Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Suppl 4, 2013

Research Article

A NOVEL CHROMATOGRAPHIC SEPARATION TECHNIQUE USING UPLC-MS/MS FOR DETERMINATION OF DESLORATIDINE AND 3-HYDROXY DESLORATIDINE IN HUMAN PLASMA AND ITS APPLICATION

V. PRAVEEN KUMAR[ab]*, PRATIMA ASHAWAT[b], ASHISH SAXENA[a], MANOJ BOB[a], BIPIN PURWAR[a], M.S.ASHAWAT[c], RAVISEKHAR KASIBHATTA[a]

[a]Lupin Bioresearch Centre, Pashan Pune 411021, Maharashtra State, [b]Department of Science, Pacific University, Udaipur, Rajasthan, [c]Rungta College of Pharmaceutical Sciences and Research , Bhilai CG, Rajasthan, India. *Email: praveenvittala@lupinpharma.com

Received: 07 Oct 2013, Revised and Accepted: 28 Oct 2013

ABSTRACT

Objective: A bioanalytical method was developed and validated using Ultra Performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS) technique for the determination of Desloratidine and 3-OH Desloratidine in human plasma.

Methods: The samples were extracted using solid-phase extraction (SPE) technique wherein Desloratidine-D5 and 3-OH desloratidine-D4 have been used as the internal standards. The use of simultaneous time and flow gradient Liquid chromatography (LC) method has enabled to achieve 0.20 minute resolution between desloratidine and 3-OH desloratidine alongwith their respective internal standards using a Thermo Hypruity C_{18} (50mm) column. The developed method was specific and sensitive having no interfering peaks in the drug free plasma.

Results: The method was validated for a linear range of 50-10000 pg/mL for Desloratidine and 3-OH Desloratidine with a correlation coefficient ≥ 0.99. The limit of detection (LOD) of 10 pg/mL for Desloratidine and 3-OH Desloratidine with a signal-to noise (S/N) >10 was achieved, thereby giving a possibility of much lower detection for even low dossiers. Inter-run precision (%CV) ranged from 1.62 to 4.90% for desloratidine and 2.05 to 5.93% for 3-OH desloratidine. Inter-run accuracy(%Bias) ranged from -5.54 to -2.55% for desloratidine and -0.48 to -8.04% The overall recoveries for Desloratidine, Desloratidine D5, 3-OH Desloratidine and 3-OH Desloratidine D4 were found to be >98%. Desloratidine and 3-OH desloratidine were found to be stable at various temperatures and for about 5 freeze-thaw cycles and reconstituted samples were stable upto 72 hours post to extraction.

Conclusion: The developed and validated method was found to be precise, reproducible and a high throughput of analyzing more than 400 samples per day could be achieved with a shorter run time of 3.0 minutes. The developed method was applied for the determination of Desloratidine, 3-OH Desloratidine following a single oral administration of a 5mg Desloratidine tablets in healthy human volunteers.

Keywords: Desloratidine, 3-OH Desloratidine, Time and Flow Gradient, SPE, UPLC-MS/MS, Human plasma.

INTRODUCTION

Desloratidine is a tricylic antihistamine which is a selective peripheral histamine H1-antagonist and also antagonist to subtypes of muscarinic acetylcholine receptor. It is an active and major metabolite of Loratidine with better efficacy as compared to Loratidine [1] [Figure-1]. Desloratidine is used as medicine for treatment of allergic symptoms by blocking the histamine activity [2].

