Project:

Measuring Total Drug Loading – LC-MS/MS

Verification of expected total drug loading in nanomedical controlled release systems by liquid chromatography–tandem mass spectrometry

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1 Introduction

Controlled release systems for delivery of drugs have a number of properties that can be very advantageous to the treatment of medical conditions. Targeting of the delivery system to the desired site of therapeutic action will strongly reduce undesired systemic side effects. In addition, a sustained release could help maintain therapeutically optimal concentrations of the drug with significantly longer intervals between administrations than what is the case with free drugs. Finally, the encapsulation of drugs might help them escape first-pass and other undesired metabolism and thus bring a much larger proportion of the injected drug to the site of action.

A crucial parameter for dosing of nanoencapsulated drugs, like any administered free drug, will the total drug amount present. For nanomedical controlled release systems, the amount of drug available should be verified after encapsulation. Factors like interactions with the matrix components of the drug delivery system or instability of the drug in the matrix could affect the available drug amount. Total drug quantification will also be crucial for subsequent assays in the EU-NCL assay cascade.

2 Principle of the Method

In order to measure the encapsulated drug amount, the delivery vehicle (nanoparticle) should be dissolved and non-covalent interactions between the drug and the delivery system should be broken. That is done in this SOP by dissolving the nanoparticles in a suitable solvent, commonly an organic solvent like methanol, acetonitrile, acetone, DMSO or halogenated solvents.

Quantification of the drug in the solubilized state is performed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). This ensures precise quantification with very high specificity as the compound of interest has to match all of the following criteria:

- Retention time on a specific LC stationary phase with a specific mobile phase composition, i.e. highly defined chemical molecular interactions of the compound with the two-phase separation system
- Molecular mass
- Mass and relative intensity of molecular fragments generated when the intact molecular ion is fragmented in the gas phase in the mass spectrometer

For each drug of interest, the extraction, separation and detection parameters have to be specifically optimized. This will generally imply a certain method development work, or adaptation of previously developed methods.

3 Applicability and Limitations (Scope)

The methodology described here is applicable to all controlled release drug formulations where the drug can be fully extracted from the release formulation with a suitable solvent. This implies that it should be ensured the solvent could solubilize both the nanocarrier and the encapsulated drug in the relevant concentrations. If possible, alcohols and other solvents with a potential to react with functional groups on the drugs should be avoided.
In the quantification step, it is required that the drug can be sufficiently well separated from components of the controlled release formulation so that interferences during the ionization process are negligible. This also implies that the drug can be ionized with one or several of the available ionization methods to provide sufficient detection sensitivity, and that it can generate suitable molecular fragments for MS/MS verification. This should be obtainable for virtually all organic compounds by careful selection of separation and ionization parameters.

4 Related Documents

Table 1:

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5 Equipment and Reagents

5.1 Equipment

For extraction of drug, pipettes and vortex mixers are generally sufficient. If carrier components cannot be solubilized fully, precipitates will have to be removed by a centrifuge.

For analysis of the extracted drug, a liquid chromatography system coupled to a tandem mass spectrometer should be used. The chromatography system will typically consist of an automated liquid sampling unit, a binary or quaternary mobile phase pump, a vacuum degasser for the mobile phases, and a thermostatted column compartment, in addition to the suitable chromatography column. The mass spectrometer should be either a quadrupole-time-of-flight (QTOF) or – preferably – a triple quadrupole instrument, equipped with the suitable ionization interfaces. For most applications, an electrospray (ESI) ion source will be suitable; for some specific analytes, however, chemical (APCI) or photon-induced (APPI) ionization might be necessary.

5.2 Reagents

For extraction of drug from nanocarriers, standard organic solvents are used. The exact choice of solvent depends on the solubility of the drug in question and on the solvent’s extraction efficiency on drug from the carrier.

Mobile phases for LC-MS/MS are prepared from standard reagents (organic solvents, volatile buffers, water) with sufficient purity, typically HPLC or – preferably – LCMS grade.

For quantification, standard curves will be built using solutions with known concentrations of the free drug. Pure drug standards are preferably obtained from the sponsor, but can also be purchased as analytical standards from commercial sources.

