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Short communication

# Excipient quantitation and drug distribution during formulation optimization

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**Abstract**

An oral granules formulation experienced high drug content and increased variability when the process was scaled up from lab scale to clinical manufacturing scale. It was suspected that mannitol, due to its smaller particle size and lower density, was preferentially lost during the top spray granulation process, thereby causing active enrichment in the remaining granules. In order to troubleshoot the problem, rapidly evaluate solutions, and further optimize the formulation, a simple and rapid analytical technique was required. Since mannitol does not have a UV chromophore, conventional HPLC/UV analysis could not be used. Three alternative analytical techniques were evaluated in terms of ease of use, reproducibility, linear dynamic range and rapidity. The HPLC/RID (refractive index detector) and HPLC/ELSD (evaporative light scattering detector) provided rapid, reproducible alternate techniques to HPLC/UV, whereas LC/MS showed poor reproducibility. Analysis of the sieve samples of the granulations by HPLC/RID and HPLC/ELSD confirmed that poor active drug distribution was due to mannitol losses in the filter bag, as well as increased low size granules low in active drug content. The resultant formulation process was modified and a reduction in the initial air flow at start-up reduced losses of mannitol in the granulator filters.


*Keywords:* Mannitol; RID; ELSD; HPLC; Excipient quantitation; Formulation optimization

## 1. Introduction

In the early development phase of a new drug candidate, formulation development occurs with limited API and small batch sizes. Oftentimes, scale-up to larger clinical batch sizes results in variation of drug recovery. Due to limited API and resources, timely troubleshooting is critical to meet clinical timelines. From an analytical standpoint, HPLC/UV is the method of choice for UV absorbing compounds since it is readily available, easy to use, accurate and rapid.

An oral granules formulation for a BCS class II inhibitor was developed using a top spray granulation process consisting of active spheres granulated with mannitol, sweetener, flavoring, and binder. Two drug loadings, 25 and 75 mg/g, were required to accommodate the clinical study. The 25 mg/g potency was prepared by diluting the 75 mg/g granulation with mannitol, but high drug variability was encountered. Typically at start-up of the top spray granulation process, loss of low size granules occurs in the filter assembly. It was hypothesized that mannitol, due to its smaller particle size and lower density compared to the active drug, would be preferentially lost, thereby causing active drug enrichment in the remaining granulation. An analytical method was required to determine the distribution of mannitol in the granulation. Since mannitol does not have a UV chromophore, alternative detectors for the HPLC analysis were required for quantitation. Several detectors for non-UV absorbing compounds, such as RID, ELSD, and MS can all be coupled to an HPLC. Alternative techniques, such as electrochemical HPLC [1] has also been shown to be useful for the quantitation of mannitol along with specialized techniques such as high performance anion exchange chromatography [2], HPLC and GC analysis of derivatized species [3,4], yet these techniques are more time consuming. Since timely formulation troubleshooting was the ultimate goal, three alternative analytical techniques, LC/MS, HPLC/RID, HPLC/ELSD, were evaluated in terms of ease of use, reproducibility, linear dynamic range and rapidity.

## 2. Experimental

The oral granules formulation was analysed for active content by HPLC/UV (in-house method). Extraction of samples was performed using water (deionized Milli Q grade) and HPLC grade acetonitrile (50/50 v/v) with sonication for 75 min. The

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same sample preparation was used for both active assay and mannitol assay with target method concentration of 4 mg/mL for mannitol using the RID and 0.4 mg/mL for the ELSD, and 0.1 mg/mL for the active by UV.

The quantitation of active was performed by HPLC/UV. A waters symmetry $C_{18}$ column (4.6 mm × 150 mm, 3.5 μm particle size) was equilibrated at 40 °C on an Agilent 1100 Series HPLC. An isocratic mobile phase of sodium phosphate monobasic monohydrate (pH 2.95; 20 mM) and HPLC grade acetonitrile (73/27 v/v) at 1.2 mL/min was required. An injection volume of 5 μL was used with UV detection at 235 nm. A run time of 10 min eluted all active and related peaks. The chromatograms consisted of the API and related impurities. No interferences from mannitol were observed since mannitol is not a UV absorbing compound.

The mannitol content was assayed using an RID (Agilent Model 1362A) coupled with an Agilent 1100 Series HPLC using a Zorbax carbohydrate analysis column (4.6 mm × 150 mm, 5 μm particle size), with a column temperature of 35 °C and the RID maintained at 35 °C. A flow rate of 2.0 mL/min was used with a mobile phase of HPLC grade acetonitrile and deionized Milli Q grade water (75/25 v/v pre-mixed). The injection volume was 15 μL with a total run time of 5 min, with the mannitol eluting at approximately 3.2 min. The chromatograms consisted of a single peak with no interferences from other excipients or the API.

