Generic Method Approach for Pharmaceutical Drug Discovery and Development using Reversed-Phase Hydrophilic Interaction Liquid Chromatography with Universal Charged Aerosol Detection

Frank Steiner [1], Michael Heidorn [1], Markus M. Martin[1], Marc Yves Chalom[2], Rodrigo de Sousa Leite[2]

Thermo Fisher Scientific, [1]Chelmsford, MA, U.S.A.; [2]São Paulo, SP, BR

Overview

Purpose: Development of a robust, user-friendly, and fast generic method approach for pharmaceutical drug discovery and development.

Methods: Different ways to serially couple reversed phase and hydrophilic-interaction liquid chromatography were examined in order to achieve their best possible synergy.

Results: The combination of reversed-phase and hydrophilic interaction liquid chromatography with charged aerosol detection is a powerful approach for generic separation and detection of non-volatile hydrophilic and hydrophobic compounds in a single run.

Introduction

Liquid chromatographic (LC) methods for the assay of active pharmaceutical ingredients (API), drug candidates, intermediates, related substances, and impurities are crucial in pharmaceutical development. During early-stage drug discovery, the chemical synthesis route, the impurity profile, and even the formulation of the drug product are not completely established but are rather subject to constant modification. Thus, from pre-clinical to clinical development and, finally, to New Drug Application (NDA) submission, a drug-specific LC method must be modified several times. These samples can contain analytes of interest with a wide polarity range which makes their chromatography challenging. A very promising practice in drug discovery is the use of two separate analyses of the same sample; by reversed-phase (RP) LC on the one hand and hydrophilic interaction LC (HILIC) on the other hand.

In this work, a generic method approach is presented that addresses common changes during the drug development lifecycle and is applicable to each new drug formulation. The analysis combines gradient RPLC in the first stage and, enabled by organic solvent addition to the eluate coming from the RP column, with gradient HILIC in the second stage. Coupling HILIC to RPLC has already been introduced.[1] This approach was optimized by combining solvent and flow rate gradients, simple solvent compositions, and UHPLC technology. This allows simplified separation of both hydrophilic and hydrophobic substances within a broad elution window and in a single, short run. Charged Aerosol Detection allows near universal detection of non-volatile substances. The performance of the generic method approach is shown with a model sample and an emtricitabine degradation study.

Methods

Figure 1 shows a schematic of the generic method approach. The sample is injected onto the polar encapped reversed phase (RPaq) column. To achieve a wide polarity range, the gradient RPLC starts with pure aqueous solvent delivered by the right pump of the dual gradient pump. Highly hydrophilic compounds of the sample directly pass through the RPaq column while hydrophobic compounds are retained.

As HILIC requires high organic mobile phases, pure organic solvent was added by the left pump of the dual gradient pump to the eluent coming the RPaq column. The Thermo Scientifc™ Dionex™ Corona™ Veo™ RS charged aerosol detector operates optimally at an incoming mobile phase flow rate of 1.5 mL/min. Different flow rates of the left and right pump are needed to accomplish a high organic content of the mobile phase driving the HILIC column while the combined flow rate does not exceed 1.5 mL/min (Figures 2 and 3).

While the RPaq column is influenced by the flow gradient of the right pump, the HILIC column is constantly operated at 1.5 mL/min. The organic content of the mobile phase is kept ≥85% during the proper separation to guarantee highly volatile mobile phase conditions for the nebulizerbased Corona Veo detector.

An “artificial” mobile phase gradient is applied to the HILIC separation by using flow gradients of both pumps (Figure 3), similar to a high pressure gradient concept.

