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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.

Keywords: LC/MS-MS; Desloratadine; Quantitation; Orthogonal; Validation; UPLC; Mixed-mode SPE

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

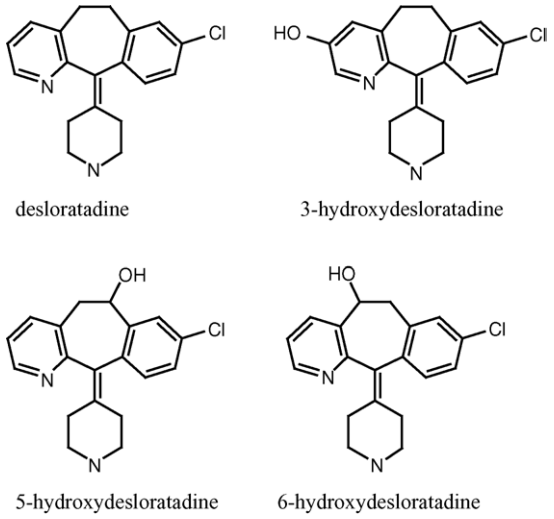

Fig. 1. Structure of desloratadine and its mono-hydroxyl metabolites: 3,5 and 6-hydroxydesloratadine.

of 100% were synthesized at Schering-Plough Research Institute (Kenilworth, NJ) (Fig. 1). Human plasma EDTA anti-coagulant was purchased from Bioreclamation Inc. (Hicksville, NY). All other chemical reagents were purchased from either Fisher Scientific (Fair Lawn, NJ) or Sigma–Aldrich Co. (St. Louis, MO). All chemical reagents were either OPTIMA Grade or Certified ACS Grade unless otherwise noted.

2.2. Instrumentation

All sample aliquoting was performed either manually or by using a Packard MultiProbe II EX liquid handling robotic system (Perkin-Elmer Life Sciences, Boston, MA, USA). Extractions were automated by using a Tomtec Quadra 96 Model 320 robotic system (Tomtec, Hamden, CT, USA). Analytes and internal standards were detected by using a Sciex API 3000 Triple Quadrupole LC-MS/MS system for the Shimadzu experiment or a Sciex API 4000 Triple Quadrupole LC-MS/MS system for the ACQUITY experiments (Applied Biosystems/MDS Sciex, Ontario, Canada). Unless otherwise noted, the mass spectrometers are operated in the positive ion multiple reaction monitoring (MRM) mode and use TurboIonSpray interfaces.

For the Shimadzu experiments, the chromatography system consisted of two Shimadzu 10ADvp LC pumps (Shimadzu Corporation, Columbia, MD, USA) controlled by a Shimadzu SCL-10A system controller. A Shimadzu DGU-14A degasser was incorporated into this system. A CTC PAL autosampler was used to perform sample injection. For the ACQUITY experiments, the medium pressure system consisted of an ACQUITY^{TM} UPLC system (Waters Corp., Millford, MA, USA) which included a column oven, autosampler, vacuum degasser and binary gradient pumps as part of the unit.

Sciex Analyst version 1.3.1 software performed data collection and peak integration. Data regression was accomplished by using the Watson version 6.4.0.03 laboratory information management system. (Thermo LabSystems, Philadelphia, PA).

2.3. Solution preparation

Stock solutions of desloratadine and 3-hydroxydesloratadine were prepared in methanol at a concentration of 100 μg/mL (corrected for purity). Intermediate and spiking solutions were prepared from the stock solutions in methanol. Claritin^{TM} free plasma was used to prepare standard and QC pools. A total of nine (ten for ACQUITY experiments) standard points and four QC concentrations were used to span the concentration range of 25 pg/mL to 10 ng/mL. The pools were stored at −20 °C until used. $^{2}H_4$-desloratadine and $^{2}H_4$-3-hydroxydesloratadine internal standard working solutions were prepared at a nominal concentration of 4 ng/mL in methanol.

3. Results and discussion

3.1. Mass spectrometry

Desloratadine, 3-hydroxydesloratadine, and their respective internal standards were infused at 1 μg/mL concentration via a syringe pump to establish optimized voltages for the declustering potential, focusing potential, collision energy, collision cell exit potential, and ionspray voltage. Collision cell gas pressure was also optimized. The MRM mass transitions of m/z 311 → m/z 259, m/z 315 → m/z 263, m/z 327 → m/z 275, and m/z 331 → m/z 279 were used to monitor desloratadine, $^{2}H_4$-desloratadine, 3-hydroxydesloratadine, and $^{2}H_4$-3-hydroxydesloratadine, respectively.

3.2. Sample extraction

Waters Oasis® HLB MCX 96-well solid phase extraction plates were selected as the SPE phase. This mixed mode copolymer phase incorporates a reverse-phase HLB copolymer and uses sulfonic acid moieties to supply the strong cationic exchange functionality. Samples containing desloratadine and 3-hydroxydesloratadine were extracted with the following protocol. The 10 mg Oasis® HLB MCX 96-well extraction plate was conditioned with 400 μL of methanol followed by 400 μL of 2% formic acid solution. A 250 μL aliquot of sample was diluted with 500 μL of 2% formic acid solution and then applied to the solid phase extraction plate. The sample was eluted using vacuum (<5 psi negative pressure) and the extraction plate was washed sequentially with 400 μL of 2% formic acid solution followed by 400 μL of methanol:acetonirile (1:1, v:v%) solution. Analytes were eluted with two 200 μL aliquots of methanol:aceontrile:water:ammonia (45:45:10:4, v:v:v:v%) solution. The eluent was then dried under a stream of nitro-

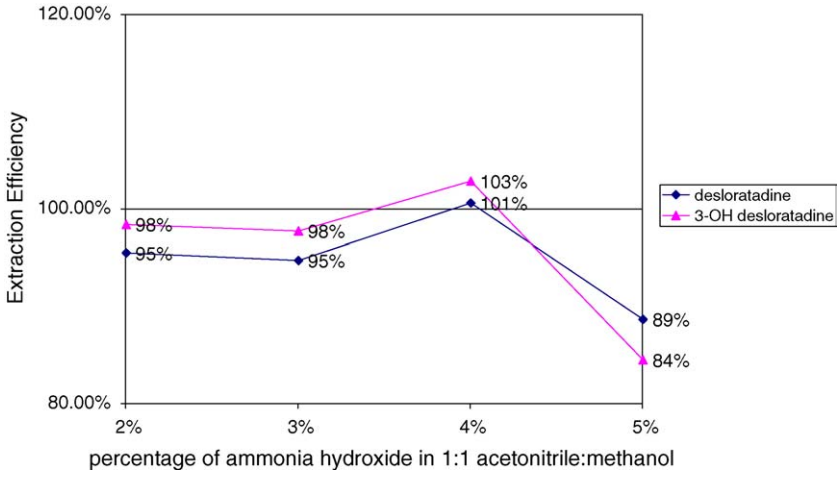

Fig. 2. 2D experiments of extraction efficiency as a function of percent ammonia in elution solution.

gen and reconstituted in 150 μL of mobile phase that was subjected to LC-MS/MS analysis.

Each step of the extraction process was optimized with various "2D" experiments [5,14]. Figs. 2 and 3 summarizes the results of these experiments. The *x*-axis is the parameter undergoing optimization while the *y*-axis shows the extraction efficiency. The extraction efficiency was calculated as the ratio of the response of an extracted sample divided by the response of a post spiked extracted blank sample. Each of the data points in the graph are an average of three replicate extractions.

The piperdine and pyridine functional groups common to both desloratadine and 3-hydroxydesloratadine have a pK_a of 9.7 and 4.3, respectively. During sample application and dilution, it can be assumed that both desloratadine and 3-hydroxydesloratadine become completely ionized given that the pH of 2% formic acid used as the diluent during sample extraction is at least 2 log units removed from the pK_a of the piperdine functional group. Moreover, during the aggressive 100% organic wash the charged desloratadine and 3-hydroxydesloratadine were retained on the polymeric solid phase extraction plate by ionic interaction alone. During the elution step, 4% ammonia in the elution solvent served to neutralize both the piperdine and pyridine functional groups thereby releasing desloratadine and 3-hydroxydesloratadine from the solid phase sorbant.