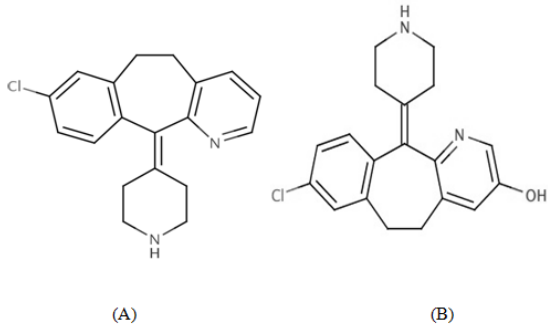


Fig. 1: Structure of Desloratidine (A) and 3-OH Desloratidine (B)

Desloratidine (8-chloro-6, 11-dihydro- 11-(4-piperdinylidene)-5H-benzo [5, 6]-cyclohepta-[1, 2-b] pyridine) is extensively metabolized to 3-hydroxy desloratidine (an active metabolite) which is subsequently glucuronidated and excreted from the body. The Pharmacokinetics of 5 mg dosage of Desloratidine is found to be equivalent to 10 mg of Loratidine in efficacy with a maximum concentration (C_{max}) of about 4000 pg/mL and time of maximum concentration (T_{max}) between 3-4 hours. [3]

Many analytical methods have been developed for both in-vitro, in-vivo analysis, determination in animal model plasma of rat and dog plasma for desloratidine which includes Spectrophotometeric, Spectroflurometric (derivatization with 2,4 DNP) techniques (UV) and HPLC techniques with the lowest limit of quantification (LLOQ) greater than 100ng [4,5]. The use of HPLC coupled with Mass Spectrometer for quantification of desloratidine and its metabolites for performing pharmacokinetic study in healthy volunteers for a dossier of 10mg formulation [6]. The use of two separation techniques using HPLC & UPLC instruments were done by *Shen JX etal*, using different time gradient chromatography separation wherein the use of sub-2µ column of ACQUITY – Waters had reduced the run time without compromising on the resolution between two metabolites[7].

In sample preparation extraction techniques using liquid-liquid extraction(LLE), Solid phase extraction(SPE) with ion-exchange sample preparation were developed by scientists around the world [8]. On review it is understood that better sample clean up and high extraction recovery was observed in SPE extraction technique [9]. Micro-extraction using Hybrid SPE technique with Zirconium coated direct sample precipitation and passing through the Hybrid-SPE cartridges and using 96 well SPE Plate extractions [10, 11] for low sample volume and with high throughput have been developed [12]. In none of the articles a proper resolution between desloratidine and 3-OH desloratidine could be achieved and the main focus of this article was to obtain a proper resolution.

This paper describes development and validation of an analytical method for the quantification of desloratidine and 3-OH desloratidine in human plasma. The use of simultaneous time and flow gradient technique ensured a good resolution of about 0.20 minute between desloratidine and 3-OH desloratidine is uniqueness of this as compared with the earlier published literature [Table-1].

Table 1: Comparison of the current established method against available analytical methods developed for estimation of Desloratidine and 3-OH Desloratidine in biological matrix

S. No.	Biological Matrix (processing volume)	Extraction Procedures	Chromatographic Separation	Resolution between DES & 3-OH DES	Analytical run time	LOQ	Detection technique	Reference
1	Plasma (1mL)	Derivatization - SPE	Isocratic	No	8 min	20ng/mL	UV detector	[4]
3	Plasma (250µl)	SPE-Micro Elution-Ion Exchange	Gradient (Sub-2µ column)	0.11 min	4.50 min	25pg/mL	UPLC-MS/MS	[7]
3	Plasma (0.5mL)	SPE	Isocratic	No	5 min	100pg/mL	LC-MS/MS	[8]
4	Plasma (300µL)	Hybrid-SPE-PPT	Isocratic	MNQ	15 min	(*)	LC-MS/MS	[9]
5	Plasma (500µl)	SPE-Micro elution	Gradient	0.16 min	4.00 min	25pg/mL	LC-MS/MS	[10]
6	Plasma	LLE	Gradient	MNQ	10 min	25pg/mL	LC-MS/MS	[12]
7	Plasma (500µL)	SPE	Gradient (flow and time)	0.18 min	3.00 min	50pg/mL	UPLC-MS/MS	Present method

LLE- liquid-liquid extraction; SPE- solid-phase extraction; LOQ- lower limit of quantification, DES-Desloratidine; 3-OH DES- 3-Hydroxy Desloratidine; * - Not available; MNQ- Metabolite not Quantified.

