HPLC and UHPLC

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How to develop better drug impurity analysis methods

Determining impurity profiles in pharmaceuticals is critical to addressing the safety, quality, and purity of drug substances and finished products. Impurities can affect the safety and efficacy of medicines and biologic drugs, resulting in the potential recall of marketed drugs. This list provides top method development considerations to help you succeed.

Method tips

Map out all sources of API impurities

Two important sources include degradation-related impurities and process-related impurities.

Know what percent of impurities you need to measure and the threshold limits

According to the International Conference on Harmonization guidelines, the drug substance reporting threshold is 0.05% for maximum daily doses </= 2g/day and 0.03% for maximum daily doses >2g/day.

Use internal AND external standards during your analysis

A robust impurity analysis method needs internal and external standards. Internal standards are added before sample preparation/clean-up to help you gauge analyte recovery, and external standards are analyzed separately from the sample to generate calibration curves for quantitation by analyte response.

Have a backup plan if certified standards are unavailable for unknown impurities

The answer is to use a universal detector that gives a uniform response independent of a compound's physicochemical properties. With modern detection technology, you can use a single calibrant to quantify multiple analytes when individual standards are unavailable.

Wash and equilibrate your LC system regularly to help ward off ghost peaks

Ghost peaks appear when compounds from previous samples contaminate your column or system components. To help ward off ghost peaks, best practice includes implementing regular washing cycles after each cycle to clean your column, tubing, injection port, and syringe.

Use a column selection guide to find the best column for your application

Finding the right column stationary phase for your method is essential. Leveraging the right selectivity can increase your resolution, robustness and accuracy, and choosing the correct particle morphology can increase your throughput and minimize solvent use and run time.

Leverage 2D-LC to help eliminate coeluting analytes

Hidden degradation products may coelute with your main drug peak due to structural similarity. Employing a second-dimensional separation using 2D-LC can reveal hidden impurities and eliminate method performance issues resulting from coelution.

Ensure your detector has sufficient dynamic range to assess all impurity profiles in one run

Your detector typically needs a wide detection range of at least three orders of magnitude to quantify your API and low-level impurities simultaneously, with accuracy and precision. The ability to detect all compounds of interest at required threshold limits in a single run will save your lab solvent, time, and stress.

Use complementary detection techniques to increase sample knowledge and method robustness

Double or triple detection method extends the scope of compounds you can measure in a single sample. You can quantify non-volatiles and semi-volatiles without chromophores with charged aerosol detection, UV-Vis for volatile and non-volatile chromophores, and MS to confirm analyte identity.

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Questions for consideration

Can your detector measure API and impurities in a single run?

Certain impurities might not have a chromophore or be able to generate gas-phase ions. When UV-Vis detectors or mass spectrometers are incompatible with your analytes, near-universal detectors like the **charged aerosol detector** are a great solution.

Does your detector have sufficient sensitivity to assess impurity profiles?

A good rule of thumb is the LODs and LOQs for your detector should lie in the low nanogram range when measuring APIs in the microgram range.

Is your detector response sensitive to changes in eluent composition?

For nebulizer-based detectors, the organic content of the mobile phase can affect the uniformity (consistency) of response during gradient elution. You can easily overcome restore response uniformity by applying an **inverse gradient**.

Is your detector response sensitive to analyte physiochemical properties and concentration?

A consistent uniform response is important when quantifying analytes for which individual calibration standards are unavailable. Physicochemical properties like refractive index, light absorption, and fluorescence can affect your **detector response** leading to an inaccurate calculation of true concentration.

Are matrix effects interfering with your sample detection?

Matrix effects may be contaminants from drug production or other chemicals creating interfering peaks that make automated peak integration difficult and can trigger out-of-specification results. You can minimize the impact matrix interferences by performing online or offline sample preparation steps, which are simple filtrations or **solid phase extraction**.

Do you detect large variances in concentration between duplicates/triplicates?

Your vial choice might be the root cause if your data has a high standard deviation. Low-quality glass gives a larger variance because the analytes in your sample may adhere to microscopic scratches or free silanols in the glass wall. Using **higher-quality gold-grade vials** can help lower variance when quantifying trace impurities.

Literature resources

- **Analysis of pharmaceutical impurities using multi-heartcutting 2D LC coupled with UV-charged aerosol MS detection**
- **High-Performance Liquid Chromatography Methods for Determining the Purity of Drugs with Weak UV Chromophores – A Review**
- **Recent applications of the Charged Aerosol Detector for liquid chromatography in drug quality control**
- **Charged aerosol detection in pharmaceutical analysis**
- **Matrix effects demystified: Strategies for resolving challenges in analytical separations of complex samples**
- **Ghost peaks in reversed-phase gradient HPLC: a review and update**

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