Fig. 2 summarizes an experiment where the extraction efficiency was monitored as a function of percent ammonia in the elution solvent. An amount as low as 2% ammonia was sufficient to recover desloratadine and 3-hydroxdesloratadine from the stationary phase. At 5% ammonia, a decrease in extraction efficiency was observed. There are two possible explanations for this result: first, it is possible that a higher percentage of ammonia releases undesirable lipids and

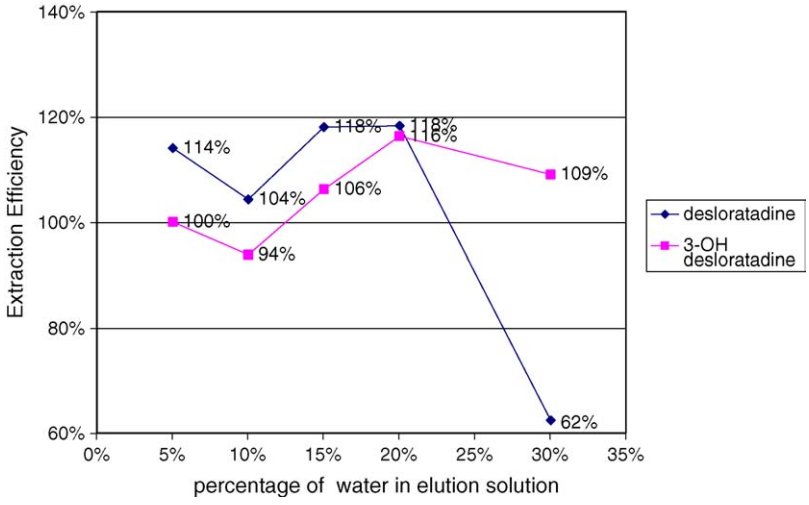

Fig. 3. 2D experiment of extraction efficiency as a function of percent water in elution solution.

endogenous proteins that could result in a higher degree of ion suppression, and second, it is possible that ammonia or other additives could be reconstituted and this in turn could affect the ionization efficiency of the analyte. In the final protocol, 4% ammonia was chosen to minimize the apparent variability between different lots of extraction plates. Fig. 3 summarizes an experiment wherein extraction efficiency is studied as a function of percent water in elution solution. As depicted, the maximum response was obtained for both desloratadine and 3-hydroxydesloratadine at aqueous concentrations in the elution solution of no more than 20%. In the final protocol, an aqueous content of 10% was chosen to minimize the apparent variability between different lots of extraction plates.

3.3. Chromatography

As noted in a previous article [12], in addition to 3-hydroxydesloratadine, two other isobaric mono-hydroxylated metabolites are present in an extracted sample requiring significant chromatographic resolution. For the Shimadzu experiment, this was accomplished by using a slow gradient profile employing a 5 μm, 2.1 mm × 50 mm Waters Atlantis™ C18 column. For the ACQUITY experiment, a Waters ACQUITY C-18, 1.7 μm, 2.1 mm × 50 mm UPLC column was used. The gradient elution programs used for both methods are summarized in Table 1. Mobile phase A consisted of 10 mM ammonium formate with 0.2% formic acid and mobile phase B consisted of 10 mM ammonium formate in methanol with 0.2% formic acid. Figs. 4 and 5 shows the data for the MRM channel of m/z 327 → m/z 275 following a 5 μL injection of a neat mixture of 3-, 5-, and 6-hydroxydesloratadine for both LC systems. The 5- and 6-hydroxydesloratadine components are resolved from the active metabolite 3-hydroxydesloratadine at retention times of 3.2 min and 1.35 min, respectively, on the Shimadzu and the ACQUITY systems. To evaluate the signal to noise ratio of each chromatography system, an aliquot of an extracted LLOQ sample was injected onto each system. Fig. 6 represents the typical response observed on the Shimadzu system following an injection of a 30 μL aliquot of the reconstituted extract. At the LLOQ, this represents an on-column injection of 0.955 pg for desloratadine and 1.05 pg of 3-hydroxydesloratadine, respectively [calculated based on 0.025 ng/mL × 250 μL (plasma volume)/150 μL (reconstitution volume) × average recovery (average of QCL and QCH post spiked recovery from Table 11) × 30 μL (injection volume)]. Fig. 7 represents the typical response observed on the ACQUITY system following the injection of 15 μL of extract. Because of the higher sensitivity afforded by the API 4000 system and the improved resolution of the UPLC column, only half the injection volume was necessary to achieve significantly better signal/noise ratio as compared to the Shimadzu system. At the LLOQ, this represents a total column load of 0.478 pg desloratdine and 0.525 pg of 3-hydroxydesloratdine calculated based on

Table 1
HPLC Gradient programs for Shimadzu and ACQUITY experiments

Total time (min)	*B* (%)	*A* (%)	Flow rate (μL/min)
(a) Shimadzu HPLC Gradient program			
0.50	20	80	250
3.30	90	10	250
3.80	90	10	250
3.81	20	80	250
4.20	20	80	250
(b) ACQUITY UPLC Gradient program			
0.25	20	80	500
1.65	90	10	500
1.90	90	10	500
1.91	20	80	500
2.10	20	80	500

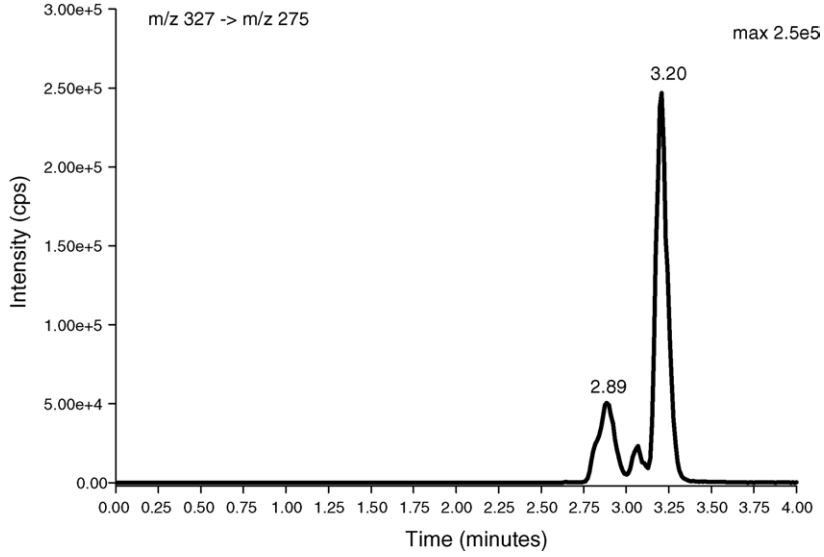

Fig. 4. Multiple reaction monitoring chromatogram produced by injecting 5 μL of 1 μg/mL neat solution of a mixture of 3-hydroxydesloratadine, 5-hydroxydesloratadine, and 6-hydroxydesloratadine on a Shimadzu HPLC system. 3-Hydroxydesloratadine has a retention time of 3.2 min.

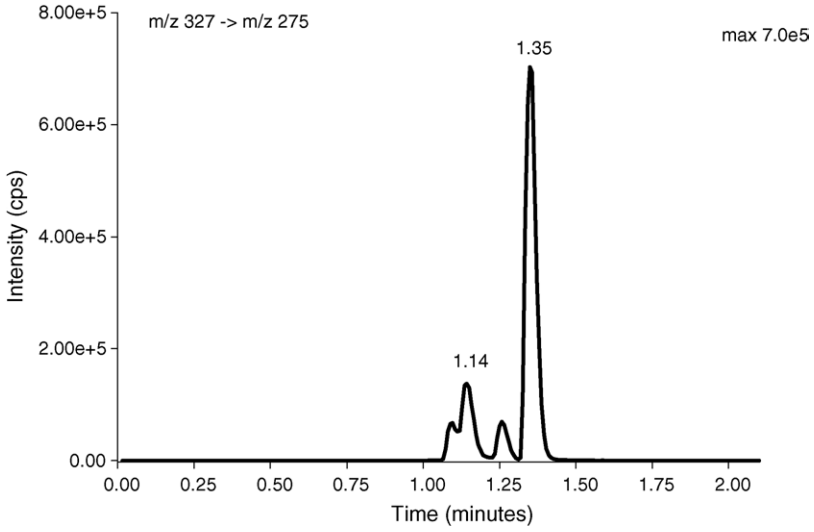

Fig. 5. Multiple reaction monitoring chromatogram produced by injecting 5 μL of 1 μg/mL neat solution of a mixture of 3-hydroxydesloratadine, 5-hydroxydesloratadine, and 6-hydroxydesloratadine on an ACQUITY UPLC system. 3-Hydroxydesloratadine has a retention time of 1.35 min.