The use of Ultra Performance Liquid Chromatography instrument as a front end has enabled a shorter run time of 3.00 minutes. Sample extraction using solid phase extraction technique had a better sample clean extraction having no matrix effect and plasma extraction recovery of >98% shows an added advantage to the other commonly employed techniques with a lowest limit of quantification (LLOQ) 50 pg/mL for both desloratidine and 3-OH desloratidine.

MATERIAL AND METHODS

Chemicals and reagents

The working standards of Desloratidine, Desloratidine D5, 3-OH Desloratidine and 3-OH Desloratidine D4 were procured from Clearsynth (Mumbai, India). High purity water was prepared in-house using a Milli-Q water purification system obtained from Millipore (Bangalore, India). Gradient grade methanol and acetonitrile were purchased Merck, Germany. Ammonium acetate (AR grade) and Formic acid (MS grade) was purchased from Merck. Drug free (blank) buffered human plasma was obtained from Drug Monitoring Research Institute (Mumbai, India).

Calibration curves

Stock solutions of Desloratidine, Desloratidine D5, 3-OH Desloratidine and 3-OH Desloratidine D4 were prepared at concentration of 100 µg/mL in 50% methanol:water (v/v) mixture as diluent. Spiking standard solutions were prepared in serial dilution from stock solutions using same diluent. Independent stocks were weighed for preparation of Calibration Curve (CC) standards and Quality Control (QC) samples. These spiking standard solutions were used to prepare the calibration curve and quality control (QC) samples in human plasma.

A nine-point standard calibration curve for desloratidine and 3-OH desloratidine were prepared by spiking 2% spiking standard solution into interference free blank plasma. The calibration curve ranged from 50-10000 pg/mL for desloratidine and 3-OH desloratidine. Quality control samples were prepared at four concentration levels: 51.621 & 51.684 pg/mL for Lowest Limit of Quantification Quality Control (LLOQQC), 150.705 & 150.990 pg/mL for Low Quality Control (LQC), 4075.890 & 4080.818 pg/mL for Medium Quality Control (MQC) and 7838.189 & 7847.426 pg/mL for High Quality Control (HQC) levels for both desloratidine and 3-OH desloratidine in a manner similar to the standard from the stock solution.

Sample preparation

A volume of 500 µL aliquot of desloratidine and 3-OH desloratidine spiked human plasma sample was mixed with 50 µL of internal standard working solution (50 ng/mL & 30 ng/mL of desloratidine D5 and 3-OH desloratidine D4 respectively). An equal volume of 2% Ortho-Phosphoric acid was added and vortexed to mix. Sample mixture was loaded onto a Strata-X (1 cm^3/30 mg), extraction cartridge which was pre-conditioned with 1 mL methanol and equilibrated by 1 mL water. The extraction cartridge was washed with 1 mL water followed by 1 mL of 20% methanol in water. Desloratidine and 3-OH desloratidine were eluted with 1 mL of elution solution (Acetonitrile: Methanol: 10 mM-Ammonium acetate (pH~3.50)-45:45:10 (v/v/v) under low pressure.

Liquid Chromatography & MS Parameters

Desloratidine and 3-OH desloratidine were analyzed using XevoTQ-S mass spectrometer, a triple stage quadrupole mass analyzer with photo multiplier detector, equipped with Electrospray ionization (ESI) source (Waters Ltd. UK). Chromatographic separation was done using Hypurity Advance 50x4.6mm, 5µ of (Thermo Ltd. UK). Mobile phase consisting of methanol-acetonitrile (1:3, v/v)-[Pump –A] and 10 mM ammonium acetate (pH ~3.5 ± 0.05) in water-[Pump-B]. Gradient elution was run starting with an initial condition of 40% of A and 60% of B for 0.1 minute, later the gradient of flow was changed from 0.60-0.90ml/min and simultaneously the mobile phase flow composition was linearly changed to 60% of A and 40% of B upto 1.2 minutes, the same composition was maintained for 2.0 minutes, later the initial composition set for column at 2.5 minute and was maintained upto 3.0 minutes (Table-2a).