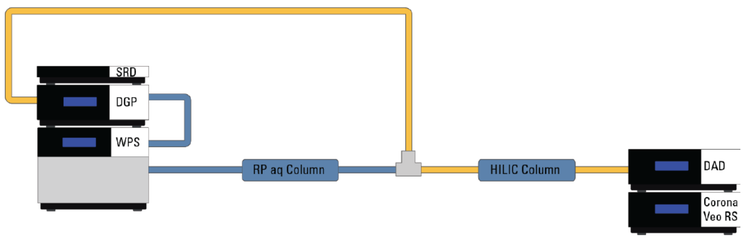

FIGURE 1. Instrumental Setup

HPLC: Thermo Scientific™ Dionex™ UltiMate™ 3000 x2 Dual RS System including WPS-3000TRS Autosampler, TCC-3000RS Column Compartment, DAD-3000RS UV Detector, and a Corona Veo RS Charged Aerosol Detector

Columns: Thermo Scientific™ Hypersil GOLD™ 1.9 µm, 2.1 × 100 mm
Thermo Scientific™ Acclaim™ HILIC-10, 2.2 µm, 2.1 × 150 mm

Tray temperature: 10 °C
UV Detector:
Flow cell volume: 2.5 µL
Wavelengths: 220–380 nm
Data Collection rate: 50 Hz

Oven temperature: 40 °C
Charged Aerosol Detector:
Filter Constant: 5
Power Function: 1.0
Collection rate: 100 Hz

Data Analysis

Thermo Scientific™ Dionex™ Chromeleon™ 6.8 Chromatography Data System CDS software, RS13

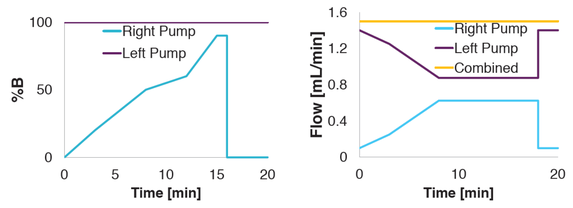

FIGURE 2 and 3. Mobile Phase and Flow Rate Gradient.

Results

Separation of Hydrophilic and Hydrophobic Compounds

A model sample was created by dissolving glucose (1), maltose (2) [both at 100 µg/mL], amiloride (3), triamterene (4), chlorthalidone (5), furosemide (6), bumetanide (7), and ethacrynic acid (8) [each at 50 µg/mL] in water. For all chromatograms, the injection volume was 3 µL.

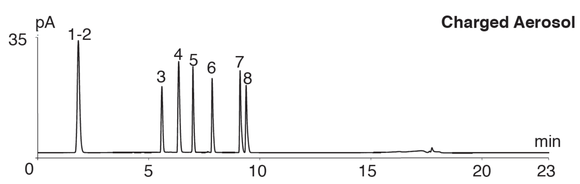

FIGURE 4. RPLC of Model Sample

Note: The same instrumental setup as shown in Figure 1 (both pumps) and the generic method was used but bypassing the HILIC column. The T-piece was directly connected to the UV detector and Corona Veo RS.

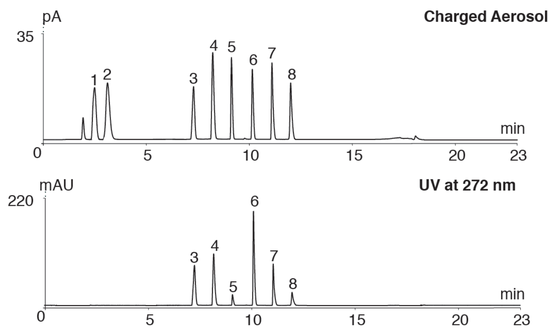

FIGURE 5A and 5B. RP-HILILC of Model Sample

Degradation of Emtricitabine

Emtricitabine USP reference standard s was dissolved in 10% concentrated hydrochloric acid at a concentration of 500 µg/mL. The sample was analyzed immediately (black), after 5 hours (**turquoise**), after 23 hours (**orange**), and after 52 hours (**purple**) with storage at 60 °C. The injection volume was 2 µL each.

