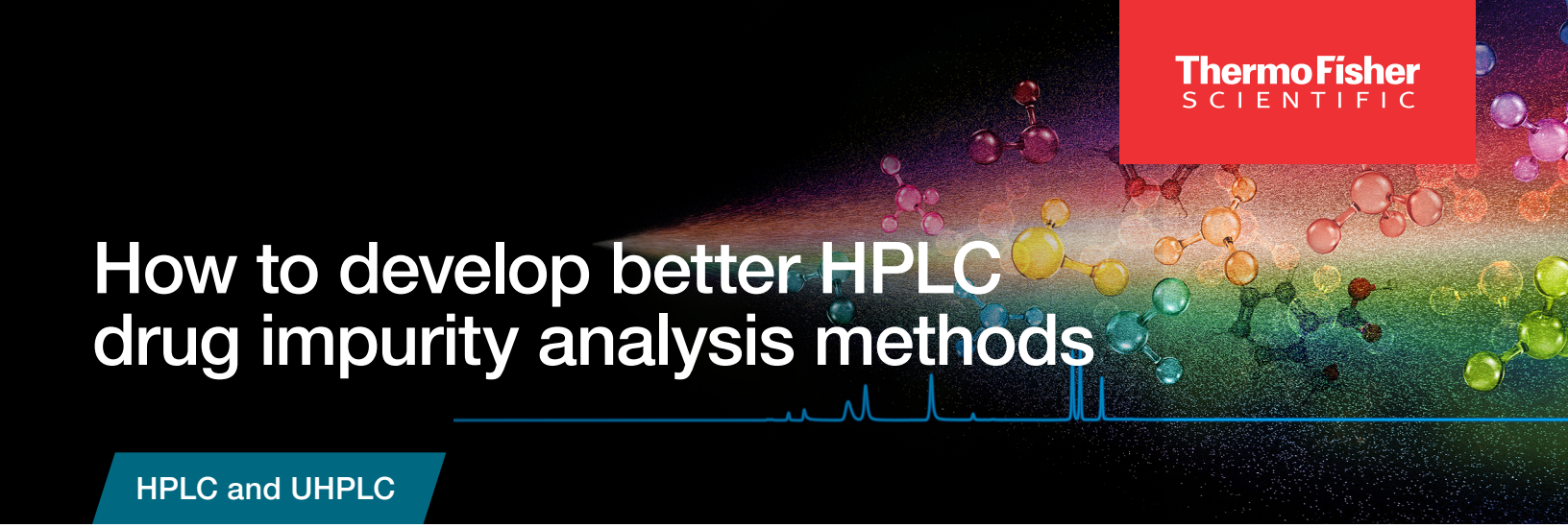

How to develop better HPLC drug impurity analysis methods

HPLC and UHPLC

Accurately determining impurity profiles in pharmaceuticals is critical to addressing the safety, quality, and purity of small molecule drugs and biologics. Impurities can affect drug safety and efficacy, resulting in the potential recall of on-market products. This list provides top considerations to help you develop accurate and robust HPLC methods.

Method tips

Map out all sources of API impurities ⚠

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

Know what percentage of impurities and threshold limits you need to measure ⚠

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

Conduct forced-degradation studies to establish detailed degradation pathways ⚠

Stress testing under heat, light, oxidative, and hydrolytic conditions reveals potential degradation products. Use HPLC analysis to separate and identify degradation products and get insights into the mechanistic pathway.

Assess impurity stability in prepared samples ⚠

Prepared samples can degrade, adsorb, or react with containers, producing storage- or handling-related impurity changes. Assessing benchtop and autosampler stability across dedicated time points can help reveal the impurity source.

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. Choosing the correct particle morphology can boost your throughput, minimize solvent use, and shorten run time.

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/cleanup to help you gauge analyte recovery. 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 near-universal detector that gives a uniform response independent of analyte physicochemical properties. With charged aerosol detection, you can use a single calibrant to quantify multiple analytes when individual standards are unavailable.

🎯 Impacts quantitative accuracy ⚠ QC testing failure risk ⏳ Affects method throughput

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Match sample solvent strength to the initial mobile phase 🎯

Injection solvents that are stronger than your eluent can distort early eluting impurity peaks, compromise the quantitation of your early eluting impurities, and mask or mis-quantify trace impurities.

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.

Use complementary detection to increase sample knowledge and method robustness ⏳

Double or triple detection methods will extend 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.

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.

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 dynamic range and sensitivity to measure API and impurities simultaneously? ⏳

Detectors that can accurately quantify APIs and low-level impurities in a single run typically require a dynamic range of at least four orders of magnitude and LOQs in the low-nanogram range when APIs are present at microgram levels.

Is your detector response sensitive to analyte volatility? 🎯 ⏳

When using evaporative detectors like charged aerosol detection, applying stricter temperature control during particle charging can allow you to expand the accuracy and quantification range of semi-volatiles.

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 this issue and restore response uniformity by applying an inverse gradient to improve quantitation and reproducibility.

Is your detector response sensitive to analyte physicochemical properties and concentration? 🎯

A consistent uniform response is important when quantifying analytes for which individual calibration standards are unavailable. Physicochemical properties, such as 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? ⚠️

You can minimize the impact of matrix interferences that create interfering peaks by performing online or offline sample preparation steps before injection, such as simple filtrations or solid phase extraction, and diverting eluted salts to waste that remains in solution.

🎯 Impacts quantitative accuracy ⚠️ QC testing failure risk ⏳ Affects method throughput

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.

Is your method robust and easy to transfer across labs and instruments? 🎯⏳⚠️

To assess robustness, determine if minor changes in pH, temperature, or flow rate affect impurity peak resolution. For method transfer, highly manual and complex methods are likely to be difficult to transfer, increasing the risk of human error and long-term performance drift.

Literature resources

- Impurity profiling of PEGylated myristoyl diglyceride, DMG-PEG 2000, a functional excipient used in mRNA lipid nanoparticle formulations
- Applications of Hydrophilic Interaction Chromatography in Pharmaceutical Impurity Profiling: A Comprehensive Review of Two Decades
- 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
- Matrix effects demystified: Strategies for resolving challenges in analytical separations of complex samples
- Recent applications of the Charged Aerosol Detector for liquid chromatography in drug quality control
- Ghost peaks in reversed-phase gradient HPLC: a review and update
- Charged aerosol detection in pharmaceutical analysis

Technical resources

- Quantifying more with less: Implementing charged aerosol detection to improve drug safety
- A reliable UHPLC/UV/CAD/MS multidetector method for routine quantification and library matching of extractables and leachables in pharmaceutical-grade plastics
- Characterization of polysorbate 80 in (bio) pharmaceuticals using HPLC-CAD
- Characterization of four saturated fatty acids using gradient HPLC-CAD highlighting optimized evaporation temperature control features
- Evaluation of custom injection programs and larger internal diameter capillary for strong solvent sample effects mitigation in liquid chromatography
- A Look at the Past & Future Impacts of HPLC-CAD Technology
- HPLC-CAD Learning Center

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