The ELSD (SEDEX 55, Richard Scientific) was coupled with a HP 1090 Series HLPC using a Phenomenex Rezex RCU–USP Sugar Alcohols column (4.0 mm × 250 mm, 8 μm particle size), with a column temperature of 85 °C and the ELSD maintained at 30 °C. A flow rate of 0.3 mL/min was used with a mobile phase of water (100%). The injection volume was 15 μL with a total run time of 12 min, with the mannitol eluting at approximately 8.0 min. The chromatograms consisted of a single peak with no interferences from other excipients or the API.

The same sample preparation as for the active quantitation was used for all LC/MS quantitation trials using an Agilent HP1100 coupled to an LCQ Deca Ion Trap mass spectrometer. The same column and chromatographic conditions used for the RID evaluation were used for the LC/MS, except that the flow was split resulting in a flow of 0.4 mL/min to the MS source. The LCQ Deca was operated in negative ion mode using electrospray ionisation (ESI). Infusion of mannitol standard allowed for optimization of the signal with a spray voltage of 4.5 kV and capillary temperature of 200 °C. Xylitol was used as an internal standard.

## 3. Results/discussion

### 3.1. LC/MS

The MS was coupled to an HPLC (HP1100) with a PDA (for UV compounds) and analysed in negative ion mode. A mannitol standard solution (0.01 mg/mL in $H_2O$:AcN (50/50 v/v) was injected and poor injection reproducibility was achieved. The addition of an internal standard (0.01 mg/mL xylitol) to reduce injection variability did not improve the results with relative standard deviations exceeding 10%. The dynamic linear range of the MS detector was found to be very limited. Mannitol standard solutions above 0.1 mg/mL saturated the detector, while concentrations at 0.005 mg/mL were very variable resulting in a R.S.D. of 17.4%. A linear regression plot of the calibration data demonstrates that under the conditions used, the LC/MS was unsuitable for analyzing mannitol due to the high variability and inferior correlation coefficient of 0.9959 and further optimization would be necessary to reduce the variability.

### 3.2. RID

The RID was coupled to a HP1100 HPLC, and mannitol standards were injected onto a Zorbax carbohydrate column. While the chromatography was optimized for injection volume, mobile phase flow, and column temperature, no optimization was required for the detector. There were no interferences from the active or other excipients. Good method linearity was obtained with a correlation coefficient of 0.9997. The RID also had a large linear dynamic range of 0.05–10 mg/mL. Its limit of detection (LOD) was 0.025 mg/mL, with the limit of quantitation (LOQ) being 0.050 mg/mL. The injection precision for $n = 6$ injections of standard was 0.46% with the RID.

### 3.3. ELSD

Alternatively, the ELSD was coupled to a HP 1090 HPLC, and mannitol standards were injected onto a Phenomenex Rezex RCU–USP Sugar Alcohols column (polymer based column). The Zorbax carbohydrate column used for the RID was unsuitable for the ELSD due to column bleed which resulted in a high background drift due to the greater sensitivity of the ELSD compared to RID [5,6]. A significant amount of time was spent optimizing the detector temperature and attenuation, as well as, the injection volume, mobile phase flow, and column temperature. No interferences from the active or other excipients were observed in the chromatogram. The detector response is non-linear, and was linearized through a log versus log plot of the data. Good method linearity was obtained with a correlation coefficient of 0.9999. The ELSD had a smaller linear dynamic range of 0.01–0.4 mg/mL. Its limit of detection (LOD) was 0.005 mg/mL, with the limit of quantitation (LOQ) being 0.01 mg/mL. The injection precision for $n = 8$ injections of standard was 2.5% with the ELSD.

### 3.4. Comparison of RID and ELSD

Good method linearity was obtained for both the RID and ELSD. Their coefficient of precision exceeded 0.999 for both curves (Table 1). The larger linear dynamic range of the RID (0.05–10 mg/mL versus 0.01–0.4 mg/mL for ELSD) could provide more versatility for the analysis of potentially widely different levels of mannitol in sieve fractions of the formulation. The ELSD has a greater sensitivity, allowing it to detect very low levels of mannitol. The injection precision of standard was significantly better with the RID. The differences in injection precision may be related to the age of the HPLC, with the Agilent 1100 LC used with the RID having greater precision than

Table 1
Comparison of mannitol calibration by RID and ELSD

| Detector type | RID | ELSD |
|---|---|---|
| Linearity ($r$) | 0.9997 | 0.9999 |
| Injection precision (%R.S.D.) | 0.46 | 2.5 |
| Limit of detection (mg/mL) | 0.025 | 0.005 |
| Limit of quantitation (mg/mL) | 0.050 | 0.010 |

the HP 1090 LC used with the ELSD. The RID was on loan for a 2 weeks period and method development and validation was performed within 1 week demonstrating its simplicity, rapidity, and accuracy. The ELSD (in-house) expended substantially longer time to set up and use and required much longer equilibration times and optimization to obtain reproducible data.