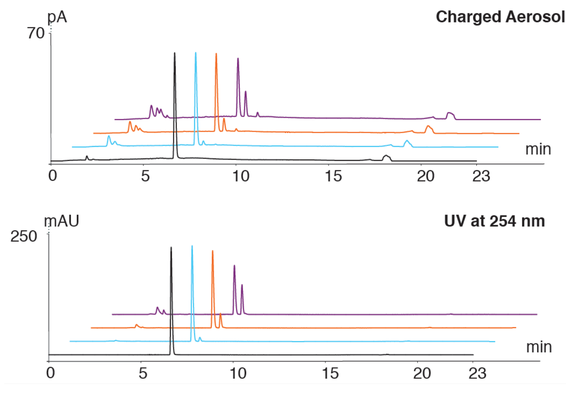

FIGURE 6A and 6B. Emtricitabine Samples

Discussion

Instrumental Setup and Concept

The analysis of pharmaceutical samples with ingredients that may cover a wide polarity range often requires a combination of methods, e.g. RPLC for hydrophobic and NPLC or HILIC for hydrophilic substances.1 Contrary to NPLC, mobile phases of HILIC are compatible to RPLC in terms of miscibility. However, HILIC is operated at high organic content eluents while RPLC gradients usually start at low organic content (or even purely aqueous) mobile phases.

The key novelty of our approach is the use of flow-rate gradients on the two pumps in action. This concept enables a narrow elution window of highly organic content with constant flow on the HILIC column, while applying a widerange gradient starting with purely aqueous mobile phase on the RP column. A consequence of this concept is that the RP column is eluted in a flow gradient in addition to the solvent gradient. This flow gradient is mandatory if the RPLC starts with low organic content (below 50%) while HILIC has to be operated with high organic content (above 85%) of the mobile phase each. This is a principle requisite to tune the entire setup to best performance for very polar compounds, as they are often present in pharmaceutical formulations. Adverse effects of starting the RP run in the B-term of a UHPLC column are almost fully compensated by the focusing effect of the overlaid elution gradient.

The in-line dilution may be considered a problem as it increases detection limits for concentration-sensitive detectors. In general, detectability is not a major issue for most applications in drug discovery and development1. The CAD is a mass-sensitive, nebulizer-based detector which is not affected by this dilution. The high organic content supports consistent nebulization at high yield and results in an almost uniform detector response at best possible sensitivity.

Single Run Separation of Hydrophilic and Hydrophobic Compounds with RP-HILIC.

In Figure 4, glucose and maltose were neither retained nor separated by RPLC. When using the generic method approach with RP-HILIC (Figure 5A), both carbohydrates were separated and even the selectivities of the hydrophobic compounds were changed according to their polar retention contribution. A comparison to the UV chromatogram (Figure 5B) clearly shows that the CAD detector response is close to uniform and enables approximate quantitation without individual calibration.

Degradation Studies of Emtricitabine

Emtricitabine is used for the treatment of HIV infection in humans. Baseline-separation is provided by the generic method approach which enables direct quantitation of emtricitabine and its degradation products without any further method development. The charged aerosol chromatogram (Figure 6A) showed the complete range of degradation products, unlike the UV trace (Figure 6B). If volatile substances with chromophores were involved, UV would be complementary to charged aerosol detection. If an MS/MS was combined to the setup, even a simultaneous identification of the degradation products would be possible.

Conclusions

- Intelligent RP-HILIC combination allows analysis of hydrophilic and hydrophobic compounds within a single run.
- RP-HILIC-UV-CAD has potential to become the generic method approach of choice for screening in pharamceutical research and development.
- The generic method setup can be applied to modify existing RPLC screening methods and increase their generic capabilities.
- Nearly uniform response for non- and semi-volatile compounds with CAD, additional use of DAD as a verification and complimentary detection tool.

References

1. Louw, S.; Pereira, A.S.; Lynen, F.; Hanna-Brown, M.; Sandra, P. *J. Chromatogr. A.*, **2008**, 1208, 90.

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