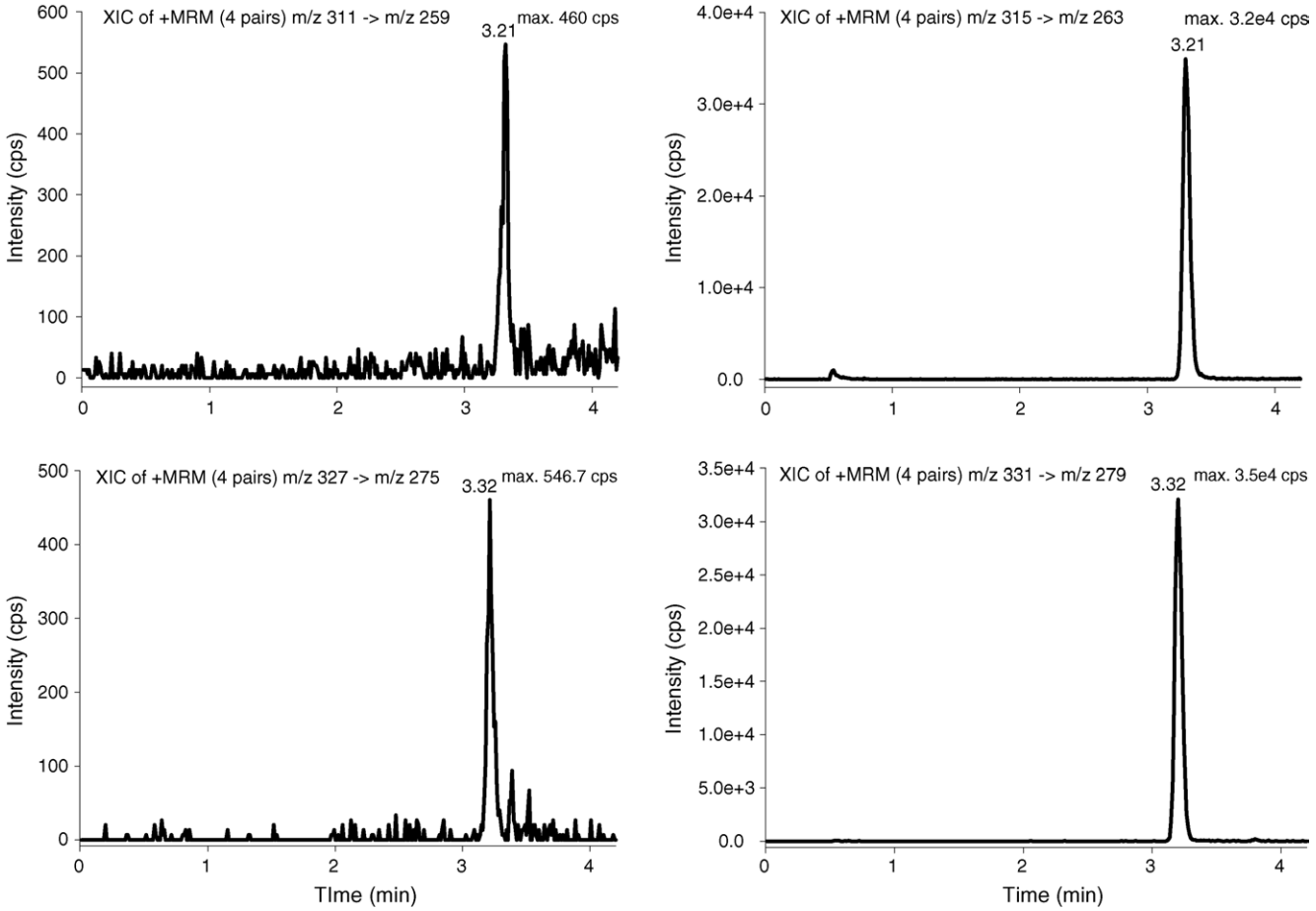

Fig. 6. Multiple reaction monitoring chromatogram produced by injecting 30 μL of an reconstituted LLOQ sample on the Shimadzu HPLC system. From the top traces to the bottom traces are the MRM channels for monitoring desloratadine, $^{2}H_4$ desloratadine, 3-hydroxydesloratadine, and $^{2}H_4$ 3-hydroxydesloratadine.

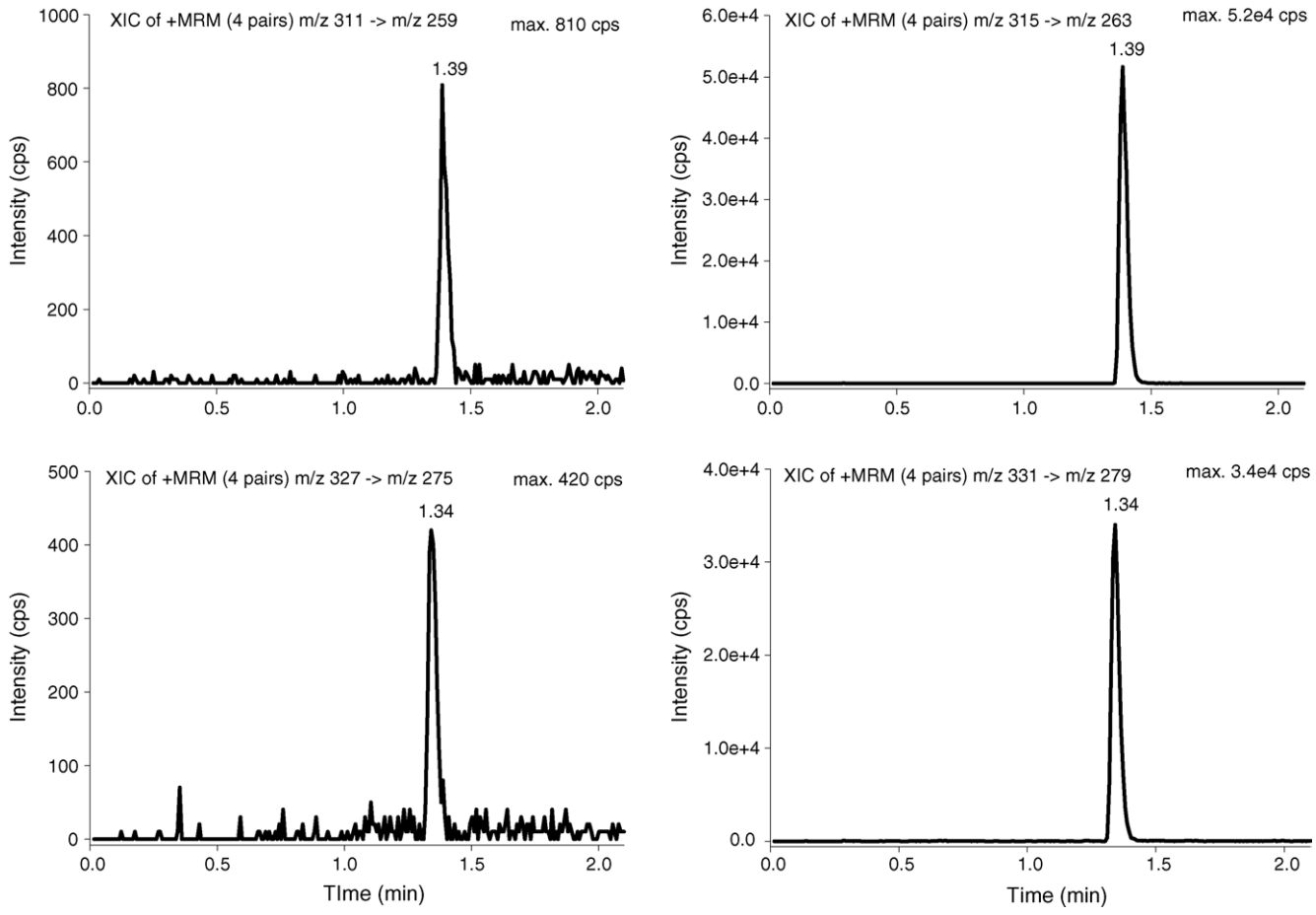

Fig. 7. Multiple reaction monitoring chromatogram produced by injecting 15 μL of an reconstituted LLOQ sample on the ACQUITY HPLC system. From the top traces to the bottom traces are the MRM channels for monitoring desloratadine, $^{2}H_4$ desloratadine, 3-hydroxydesloratadine, and $^{2}H_4$ 3-hydroxydesloratadine.

0.025 ng/mL × 250 μL (plasma volume)/150 μL (reconstitution volume) × average recovery (average of QCL and QCH post spiked recovery from Table 11) × 15 μL (injection volume).

In order to evaluate the contribution of UPLC to the improved sensitivity observed on the ACQUITY system, 10 μL aliquots of a 10 μg/mL desloratadine and 3–hydroxydesloratadine neat solution were injected onto either a Shimadzu or an ACQUITY system coupled to an API 4000 detector. As depicted in Fig. 8, the peak width was significantly improved for both desloratadine and 3-hydroxydesloratadine on the ACQUITY column. Specifically, the peak width was only 0.15 min and 0.16 min wide for desloratadine and 3-hydroxydesloratadine, respectively, on the ACQUITY UPLC system, whereas the peak width was 0.37 min and 0.32 min wide for desloratadine and 3-hydroxydesloratadine, respectively, on the Shimadzu HPLC system. Nevertheless, only a marginal improvement in sensitivity (peak height) was the result. This observation may be explained on the basis of the UPLC C18 column being less hydrophobic than the column on the HPLC system resulting in earlier elution of both desloratadine and 3-hydroxydesloratadine during the gradient profile. The later elution on the Shimadzu HPLC system occurs at a higher percentage of organic modifier resulting in improved sensitivity under electrospray conditions.