Both the RID and ELSD were found to be suitable detectors in analyzing for mannitol. A slight preference for the RID was noted due to its ease of use and robustness, although it did not perform as well as the ELSD in terms of sensitivity. The ELSD provided lower detection limits. Since timely formulation support was the main criteria, the greater sensitivity of the ELSD was not required due to the high levels of mannitol present, and in fact, required an additional dilution step to be within the lower linear range for mannitol quantitation. The RID was preferred since active quantitation by HPLC/UV and mannitol quantitation by RID was performed from the same stock sample solutions. Under the conditions and timeframe analysed, the LC/MS was found to lack precision and had poor linear response and a limited linear dynamic range.

### 3.5. Analysis of formulation

With a method to reproducibly quantitate mannitol established, the next step in determining the cause of higher active content and variability was to measure the distribution of mannitol in sieve fractions of the oral granules formulation. In batch A, 25 mg/g formulation, the blend uniformity ($n = 10$ samples) showed high active content at 112.5% label claim (LC) and a wide range and variability, R.S.D.% = 5.9%, range = 101.0–117.8%LC. This formulation contained 75.35% mannitol and 13% active drug and 11.65% other excipients. The sieve fractions were analysed by HPLC/UV for quantitation of the active drug and by HPLC/RID and HPLC/ELSD for quantitation of mannitol, using one sample preparation (ELSD required a second dilution for its linear range). The sieve assays for the RID and ELSD were slightly different since the two sets of samples were sampled from the drum at different times. As seen in Tables 2a and 2b, the smaller particle size fractions (sieve # 120 and 230) contained high levels of mannitol and low levels of active, relative to the other sieve fractions. The filter assembly also showed high mannitol content but low active drug content. The filter assembly sample was only analysed by RID for the out of specification batch because the sample was unique and could not be reproduced. The batches that conformed to specifications did not generate any significant amount of low size granules in the filter assembly. The pan contained no sample most likely due to loss into the filter bag assembly on start-up and throughout the granulation process.

Table 2a
Sieve assay results for batch A (out of specification) by RID and UV

| Sieve # | Sieve size (μm) | Weight (g) | Dilution (mL) | Mannitol (%LC) | Active (%LC) |
|---|---|---|---|---|---|
| 14 | >1400 | 0.7884 | 100 | 93.6 | 115.7 |
| 18 | >1000 | 5.4062 | 750 | 93.2 | 128.1 |
| 20 | >850 | 5.7119 | 750 | 92.2 | 136.4 |
| 25 | >710 | 6.5756 | 800 | 90.6 | 140.9 |
| 35 | >500 | 11.8478 | 1500 | 91.0 | 141.9 |
| 60 | >250 | 9.6040 | 1200 | 101.4 | 79.0 |
| 120 | >125 | 5.9137 | 750 | 117.3 | 9.1 |
| 230 | >63 | 3.9402 | 500 | 126.9 | 1.0 |
| PAN | – | na | na | na | na |
| Filter | – | 1.9724 | 250 | 122.7 | 4.8 |

na: no sample available.

Table 2b
Sieve assay results for batch A (out of specification) by ELSD and UV

| Sieve # | Sieve size (μm) | Weight (g) | Dilution (mL) | Mannitol (%LC) | Active (%LC) |
|---|---|---|---|---|---|
| 14 | >1400 | 0.8562 | 100 | 86.3 | 118.6 |
| 18 | >1000 | 7.1201 | 2000 | 94.3 | 131.6 |
| 20 | >850 | 6.9014 | 2000 | 94.8 | 139.3 |
| 25 | >710 | 7.9653 | 2000 | 95.1 | 142.7 |
| 35 | >500 | 11.9637 | 2000 | 91.2 | 145.7 |
| 60 | >250 | 9.5650 | 2000 | 101.9 | 85.9 |
| 120 | >125 | 3.0546 | 500 | 113.3 | 9.6 |
| 230 | >63 | 2.1195 | 500 | 126.0 | 1.3 |
| PAN | – | na | na | na | na |
| Filter | – | na | na | na | na |

na: no sample available.