When empty nanocarriers can be obtained from the sponsor, it is highly advantageous to run these as negative controls in the experiments to exclude the possibility of any matrix interferences.
Where available and not prohibitively expensive, a standard of the drug labeled with stable atomic isotopes (e.g. $^2\text{H}$, $^{13}\text{C}$, $^{15}\text{N}$) should be used to ensure exact quantification. Such labeled compounds can often be delivered by the sponsor, otherwise they have to be purchased commercially or synthesized on demand. The cost of the latter option can potentially be very high.

5.3 Reagent Preparation
All drug calibration series and LC-MS/MS mobile phases are prepared according to normal procedures. Calibration samples are prepared in the same solvent matrix as the samples to be measured.

6 Procedure

6.1 General remarks
The method described here involves specific method development for each new drug, both for drug extraction and LC-MS/MS analysis. New formulations of drugs for which a method for quantification already exists, might require only minor modifications of the existing method.

Stability data on both the nanoformulation and the free drug in different aqueous and organic solvents should be requested from the sponsor. The same applies to solubility data, as this will make the initial choice of extraction protocol as effective and rational as possible.

6.2 Flow chart
A flow chart outlining the overall method is shown in Figure 1. A separate flowchart describing the general principles for LC-MS/MS method development is shown in Figure 2.
Figure 1. Brief outline of work flow for complete extraction and analysis.
Figure 2. Brief outline of work for LC-MS/MS method development.
6.3 Reporting

The measurements report should include the following:

- Product number, CAS number and batch number of drug standard(s) used, including isotope labeled compounds if applicable
- Choice of solvent for extraction of drug from nanocarriers, and volumes used (solvent and nanomedicine sample)
- Predicted drug concentration (from sponsor)
- Any observations made during drug extraction (precipitation, color changes, other phenomena)
- The drug concentrations of the calibration samples, and the linearity of the calibration curve reported as the R² value
- The measured concentration of the QC samples
- The measured concentration of the drug sample, including variability between the replicates. Also, if dilution parallels of the sample are analyzed,
- Analytical hardware used, and instrument settings including LC gradient and ionization parameters. This can take the form of a standard instrument-generated run report.

7 Quality Control, Quality Assurance, Acceptance Criteria

Calibration curves for drug quantification should encompass (at least) 5 concentration points. The curves should be measured in triplicate throughout the run sequence; one complete calibration curve at beginning of sequence, one at the end of the sequence, and one curve distributed as single calibration points during the sample sequence. The analyzed sample concentration should fall between two calibration points. Samples should be extracted and measured in triplicate. Both the calculated mean concentrations and corresponding standard deviations, retention times and peak area values have to be reported both for the calibration points and sample measurements. For the calibration curves, the curve fit linearity (R² value) should be given.

It is strongly advisable that stable isotope labeled internal drug standards is used during the quantification to correct for matrix effects and sample preparation losses. The labeled standard should be added in the same concentration to samples and calibration curves, and should follow the analyte (drug) during the sample workup. If an internal standard is not available, it is necessary to analyze at least two dilution parallels of the sample and check that measured concentration is in concordance with the dilution factor. This reveals possible matrix effects, as matrix interferences in LC-MS/MS don’t scale linearly with dilution.

QC samples should be prepared the same way as the calibration samples, but in a separate preparation (weighing, dissolution). Specifically, the QC samples should be prepared by a different person, who should only get as input the solvent, the non-dissolved drug and the desired end concentrations; all calculations should be performed separately by the QC preparing person. At least two QC samples with different drug concentrations should be run. These should be analyzed at least two times during the sampling sequence to reveal instrument drift. If no preexisting stability data is available (from literature or sponsor) for the drug in the chosen solvent, QC samples should be
prepared at least 2 time points and run in the same LC-MS/MS sequence to assess stability of the analyte.

8 Health and Safety Warnings, Cautions and Waste Treatment
Samples should be prepared with care to protect the sample from contamination and to minimize exposure to personnel; appropriate safety precautions and protective gear such as gloves, lab coat and goggles must be worn. After the measurement, the carrier containing samples should be discharged as appropriate for nanomaterials. Eluted LC mobile phase containing test drug should be discharged as appropriate for the active ingredient.

9 Abbreviations
SOP Standard Operating Procedure
LC Liquid Chromatography
MS/MS Tandem mass spectrometry
DMSO Dimethyl sulfoxide