3.4. Method validation

3.4.1. Study design

Bioanalytical validation was assessed with three core analytical runs. Each analytical run included nine (ten for ACQUITY experiment) calibration standards ($n = 2$ at each concentration) and quality control samples prepared at four concentrations ($n = 6$ at each concentration). Each run also contained two zero standards (blank human matrix samples with IS) and two control blanks (blank human matrix samples without IS). For each core analytical run, at least two-thirds of the calibration standards must have individual accuracy within ±15% of the nominal value (±20% at the LLOQ). For each of the three core analytical runs, at least one of the two calibration standards at both the LLOQ and upper level of quantitation (ULOQ) must meet this criterion. If a calibration standard does not have accuracy within ±15% of the nominal value (±20% at the LLOQ), it is omitted from the calibration curve regression. For each validation run, the

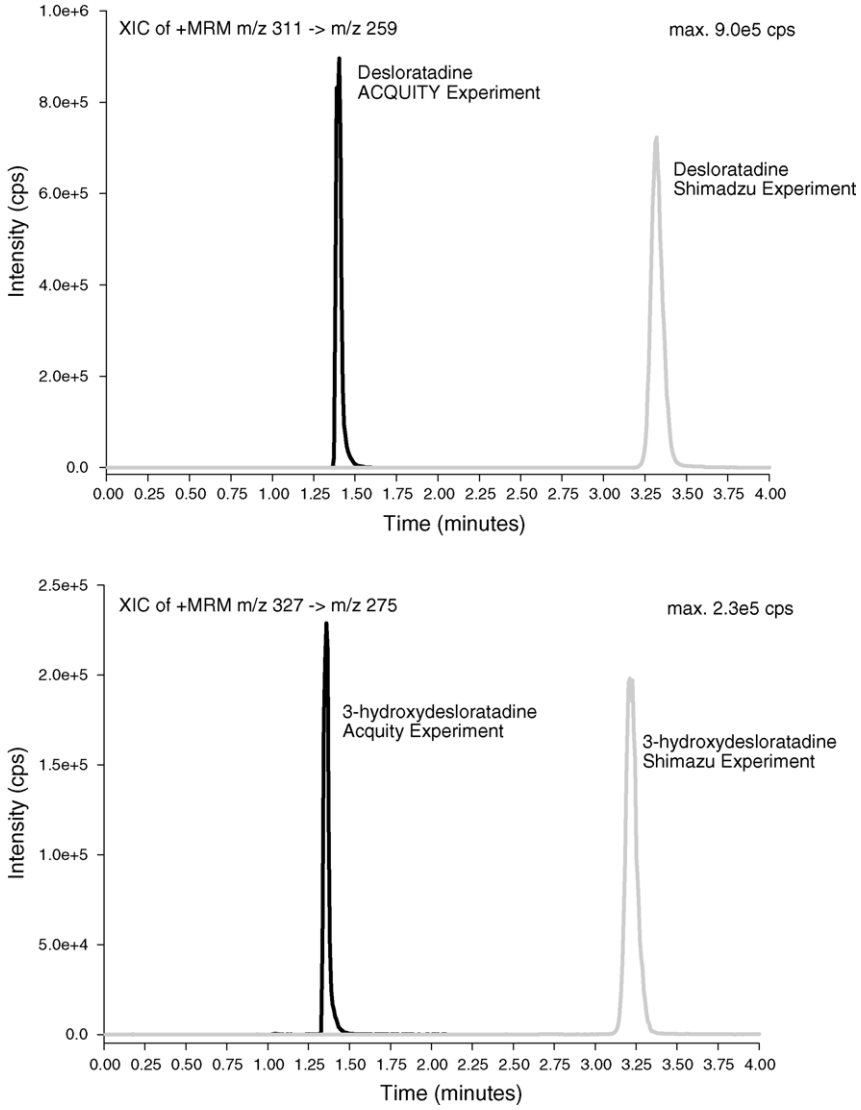

Fig. 8. Comparison of 10 μL injections of a 10 μg/mL desloratadine and 3-hydroxydesloratadine neat solution made on both Shimadzu and ACQUITY HPLC systems connected to an API 4000 system. From the top traces to the bottom traces are the MRM channels for monitoring desloratadine and 3-hydroxydesloratadine.

coefficient of determination (r^2) of the calibration curve must be greater than 0.98. For the three core validation runs, the within-run and between-run mean accuracy at each QC level should be within ±15% (±20% at the LLOQ). For each run, at least two-thirds of the QC samples at each concentration level must have individual accuracy within ±15% (±20% at the LLOQ). The within-run and between-run precision should be ≤15% (≤20% at the LLOQ).

Selectivity of the method was assessed by screening six sources of blank plasma for interference at the retention times of desloratadine, $^2H_4$-desloratadine, 3-hydroxydesloratadine, and $^2H_4$-3-hydroxydesloratadine. The selectivity of the method was also assessed to ensure that there was no interference between desloratadine, 3-hydroxydesloratadine, and their respective internal standards. To be acceptable, the response at the expected retention time of desloratadine and 3-hydroxydesloratadine must be less than 20% of the mean peak response calculated from the analysis of the LLOQ QC samples. The response at the expected retention time of $^2H_4$-desloratadine and $^2H_4$-3-hydroxydesloratadine must be less then 5% of the mean peak response of the IS in the LLOQ QC samples.

Integrity of dilution was assessed to determine whether a sample with a concentration greater than the ULOQ can be diluted with matrix for accurate quantitation within the range of the calibration curve. To be acceptable, the mean accuracy of at least three replicates should be within ±15% and two-thirds of the dilution QC samples should have individual accuracy within ±15%.

The stability of desloratadine and 3-hydroxydesloratadine was assessed by analyzing QC samples at low and high concentrations ($n = 6$ at each concentration) that were stored

Table 2
Back-calculated desloratadine concentrations in human plasma calibration standards and calibration curve parameters, Shimadzu experiment

Concentration (ng/mL)	STD 1 0.0250	STD 2 0.0500	STD 3 0.100	STD 4 0.500	STD 5 1.50	STD 6 2.00	STD 7 5.00	STD 8 8.00	STD 9 10.0	A[a]	B[a]	C[a]	r^2
Core run 1	0.0237	0.0528	0.0993	0.494	1.39	1.94	4.82	8.02	No value[b]	−0.00112	0.500	−0.000440	0.9983
	0.0247	0.0531	0.103	0.514	1.53	1.98	4.99	8.23	10.1				
Core run 2	0.0261	0.0436	0.105	0.502	1.41	1.97	4.88	7.97	9.61	0.00141	0.483	0.00135	0.9974
	0.0247	0.0510	0.103	0.492	1.54	2.11	5.03	8.22	10.3				
Core run 3	0.0248	0.0452	0.0945	0.495	1.46	2.12	4.89	8.06	9.50	0.00190	0.483	0.000163	0.9976
	0.0266	0.0491	0.105	0.517	1.54	2.02	4.90	8.23	10.3				
n	6	6	6	6	6	6	6	6	5	3	3	3	3
Overall mean	0.0251	0.0491	0.102	0.502	1.48	2.02	4.92	8.12	9.96				
S.D.	0.00106	0.00397	0.00407	0.0108	0.0679	0.0755	0.0773	0.119	0.382				
%CV	4.2	8.1	4.0	2.2	4.6	3.7	1.6	1.5	3.8				
%DIFF	0.4	−1.8	2.0	0.4	−1.3	1.0	−1.6	1.5	−0.4				

[a] $y = Ax^2 + Bx + C$, where y is the peak area ratio of desloratadine to IS, x is the concentration[2] of desloratadine, and A, B, and C are calibration curve parameters.
[b] Insufficient sample, deactivated.

Table 3
Back-calculated 3-hydroxydesloratadine concentrations in human plasma calibration standards and calibration curve parameters, Shimadzu experiment

Concentration (ng/mL)	STD 1 0.0250	STD 2 0.0500	STD 3 0.100	STD 4 0.500	STD 5 1.50	STD 6 2.00	STD 7 5.00	STD 8 8.00	STD 9 10.0	A[a]	B[a]	C[a]	r^2
Core run 1	0.0291	0.0447	0.0904	0.484	1.47	2.11	4.89	7.82	No value[b]	0.00729	0.540	0.00125	0.9920
	0.0246	0.0425	0.101	0.515	1.59	2.20	5.44	8.03	9.55				
Core run 2	0.0278	0.0511	0.100	0.474	1.50	2.06	4.80	7.45	9.99	0.00337	0.574	−0.000400	0.9969
	0.0222	0.0491	0.0986	0.505	1.55	2.04	5.10	8.19	10.3				
Core run 3	0.0258	0.0519	0.0977	0.493	1.47	2.11	5.03	7.94	9.31	0.00365	0.564	−0.000894	0.9971
	0.0253	0.0474	0.0874	0.504	1.60	2.08	4.97	8.22	10.3				
n	6	6	6	6	6	6	6	6	5	3	3	3	3
Overall mean	0.0258	0.0478	0.0959	0.496	1.53	2.10	5.04	7.94	9.89				
S.D.	0.00243	0.00367	0.00558	0.0151	0.0583	0.0562	0.223	0.284	0.447				
%CV	9.4	7.7	5.8	3.0	3.8	2.7	4.4	3.6	4.5				
%DIFF	3.2	−4.4	−4.1	−0.8	2.0	5.0	0.8	−0.8	−1.1				

[a] $y = Ax^2 + Bx + C$, where y is the peak area ratio of 3-hydroxydesloratadine to IS, x is the concentration[2] of 3-hydroxydesloratadine, and A, B, and C are calibration curve parameters.
[b] Insufficient sample, deactivated.