Table 3a
Sieve assay results for batch B (conformed to specification) by RID and UV

| Sieve # | Sieve size (μm) | Weight (g) | Dilution (mL) | Mannitol (%LC) | Active (%LC) |
|---|---|---|---|---|---|
| 14 | >1400 | 0.7294 | 200 | 96.3 | 107.0 |
| 18 | >1000 | 9.5476 | 2000 | 95.0 | 106.0 |
| 20 | >850 | 9.4928 | 2000 | 95.2 | 105.1 |
| 25 | >710 | 10.0134 | 2000 | 97.1 | 104.7 |
| 35 | >500 | 12.8698 | 2000 | 99.7 | 102.4 |
| 60 | >250 | 4.9506 | 1200 | 113.7 | 82.7 |
| 120 | >125 | 1.9492 | 500 | 161.9 | 17.8 |
| 230 | >63 | na | na | na | na |
| PAN | – | 0.2267 | 50 | 176.3 | 2.7 |
| Filter | – | na | na | na | na |

na: no sample available.

Table 3b
Sieve assay results for batch B (conformed to specification) by ELSD and UV

| Sieve # | Sieve size (μm) | Weight (g) | Dilution (mL) | Mannitol (%LC) | Active (%LC) |
|---|---|---|---|---|---|
| 10 | >2000 | 0.4206 | 100 | 96.4 | 103.6 |
| 14 | >1400 | 2.9920 | 1000 | 98.9 | 106.5 |
| 18 | >1000 | 14.9989 | 2000 | 97.6 | 105.6 |
| 20 | >850 | 12.3062 | 2000 | 100.6 | 106.9 |
| 25 | >710 | 9.3375 | 2000 | 102.3 | 105.5 |
| 35 | >500 | 7.5753 | 2000 | 106.0 | 102.3 |
| 60 | >250 | 1.7037 | 500 | 118.6 | 80.5 |
| 120 | >125 | 0.7099 | 250 | 172.3 | 13.4 |
| PAN | – | 0.1396 | 50 | 176.4 | 2.7 |
| Filter | – | na | na | na | na |

na: no sample available.

The results indicated that low size granules are preferentially lost to the filter bag assembly and since the low size granules are enriched in mannitol relative to the active drug, the remaining granulation is enriched in active. Presence of larger amount of low size granules will also increase the possibility of segregation during sampling thus increasing the variability in the blend/content uniformity samples.

In an effort to optimize the process, the mannitol used to dilute the lower dose formulation was replaced with a mixture of mannitol and sugar spheres (bulking agent) resulting in 13% active, 49.35% mannitol, 26% sugar spheres and 11.65% other excipients. In addition, the initial air flow at start-up was reduced to further minimize losses of mannitol. This formulation, batch B, was analysed and the mean content uniformity was 100.5% with a low R.S.D. at 3.2% and a tight range of 94.6–105.9%, well within specifications. Analysis of the sieve fractions (Tables 3a and 3b) demonstrate that although the smaller particle size are enriched in mannitol, less of the low size granules are found for this formulation. Consequently no granulation was found in the filter bag assembly and only small amounts in the pan.

## 4. Conclusion

When drug content variability fails acceptable USP limits, formulation optimization with limited resources becomes a challenging task. Timely analytical support during formulation development and scale-up can assist the formulator in determining areas for optimization and can offer direction for future formulation development. A method allowing for single sample preparation and tandem analysis of active content and non-UV absorbing content (mannitol) was found to be advantageous through the use of conventional HPLC/UV along with either an RID or an ELSD. Both the RID or ELSD detectors can provide quantitative results for the sieve fractions depicting where the fractions contain higher levels of mannitol, and therefore isolating the losses during the manufacturing process. The initial batch (formulation A) contained mannitol enrichment in the low size granules and in the filter bag. Since the original formulation for the low dose contained mannitol as the filler, a modification to also include a denser bulking agent such as sugar spheres was made. Formulation B was made with the modifications (reducing mannitol content and reducing initial air flow), and the results met USP acceptance criteria. Consequently, the granulation process required control of the generation of low size granules to ensure a better blend uniformity.

The RID method development, validation, and analysis of samples was very rapid (within 2 weeks) thereby reducing development time. Although a limitation of the RID was the level of sensitivity achieved (LOQ 0.05 mg/mL), unsuitable for determining low level degradates, as shown in this example, it can be successfully used to monitor excipient levels and can provide formulation support in a timely manner. The ELSD method development was longer due to the required detector optimization. It is, however, more sensitive than the RID (LOQ 0.01 mg/mL). LC/MS was also investigated as an alternative method of analysis for mannitol, yet the variability obtained

during injection precision experiments showed that either the technique is semi-quantitative or further optimization would be required. The RID and ELSD, in comparison, were more favorable since the time required for method development, validation and analyses were quite rapid. The RID and ELSD can be used to quantitate many different pharmaceutical additives such as starches, PEGs, polymers, and lipids, and are known as a universal detectors.

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