control. Typically when handling a new API, in absence of a specific OEL and detailed supporting toxicological data, a default control banding of HBB4 is often employed.

A method has been developed that successfully measures sorbitol, mannitol and glucose captured on glass fibre air sampling filters. Based upon the validation approach used, absolute instrumental LOD of 7, 7 and 14 ng respectively were determined. Spike recoveries over a range of concentrations were typically 90–110%; within run and between run precision were typically <3%; extracted test solutions were found to be stable for at least 7 days prior to analysis and a method LOQ was determined to be ca. 30 ng.

The ISPE guide [1] defines the measurement sensitivity as the analyte mass that needs to be quantifiable for the evaluation of a containment system to a desired percentage of a stated CPT. In order to determine whether the method sensitivity is suitable, the following equation, as defined in the ISPE guide [1], can be used: $\text{Sensitivity(ng)} = \frac{(\%S \times \text{CPT} \times 1000\,\text{ng}/\mu\text{g} \times Q \times t)}{(1000\,\text{L m}^{-3})}$ where:

$\%S$ = percentage of the CPT that is desired to be quantitated/100
CPT = containment performance target ($\mu g/m^3$)
Q = air sampler pump flow rate ($L\,min^{-1}$)
t = sampling time (min)

As an example, for a method LoQ of ca. 30 ng determined in this work, and applying the following conditions:

$\%S$ = 10% (acceptable value, Appendix 4, ISPE guide)
CPT = 1 $\mu g/m^3$ (health hazard band 4)
Q = 2 $L\,min^{-1}$ (nominal sampling rate for an IOM air sampler)

A minimum sampling time of 150 min would be required to reach the desired method sensitivity to undertake containment assessment against this CPT value of 1 $\mu g/m^3$ with quantification to a tenth of this limit. By switching to a higher flow rate sampler, such as the GSP10 device, similar method sensitivity would be achievable in a shorter sampling time of 30 min. This could be advantageous in assessing containment performance arising from short duration task specific workplace activities that can be extremely dusty, e.g. filling bags with powders.

5. Conclusion

An IC-PAD method has been developed that successfully measures sorbitol, mannitol and glucose captured on glass fibre air sampling filters. The method LOQ was determined to be ca. 30 ng which equates to a minimum air sampling time of 150 min if a containment assessment exercise were to be conducted against a 0.1 $\mu g/m^3$ CPT by collecting sample on a filter using an air sampler operating at 2 $L\,min^{-1}$.

Acknowledgement

Funding from the HSL Investment Research Program is gratefully acknowledged.

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