Table 4
Analytical performance of desloratadine quality control samples in human plasma, Shimadzu experiment

Run date	Curve number	LLOQ (0.0250 ng/mL)	%DIFF	QCL (0.0750 ng/mL)	%DIFF	QCM (1.00 ng/mL)	%DIFF	QCH (7.50 ng/mL)	%DIFF
	1	0.0241	−3.6	0.0775	3.3	0.971	−2.9	7.29	−2.8
		0.0348	39.2	0.0750	0.0	0.991	−0.9	7.19	−4.1
Core run 1		0.0279	11.6	0.0725	−3.3	0.943	−5.7	7.40	−1.3
		0.0239	−4.4	0.0691	−7.9	0.980	−2.0	7.55	0.7
		0.0264	5.6	0.0774	3.2	0.989	−1.1	7.85	4.7
		0.0287	14.8	0.0764	1.9	0.955	−4.5	7.61	1.5
Intrarun mean		0.0276		0.0747		0.972		7.48	
Intrarun S.D.		0.00401		0.00330		0.0192		0.239	
Intrarun %CV		14.5		4.4		2.0		3.2	
Intrarun %DIFF		10.4		−0.4		−2.8		−0.3	
n		6		6		6		6	
	2	0.0217	−13.2	0.0696	−7.2	1.00	0.0	7.12	−5.1
		0.0285	14.0	0.0647	−13.7	0.999	−0.1	7.23	−3.6
Core run 2		0.0276	10.4	0.0695	−7.3	0.938	−6.2	7.59	1.2
		0.0291	16.4	0.0680	−9.3	1.01	1.0	7.56	0.8
		0.0297	18.8	0.0779	3.9	0.991	−0.9	7.45	−0.7
		0.0299	19.6	0.0729	−2.8	0.940	−6.0	7.40	−1.3
Intrarun mean		0.0278		0.0704		0.980		7.39	
Intrarun S.D.		0.00308		0.00452		0.0321		0.185	
Intrarun %CV		11.1		6.4		3.3		2.5	
Intrarun %DIFF		11.2		−6.1		−2.0		−1.5	
n		6		6		6		6	
	3	0.0265	6.0	0.0751	0.1	1.00	0.0	7.17	−4.4
		0.0256	2.4	0.0811	8.1	1.00	0.0	7.49	−0.1
Core run 3		0.0326	30.4	0.0789	5.2	1.02	2.0	7.61	1.5
		0.0277	10.8	0.0765	2.0	1.05	5.0	7.62	1.6
		0.0258	3.2	0.0987	31.6	1.06	6.0	7.78	3.7
		0.0289	15.6	0.0729	−2.8	1.06	6.0	7.69	2.5
Intrarun mean		0.0279		0.0805		1.03		7.56	
Intrarun S.D.		0.00264		0.00935		0.0286		0.214	
Intrarun %CV		9.5		11.6		2.8		2.8	
Intrarun %DIFF		11.6		7.3		3.0		0.8	
n		6		6		6		6	
Mean concentration found (ng/mL)		0.0277		0.0752		0.994		7.48	
Inter-run S.D.		0.00310		0.00729		0.0375		0.213	
Inter-run %CV		11.2		9.7		3.8		2.8	
Inter-run %DIFF		10.8		0.3		−0.6		−0.3	
n		18		18		18		18	

Table 5
Analytical performance of 3-hydroxydesloratadine quality control samples in human plasma, Shimadzu experiment

Run date	Curve number	LLOQ (0.0250 ng/mL)	%DIFF	QCL (0.0750 ng/mL)	%DIFF	QCM (1.00 ng/mL)	%DIFF	QCH (7.50 ng/mL)	%DIFF
	1	0.0200	−20.0	0.0775	3.3	1.06	6.0	7.18	−4.3
		0.0240	−4.0	0.0758	1.1	1.10	10.0	7.32	−2.4
Core run 1		0.0242	−3.2	0.0814	8.5	1.02	2.0	7.56	0.8
		0.0254	1.6	0.0852	13.6	1.08	8.0	7.53	0.4
		0.0288	15.2	0.0794	5.9	1.09	9.0	7.60	1.3
		0.0244	−2.4	0.0782	4.3	1.06	6.0	7.43	−0.9
Intrarun mean		0.0245		0.0796		1.07		7.44	
Intrarun S.D.		0.00282		0.00333		0.0286		0.161	
Intrarun %CV		11.5		4.2		2.7		2.2	
Intrarun %DIFF		−2.0		6.1		7.0		−0.8	
n		6		6		6		6	
	2	0.0243	−2.8	0.0638	−14.9	1.02	2.0	6.88	−8.3
		0.0290	16.0	0.0662	−11.7	1.07	7.0	7.57	0.9
Core run 2		0.0252	0.8	0.0783	4.4	0.979	−2.1	7.41	−1.2
		0.0216	−13.6	0.0730	−2.7	1.03	3.0	7.37	−1.7
		0.0284	13.6	0.0780	4.0	0.965	−3.5	7.48	−0.3
		0.0242	−3.2	0.0791	5.5	0.947	−5.3	7.46	−0.5
Intrarun mean		0.0255		0.0731		1.00		7.36	
Intrarun S.D.		0.00280		0.00665		0.0462		0.246	
Intrarun %CV		11.0		9.1		4.6		3.3	
Intrarun %DIFF		2.0		−2.5		0.0		−1.9	
n		6		6		6		6	
	3	0.0228	−8.8	0.0800	6.7	0.997	−0.3	7.36	−1.9
		0.0258	3.2	0.0809	7.9	1.01	1.0	7.75	3.3
		0.0286	14.4	0.0786	4.8	1.04	4.0	7.69	2.5
Core run 3		0.0272	8.8	0.0802	6.9	1.06	6.0	7.77	3.6
		0.0282	12.8	0.101	34.7	1.07	7.0	7.70	2.7
		0.0305	22.0	0.0789	5.2	1.04	4.0	7.81	4.1
Intrarun mean		0.0272		0.0833		1.04		7.68	
Intrarun S.D.		0.00265		0.00873		0.0281		0.163	
Intrarun %CV		9.7		10.5		2.7		2.1	
Intrarun %DIFF		8.8		11.1		4.0		2.4	
n		6		6		6		6	
Mean concentration found (ng/mL)		0.0257		0.0786		1.04		7.49	
Inter-run S.D.		0.00284		0.00758		0.0434		0.230	
Inter-run %CV		11.1		9.6		4.2		3.1	
Inter-run %DIFF		2.8		4.8		4.0		−0.1	
n		18		18		18		18	

Table 6
Back-calculated desloratadine concentrations in human plasma calibration standards and calibration curve parameters, ACQUITY experiment

Concentration (ng/mL)	STD 1 0.0250	STD 2 0.0500	STD 3 0.100	STD 4 0.200	STD 5 0.500	STD 6 1.50	STD 7 2.00	STD 8 5.00	STD 9 8.00	STD 10 10.0	A[a]	B[a]	C[a]	r^2
Core run 1	0.0250	0.0488	0.0989	0.192	0.483	1.55	2.01	5.13	7.99	9.49	0.00198	0.461	0.000203	0.9988
	0.0253	0.0510	0.100	0.198	0.479	1.55	2.03	5.29	8.19	9.84				
Core run 2	0.0264	0.0472	0.0984	0.198	0.503	1.57	2.04	5.26	7.78	10.0	0.00215	0.464	0.000579	0.9985
	0.0250	0.0484	0.0980	0.196	0.477	1.56	2.01	5.13	8.14	9.55				
Core run 3	0.0251	0.0491	0.102	0.194	0.509	1.55	2.13	5.10	8.10	9.45	0.00111	0.462	0.000462	0.9987
	0.0254	0.0501	0.0976	0.187	0.499	1.45	1.98	5.21	7.98	10.1				
n	6	6	6	6	6	6	6	6	6	6				
Overall mean	0.0254	0.0491	0.0992	0.194	0.492	1.54	2.03	5.19	8.03	9.74				
S.D.	0.000532	0.00133	0.00162	0.00422	0.0137	0.0440	0.0516	0.0781	0.148	0.279				
%CV	2.1	2.7	1.6	2.2	2.8	2.9	2.5	1.5	1.8	2.9				
%DIFF	1.6	−1.8	−0.8	−3.0	−1.6	2.7	1.5	3.8	0.4	−2.6				

[a] $y = Ax^2 + Bx + C$, where y is the peak area ratio of desloratadine to IS, x is the concentration^2 of desloratadine, and A, B, and C are calibration curve parameters.

