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Orthogonal extraction/chromatography and UPLC, two powerful new techniques for bioanalytical quantitation of desloratadine and 3-hydroxydesloratadine at 25 pg/mL

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Abstract

Validation of the bioanalytical method for determination of desloratadine and 3-hydroxydesloratadine was conducted using ultra high pressure liquid chromatography (UPLC) in conjunction with mix mode solid phase extraction. The dynamic range of the assay was from 0.025 ng/mL to 10 ng/mL using 96-well solid phase extraction. On an UPLC system, the inter-run accuracy was better than 94.7% for desloratadine ($n = 18$) and 94.0% for 3-hydroxydesloratadine ($n = 18$). The between-run precision (%CV) ranged from 2.6% to 9.8% for desloratadine ($n = 18$) and 3.1% to 11.1% for 3-hydroxydesloratadine ($n = 18$). The limit of quantitation represented 0.478 pg and 0.525 pg of extracted material injected on-column for desloratadine and 3-hydroxydesloratadine, respectively. The total run time was slightly over 2 min per sample. The approach of orthogonal extraction/chromatography and UPLC significantly improves assay performance while also increasing sample throughput for drug development studies.
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1. Introduction

High-throughput bioanalytical methods are essential to support the rapid discovery and development of drugs in the pharmaceutical industry. Liquid chromatography coupled with tandem mass spectrometric detection (LC-MS/MS) is considered the benchmark analytical methodology to be employed for the quantification of new chemical entities in biological fluids [1–4]. Many of the chromatographic instrumental techniques have now matured and automation is commonplace [6–8]. Because of the high sensitivity and selectivity of LC-MS/MS, rigorous chromatographic resolution of analytes and/or tedious sample extraction protocols are typically not required even when complex biological matrices are used. Nevertheless, the development of drugs with increased potency will continue to challenge the analytical chemist to lower the level of quantitation (LLOQ). A LLOQ of 100 pg/mL or lower is a common requirement to support a clinical development program and when this is coupled with the demand for lower sample volumes the analytical chemist is challenged to develop optimized methods.

Of the three commonly used extraction techniques, "dilute and shoot" or protein precipitation is the least suited for clinical studies because of its susceptibility to ion suppression and matrix effects. Liquid–liquid extraction and solid phase extraction (SPE) are techniques that offer much cleaner sample extracts that in turn serve to make the method more robust and scalable. SPE is particularly powerful in this regard because of its unique ability to utilize a variety of retention mechanisms [5]. Moreover, the availability of SPE products in a 96-well format affords the opportunity for high-throughput analysis. The most widely used SPE sorbent phases are those that base retention and elution on polar

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and non-polar interaction mechanisms. Specifically, sorbents that incorporate C_{18} ligands are widely available and are in common use. However, the combination of polar and non-polar retention mechanisms is also the least selective because nearly everything is retained from the biological matrices along with the analyte of interest. Although this retentiveness is sometimes an advantage in cases where metabolites and parent drug have very different polarities, in general, extractions based on this type of interaction mechanism do not provide sufficiently clean extracts for clinical bioanalysis without the aid of fairly sophisticated HPLC elution profiles. On the other hand, a sorbent that utilizes an ion exchange interaction can be highly selective for molecules that contain functional groups capable of exhibiting either a positive or negative charge under appropriate acidic or basic conditions [5]. The drawback with high selectivity is that, on occasion, metabolites with a significantly different pK_a from the parent drug will be difficult to isolate. To fulfill the requirements of selectivity and retention, recently developed mix mode sorbents are now available that afford hydrophobic and hydrophilic interactions in addition to ion exchange. The advantage of incorporating both of these retention mechanisms is the potential to isolate structurally diverse analytes with adequate selectivity and with high recovery suitable for clinical bioanalysis.

Decreases in the particle size of the silica used in modern HPLC columns significantly increases the number of height equivalent theoretical plates for a given length column. More specifically, at higher flow rates the Van Deemter plot is nearly flat for a column packed with particles smaller than two microns. The practical implication of this is that flow rates can be increased without regard for the loss of resolution. However, the use of smaller particles requires much greater pressures (>6000 psi) than conventional HPLC systems are designed to handle. Although a number of research articles [9–11] have shown the advantage of the application of small particle sizes on home built HPLC systems, a commercial system that can handle high pressure applications has not been available until recently [15–17]. The Waters ACQUITY^{TM} UPLC system is a LC system designed to handle medium column back-pressures of up to 15,000 psi. By using an LC system comprised of a 2.1 cm × 5 cm, 1.7 μm LC silica/polymeric hybrid column, it is practical to use a water/methanol based mobile phase system at a flow rate as high as 0.5 mL/min without loss of resolution. Compared to the typical 0.25 mL/min flow rate used for 2.1 mm internal diameter 5 μm HPLC columns, the total flow rate can be doubled on an UPLC system without compromising resolution [19].

The development of rugged and robust liquid–liquid extraction or solid phase extraction methods is only the first step in the process. Method transfer from development to validation to production often requires substantial investment in hands on training from the method developer to production staff. Given this, method development paradigms have acquired increased importance in today's bioanalytical laboratory where standardized method development protocols can be applied to a variety of compounds. One such paradigm employed by our group centers on an orthogonal concept for sample extraction and chromatography. Specifically, mixed mode solid phase extraction is coupled with reversed phase HPLC chromatography. The advantage of such an approach is intuitive given that the extraction is ionic in nature; residual endogenous interferences resulting from extraction are unlikely to present a selectivity problem for the reverse-phase chromatography system [20,21]. Moreover, because a cleaner sample is presented to the analytical system, more flexibility exists within the chromatography system to resolve metabolites if so required.

CLARINEX® or desloratadine is a potent long-acting tricyclic histamine antagonist from Schering-Plough. Receptor binding data indicates that desloratadine shows significant interaction with the human histamine H1-receptor. Clinical studies conducted in 924 patients demonstrated that 5 mg CLARINEX once daily improved allergic rhinitis symptoms. Following oral administration of desloratdine 5 mg once daily for 10 days, a mean peak plasma concentration of 4 ng/mL was observed at peak dose. Given the low maximum plasma concentration, it was essential that the bioanalytical method be refined to an LLOQ of 25 pg/mL in order to measure both desloratadine and its major active metabolite 3-hydroxy (3-OH) desloratadine. Two previous analytical methods were published for detection of desloratadine and 3-hydroxydesloratadine using LC-MS/MS [12,13]. One method from our laboratory presented an automated 96-well solid phase extraction method that utilized a 500 μL aliquot of human heparin plasma [12]. Because this method was based on a traditional C18 extraction system coupled to a reverse-phase HPLC system, a 6 min run time was required for resolution from all interferences. In this article, we describe two experiments we have undertaken to improve throughput of this analytical method without compromising the ruggedness and selectivity of the existing assay. First, a low flow rate experiment (hereafter referred to as the "Shimadzu experiment") that integrates the "orthogonal" extraction paradigm stated above was explored at a flow rate of 0.25 mL/min. For this exercise, only 250 μL plasma was used and the run time was shortened to 4 min. Second, in a high flow rate experiment (here after referred to as the "ACQUITY" experiment), the method was further refined by increasing the flow rate from 0.25 mL/min to 0.5 mL/min on an ACQUITY^{TM} UPLC system. This improvement further reduced the total run time to just slightly over 2 min.

2. Experimental

2.1. Materials

Desloratadine with a purity of 98.3%, 3-hydroxydesloratadine with a purity of 99.3%, $^{2}H_4$-desloratadine with a purity of 99.9%, and $^{2}H_4$-3-hydroxydesloratadine with a purity

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