Table 7
Back-calculated 3-hydroxydesloratadine concentrations in human plasma calibration standards and calibration curve parameters, ACQUITY experiment

Concentration (ng/mL)	STD 1 0.0250	STD 2 0.0500	STD 3 0.100	STD 4 0.200	STD 5 0.500	STD 6 1.50	STD 7 2.00	STD 8 5.00	STD 9 8.00	STD 10 10.0	A[a]	B[a]	C[a]	r^2
Core run 1	0.0251	0.0446	0.0955	0.192	0.479	1.51	2.03	5.29	8.05	9.07	0.00254	0.384	−0.000224	0.9965
	0.0269	0.0501	0.0955	0.202	0.513	1.56	2.07	5.41	8.20	9.90				
Core run 2	0.0243	0.0520	0.108	0.190	0.490	1.53	1.96	5.18	7.81	9.86	0.00246	0.400	−0.000518	0.9976
	0.0242	0.0536	0.103	0.182	0.469	1.53	2.02	5.18	8.10	9.89				
Core run 3	0.0239	0.0503	0.0982	0.191	0.507	1.53	2.09	5.01	8.31	9.53	0.000336	0.405	−0.000553	0.9978
	0.0268	0.0477	0.104	0.190	0.485	1.47	2.05	5.40	7.84	9.80				
n	6	6	6	6	6	6	6	6	6	6				
Overall mean	0.0252	0.0497	0.101	0.191	0.491	1.52	2.04	5.25	8.05	9.68				
S.D.	0.00134	0.00319	0.00510	0.00640	0.0168	0.0299	0.0455	0.153	0.197	0.327				
%CV	5.3	6.4	5.0	3.4	3.4	2.0	2.2	2.9	2.4	3.4				
%DIFF	0.8	−0.6	1.0	−4.5	−1.8	1.3	2.0	5.0	0.6	−3.2				

[a] $y = Ax^2 + Bx + C$, where y is the peak area ratio of 3-hydroxydesloratadine to IS, x is the concentration^2 of desloratadine, and A, B, and C are calibration curve parameters.

Table 8
Analytical performance of desloratadine quality control samples in human plasma, ACQUITY experiment

Run date	Curve number	LLOQ (0.0250 ng/mL)	%DIFF	QCL (0.0750 ng/mL)	%DIFF	QCM (1.00 ng/mL)	%DIFF	QCH (7.50 ng/mL)	%DIFF
	1	0.0291	16.4	0.0697	−7.1	1.04	4.0	7.78	3.7
		0.0235	−6.0	0.0724	−3.5	1.05	5.0	7.58	1.1
		0.0253	1.2	0.0726	−3.2	1.05	5.0	7.57	0.9
Core run 1		0.0286	14.4	0.0717	−4.4	0.982	−1.8	7.34	−2.1
		0.0262	4.8	0.0684	−8.8	1.07	7.0	7.36	−1.9
		0.0263	5.2	0.0746	−0.5	1.06	6.0	7.41	−1.2
Intrarun mean		0.0265		0.0716		1.04		7.51	
Intrarun S.D.		0.00209		0.00221		0.0311		0.169	
Intrarun %CV		7.9		3.1		3.0		2.3	
Intrarun %DIFF		6.0		−4.5		4.0		0.1	
n		6		6		6		6	
	2	0.0240	−4.0	0.0708	−5.6	0.972	−2.8	7.26	−3.2
		0.0261	4.4	0.0697	−7.1	1.04	4.0	7.08	−5.6
		0.0238	−4.8	0.0687	−8.4	1.04	4.0	7.45	−0.7
Core run 2		0.0203	−18.8	0.0665	−11.3	1.01	1.0	7.53	0.4
		0.0259	3.6	0.0713	−4.9	0.992	−0.8	7.21	−3.9
		0.0252	0.8	∼0.0633	−15.6	1.01	1.0	No value	
Intrarun mean		0.0242		0.0684		1.01		7.31	
Intrarun S.D.		0.00214		0.00302		0.0267		0.183	
Intrarun %CV		8.8		4.4		2.6		2.5	
Intrarun %DIFF		−3.2		−8.8		1.0		−2.5	
n		6		6		6		5	
	3	0.0249	−0.4	0.0761	1.5	1.01	1.0	7.27	−3.1
		0.0243	−2.8	0.0729	−2.8	0.984	−1.6	7.15	−4.7
		0.0242	−3.2	0.0729	−2.8	1.04	4.0	7.39	−1.5
Core run 3		0.0232	−7.2	0.0716	−4.5	1.04	4.0	7.45	−0.7
		0.0231	−7.6	0.0749	−0.1	1.05	5.0	7.32	−2.4
		0.0260	4.0	0.0690	−8.0	1.04	4.0	7.54	0.5
Intrarun mean		0.0243		0.0729		1.03		7.35	
Intrarun S.D.		0.00109		0.00250		0.0252		0.138	
Intrarun %CV		4.5		3.4		2.4		1.9	
Intrarun %DIFF		−2.8		−2.8		3.0		−2.0	
n		6		6		6		6	
Mean concentration found (ng/mL)		0.0250		0.0710		1.03		7.39	
Inter-run S.D.		0.00204		0.00312		0.0292		0.176	
Inter-run %CV		8.2		4.4		2.8		2.4	
Inter-run %DIFF		0.0		−5.3		3.0		−1.5	
n		18		18		18		17	

Table 9
Analytical performance of 3-hydroxydesloratadine quality control samples in human plasma, ACQUITY experiment

Run date	Curve number	LLOQ (0.0250 ng/mL)	%DIFF	QCL (0.0750 ng/mL)	%DIFF	QCM (1.00 ng/mL)	%DIFF	QCH (7.50 ng/mL)	%DIFF
	1	0.0349*		0.0712	−5.1	1.01	1.0	7.82	4.3
		0.0275	10.0	0.0715	−4.7	1.05	5.0	7.60	1.3
		0.0238	−4.8	0.0727	−3.1	1.05	5.0	7.68	2.4
Core run 1		0.0267	6.8	0.0692	−7.7	1.08	8.0	7.83	4.4
		0.0282	12.8	0.0751	0.1	1.06	6.0	7.43	−0.9
		0.0234	−6.4	0.0761	1.5	1.07	7.0	7.52	0.3
Intrarun mean		0.0259		0.0726		1.05		7.65	
Intrarun S.D.		0.00219		0.00258		0.0242		0.161	
Intrarun %CV		8.5		3.6		2.3		2.1	
Intrarun %DIFF		3.6		−3.2		5.0		2.0	
n		5		6		6		6	
	2	0.0250	0.0	0.0688	−8.3	0.985	−1.5	7.17	−4.4
		0.0247	−1.2	0.0725	−3.3	1.03	3.0	7.16	−4.5
		0.0284	13.6	0.0676	−9.9	1.05	5.0	7.61	1.5
Core run 2		0.0271	8.4	0.0691	−7.9	1.02	2.0	7.18	−4.3
		0.0215	−14.0	0.0680	−9.3	1.02	2.0	7.25	−3.3
		0.0255	2.0	0.0720	−4.0	1.06	6.0	No value	
Intrarun mean		0.0254		0.0697		1.03		7.27	
Intrarun S.D.		0.00236		0.00208		0.0264		0.191	
Intrarun %CV		9.3		3.0		2.6		2.6	
Intrarun %DIFF		1.6		−7.1		3.0		−3.1	
n		6		6		6		5	
	3	0.0218	−12.8	0.0657	−12.4	0.991	−0.9	7.44	−0.8
		∼0.0303	21.2	0.0747	−0.4	1.03	3.0	7.24	−3.5
		0.0251	0.4	0.0717	−4.4	1.02	2.0	7.42	−1.1
Core run 3		0.0246	−1.6	0.0647	−13.7	1.07	7.0	7.58	1.1
		0.0237	−5.2	0.0681	−9.2	1.01	1.0	7.27	−3.1
		0.0224	−10.4	0.0694	−7.5	1.03	3.0	7.71	2.8
Intrarun mean		0.0247		0.0691		1.03		7.44	
Intrarun S.D.		0.00304		0.00374		0.0264		0.180	
Intrarun %CV		12.3		5.4		2.6		2.4	
Intrarun %DIFF		−1.2		−7.9		3.0		−0.8	
n		6		6		6		6	
Mean concentration found (ng/mL)		0.0253		0.0705		1.04		7.47	
Inter-run S.D.		0.00247		0.00315		0.0275		0.227	
Inter-run %CV		9.8		4.5		2.6		3.0	
Inter-run %DIFF		1.2		−6.0		4.0		−0.4	
n		17		18		18		17	

under various conditions. To be acceptable, two-thirds of all stability QC samples at each level should have individual accuracy within ±15% of the nominal value, and the mean accuracy at each level should be within ±15% of the nominal value. The stability of desloratadine and 3-hydroxydesloratadine in stock solutions during long-term storage at 4 °C was assessed by comparing stored stock solutions to fresh stock solutions. In addition, the stability of desloratadine and 3-hydroxydesloratadine in stock solutions at room temperature for at least 6 h was also assessed. For stock solution stability, the mean difference of the peak response of the evaluated stock solution should be ≤5% from the mean peak response of a freshly prepared stock solution.

3.4.2. Accuracy and precision (Shimadzu experiment)

For calibration standards, the between-run precision and accuracy results for desloratadine and 3-hydroxydesloratadine calculated based on three analytical core runs are listed in Tables 2 and 3, respectively. The intra-run accuracy (%DIFF) ranged from −1.8% to 2% for desloratadine and −4.4% to 3.2% for 3-hydroxydesloratadine for $n = 6$ replicate standards. Between-run precision (%CV) ranged from 1.5% to 8.1% for desloratadine and 2.7% to 7.7% for 3-hydroxydesloratadine for $n = 6$ replicate standards.

For QC samples, the between-run precision and accuracy results for desloratadine and 3-hydroxydesloratadine calculated based on three analytical core runs are listed in Tables 4 and 5, respectively. The inter-run accuracy (%DIFF) for desloratadine ranged from −0.6% for $n = 18$ QCM samples to 10.8% for $n = 18$ LLOQ samples. The inter-run accuracy for 3-hydroxydesloratadine ranged from −0.1% for $n = 18$ QCH samples to 4.8% for $n = 18$ QCL samples. The between-run precision (%CV) ranged from 2.8% to 11.2% for desloratadine ($n = 18$) and 3.1% to 11.1% for 3-hydroxydesloratadine ($n = 18$).

3.4.3. Accuracy and precision (ACQUITY experiment)

For calibration standards, the between-run precision and accuracy results for desloratadine and 3-hydroxydesloratadine calculated based on three analytical core runs are listed in Tables 6 and 7, respectively. The intra-run accuracy (%DIFF) ranged from −3.0% to 3.8% for desloratadine and −4.5% to 5.0% for 3-hydroxydesloratadine

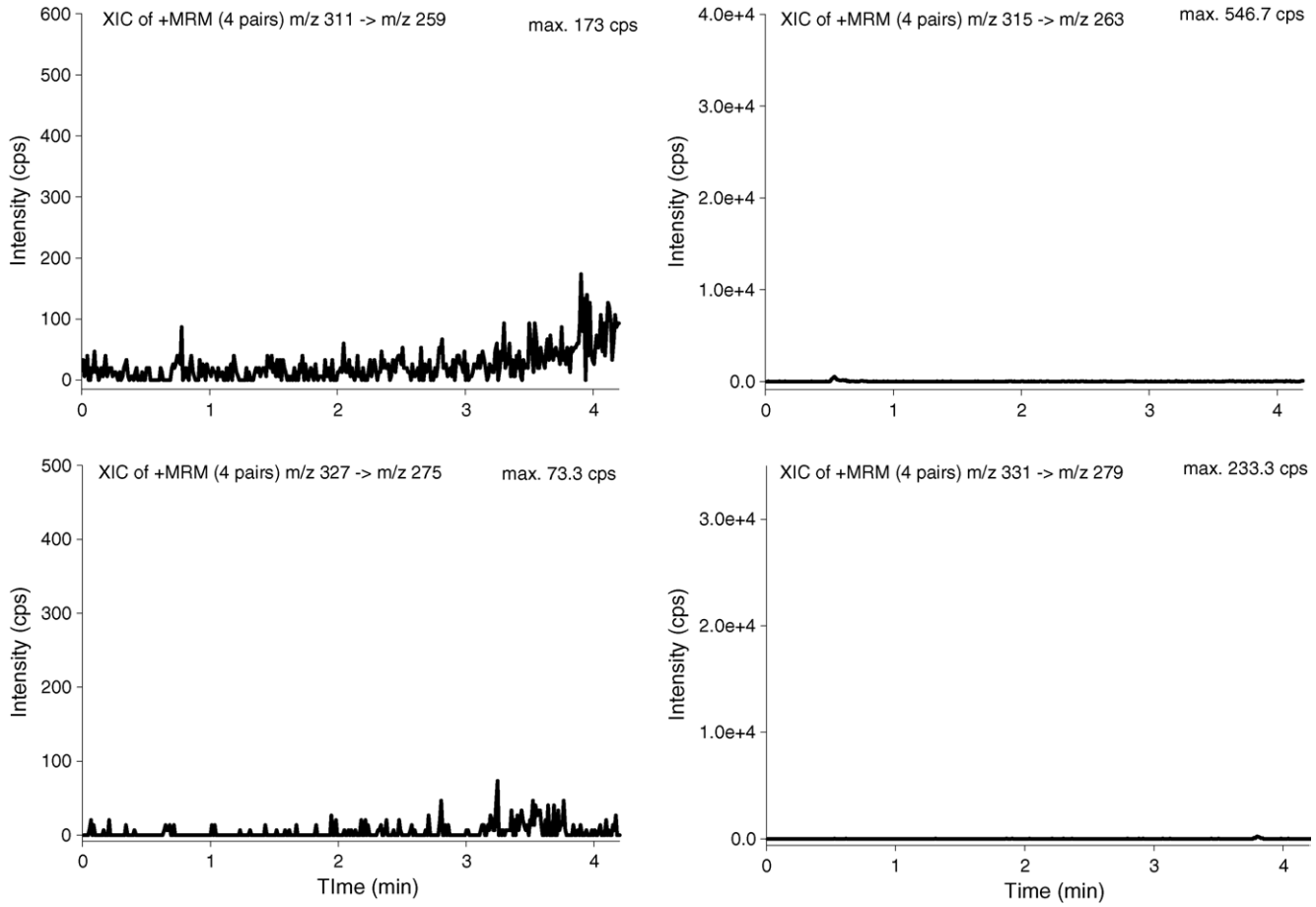

Fig. 9. Multiple reaction monitoring chromatogram produced by injecting 30 μL of an reconstituted blank sample on the Shimadzu HPLC system. From the top traces to the bottom traces are the MRM channels for monitoring desloratadine, $^{2}H_4$ desloratadine, 3-hydroxydesloratadine, and $^{2}H_4$ 3-hydroxydesloratadine.

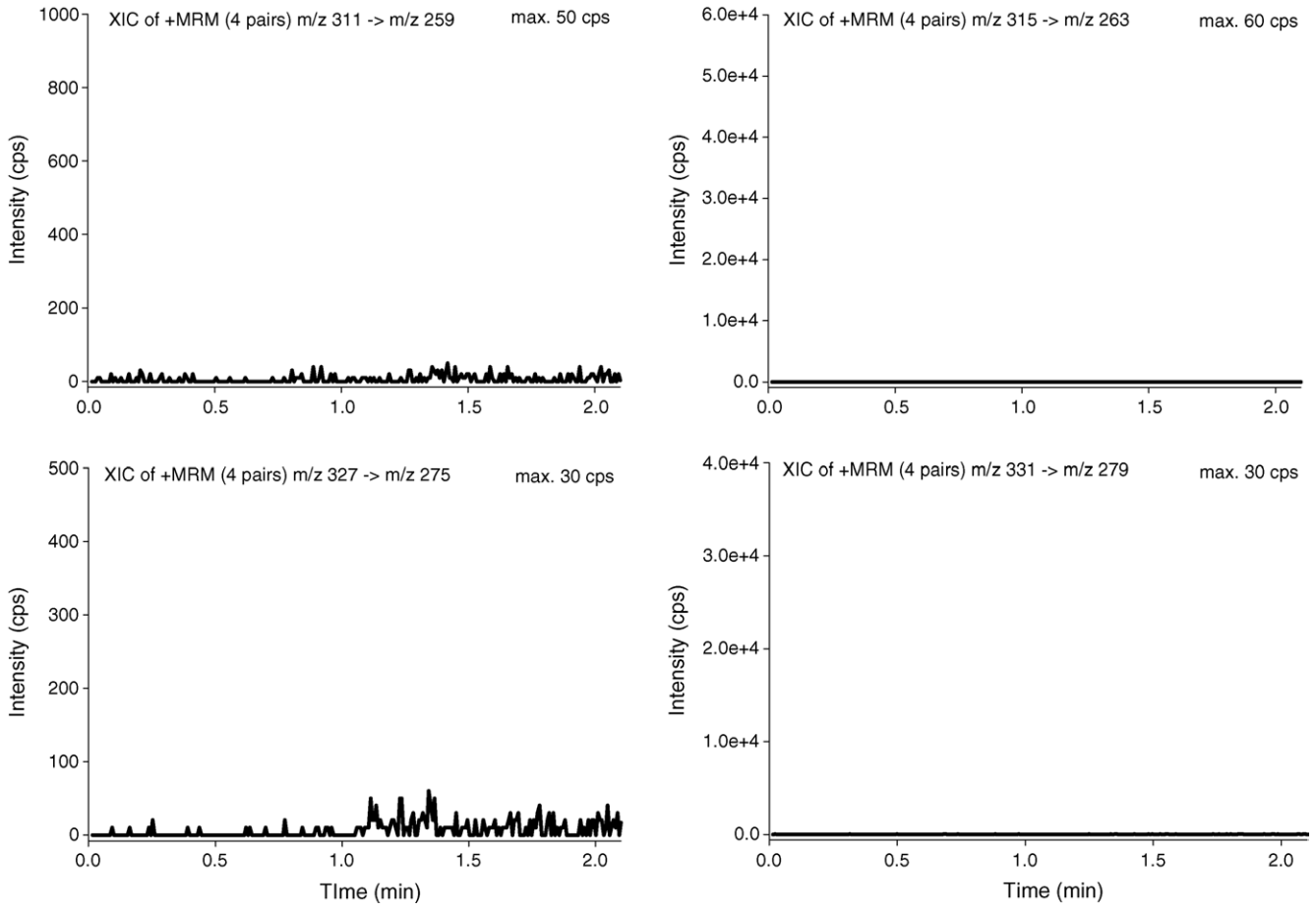

Fig. 10. Multiple reaction monitoring chromatogram produced by injecting 15 μL of an reconstituted blank sample on the ACQUITY HPLC system. From the top traces to the bottom traces are the MRM channels for monitoring desloratadine, $^{2}H_4$ desloratadine, 3-hydroxydesloratadine, and $^{2}H_4$ 3-hydroxydesloratadine.

for $n = 6$ replicate standards. Between-run precision (%CV) ranged from 1.5% to 2.9% for desloratadine and 2.0% to 6.4% for 3-hydroxydesloratadine for $n = 6$ replicate standards.

For QC samples, the between-run precision and accuracy results for desloratadine and 3-hydroxydesloratadine calculated based on three analytical core runs are listed in Tables 8 and 9, respectively. The inter-run accuracy (%DIFF) for desloratadine ranged from −5.3% for $n = 18$ QCL samples to 3.0% for $n = 18$ QCM samples. The inter-run accuracy for 3-hydroxydesloratadine ranged from −6.0% for $n = 18$ QCL samples to 4.0% for $n = 18$ QCM samples. 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 ($n = 18$).

3.4.4. Integrity of dilution

Dilution quality control (QCD) samples were prepared at a concentration of 37.50 ng/mL in blank matrix (human EDTA plasma) and diluted 10-fold with blank matrix (human EDTA plasma) ($n = 5$). Accuracy data for desloratadine and 3-hydroxydesloratadine integrity of dilution are presented in Table 10.

3.4.5. Selectivity

Blank human EDTA from six sources were screened and found to be free of interference from endogenous components or other sources at the same mass transitions and retention times as desloratadine and 3-hydroxydesloratadine and their respective internal standards. Figs. 9 and 10 show typ-

Table 10
10× Dilution Integrity for desloratadine and 3-hydroxy desloratadine in human plasma

	Desloratadine (37.5 ng/mL)	3-Hydroxydesloratadine (37.5 ng/mL)
	35.8	35.8
	36.8	35.7
	36.9	36.2
	35.4	35.3
	36.7	37.9
Mean	36.3	36.2
S.D.	0.676	1.01
%CV	1.9	2.8
%Theoretical	96.8	96.5
%DIFF	−3.2	−3.5
n	5	5

Table 11
Recovery experiments for desloratadine and 3-hydroxydesloratadine

ID	QCL neat	QCH neat	QCL	QCH	QCL post spiked	QCH post spiked	QCL	QCH
Desloratadine	QCs vs. neat				QCs vs. post spiked			
Nominal concentration (ng/mL)	0.0750 ng/mL	7.50 ng/mL	0.0750 ng/mL	7.50 ng/mL	0.0750 ng/mL	7.50 ng/mL	0.0750 ng/mL	7.50 ng/mL
	6.52E + 04	5.59E + 06	1.79E + 04	1.85E + 06	2.48E + 04	2.36E + 06	1.79E + 04	1.85E + 06
	6.36E + 04	5.53E + 06	2.13E + 04	1.86E + 06	2.68E + 04	2.81E + 06	2.13E + 04	1.86E + 06
Peak	6.88E + 04	5.97E + 06	1.70E + 04	1.58E + 06	2.40E + 04	2.25E + 06	1.70E + 04	1.58E + 06
area	7.00E + 04	5.99E + 06	1.95E + 04	2.09E + 06	2.60E + 04	2.52E + 06	1.95E + 04	2.09E + 06
	7.38E + 04	6.11E + 06	1.85E + 04	1.77E + 06	2.32E + 04	2.26E + 06	1.85E + 04	1.77E + 06
	7.24E + 04	5.97E + 06	2.04E + 04	1.99E + 06	2.50E + 04	2.40E + 06	2.04E + 04	1.99E + 06
Mean	6.90E + 04	5.86E + 06	1.91E + 04	1.86E + 06	2.50E + 04	2.43E + 06	1.91E + 04	1.86E + 06
n	6	6	6	6	6	6	6	6
Recovery			27.7	31.7			76.5	76.3
3-Hydroxydesloratadine								
	2.24E + 04	1.99E + 06	9.32E + 03	9.59E + 05	1.16E + 04	1.07E + 06	9.32E + 03	9.59E + 05
	2.04E + 04	2.00E + 06	1.01E + 04	9.31E + 05	1.25E + 04	1.23E + 06	1.01E + 04	9.31E + 05
Peak	2.16E + 04	1.81E + 06	9.86E + 03	8.19E + 05	1.21E + 04	1.04E + 06	9.86E + 03	8.19E + 05
area	2.17E + 04	2.04E + 06	9.62E + 03	9.85E + 05	1.07E + 04	1.11E + 06	9.62E + 03	9.85E + 05
	2.58E + 04	2.06E + 06	9.82E + 03	9.10E + 05	1.17E + 04	1.07E + 06	9.82E + 03	9.10E + 05
	2.22E + 04	2.09E + 06	1.02E + 04	9.55E + 05	1.15E + 04	1.10E + 06	1.02E + 04	9.55E + 05
Mean	2.24E + 04	2.00E + 06	9.82E + 03	9.27E + 05	1.17E + 04	1.10E + 06	9.82E + 03	9.27E + 05
n	6	6	6	6	6	6	6	6
Recovery			43.9	46.4			84.1	84.0

Table 12
Stability data for desloratadine and 3-hydroxydesloratadine in human plasma

Conditions	Minimum stability
Short-term room temperature (RT) stability in human plasma	162 h
Frozen stability in human plasma stored in a −20 °C freezer	401 days
Freeze-thaw stability in human plasma	5 cycles
Autosampler stability at room temperature	5 days
Stock solution stability	274 days

ical MRM chromatograms for an extracted human plasma blank sample of desloratadine and 3-hydroxydesloratadine and the internal standards $^{2}H_4$-desloratadine, and $^{2}H_4$-3-hydroxydesloratadine, for both Shimadzu and ACQUITY systems, respectively.

3.4.6. Recovery

The recovery for the analytes was assessed at the low and high QC concentrations ($n = 6$ at each concentration). Recovery was determined by comparing the mean peak response from processed QC samples to the mean peak response from unprocessed (neat) standards. In addition, the mean peak response of the extracted blank matrix samples that were spiked (post-extraction) with QC stock solution was compared to the mean peak response of processed QC samples to demonstrate extraction efficiency. The recovery for desloratadine and 3-hydroxydesloratadine is listed in Table 11. Because of a high degree of ion suppression observed for both desloratadine (36.1–41.5%) and 3-hydroxydesloratadine (52.2–55%), additional experiments were performed to establish the source of this ion suppression. The results of these studies are reported elsewhere [18].

3.4.7. Stability

The stability of desloratadine and 3-hydroxydesloratadine was assessed by analyzing QC samples at low and high concentrations (0.075 ng/mL and 75 ng/mL, respectively; $n = 6$ at each concentration) that were stored under various conditions. These experiments are summarized in Table 12.

4. Conclusion

An automated, sensitive, rugged, rapid, and reproducible LC-MS/MS method was developed by incorporating an orthogonal approach to extraction and chromatography. Mixed mode solid phase extraction was used in conjunction with UPLC on an API 4000 LC/MS-MS platform. The validation experiments met current FDA acceptance criteria for precision and accuracy. These results show that approaches detailed in this article can result in significant improvements in sample throughput. For very fast bioanalysis, operating at higher linear velocities using sub 2-μm particles clearly has advantages unmatched by conventional approaches.

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