Table 2a: Gradient elution program of Chromatographic Separation

Step	Time	Flow rate (mL/min)	Mobile Phase A	Mobile Phase Pump B
0	0.00	0.600	40	60
1	0.10*	0.600	40	60
2	1.20*	0.900	60	40
3	2.00	0.900	60	40
4	2.50	0.600	40	60
5	3.00	0.600	40	60

*Simultaneous time and flow gradient

The use of simultaneous gradient change in flow and composition has enabled a good resolution between desloratidine and 3-OH desloratidine. The column oven temperature was kept 40 ºC. The total run time for each sample analysis was 3 min. The injection volume was 5 µL and partial needle with needle overfill mode. The mass spectrometer was operated in the selected reaction-monitoring (SRM) mode. Sample introduction and ionization was ESI in the positive ion mode. The source and compound parameters of the MS for all analytes are listed in Table-2b. The data acquisition was ascertained by MassLynx 4.1 software.

Table 2b: Ion source and compound dependent parameters

Ion source				
Capillary voltage	3.50 kV			
Source temperature	150ºC			
Desolvation Temperature	550ºC			
Desolvation gas flow	1100 L/Hr			
Cone gas flow	150 L/Hr			
Polarity mode	Positive			
Compound dependent parameters				
	Desloratidine	**Desloratidine D5**	**3-OH Desloratidine**	**3-OH Desloratidine D4**
Precursor ion (m/z)	311.1	316.2	327.3	331.1
Product ion (m/z)	259.2	264.2	275.2	279.2
Cone voltage (V)	55	55	55	55
Q1-HM / LM* (amu)[a]	3.00	3.00	3.00	3.00
Q3-HM / LM* (amu)[b]	15.00	15.00	15.00	15.00
Collision energy	25	29	25	21

[a] Quadrupole 1 High and Low Mass resolution parameters [b] Quadrupole 3 High and Low Mass resolution parameters

Method Performance Assessment

The method has been validated for specificity, selectivity, sensitivity, linearity, precision, accuracy, recovery, dilution integrity, stability and matrix effect meeting the global regulatory requirements [13, 14]. Specificity was performed by analyzing the blank plasma samples from eight different sources (or donors) to test for interference at the retention time of desloratidine, 3-OH desloratidine, desloratidine D5 and 3-OH desloratidine D4. Selectivity was evaluated for each individual analyte and internal standard individually and for the most common concomitant medications which include Ranitidine, Paracetamol, Ibuprofen and Aspirin. Sensitivity was determined by analyzing six replicates of lowest limit of quantification (LLOQ) spiked in interference free blank plasma. The Linearity of the calibration standards were assessed using six calibration curves used for assay validation. The inter-run and intra-run precision and accuracy was determined by replicate analysis of HQC, MQC, LQC & LLOQQC levels.

Accuracy is determined as the percent of %Bias and was calculated using the formula $\%bias = (E-T)\times 100/T$ where 'E' is the experimentally determined concentration and 'T' is the theoretical concentration. Assay precision was calculated by using the formula $\%CV = (SD/M)\times 100$ where %CV is percent of coefficient of variation, 'M' is the mean of experimentally determined concentrations and 'SD' is the standard deviation of M.

The recovery of desloratidine, desloratidine-D5, 3-OH desloratidine and 3-OH desloratidine-D4 were determined by comparing the peak area of extracted samples at HQC, MQC & LQC levels to the peak area of post spiked equivalents.

The stability experiments were carried out at HQC, MQC & LQC levels (n=6). Stability was determined by calculating the percentage stability (%Stability) which is calculated using the formula $\%STB = (S/C)x100$ where 'S' is the mean back calculated concentration of stability samples and 'C' is the mean back calculated concentration of freshly prepared (comparison) samples. Stability was considered acceptable if the %CV was < 15% and %STB was between 85-115%.

Bench-top stability was evaluated for a minimum period of about 6 hours at room temperature and compared against freshly prepared samples. The process stability was evaluated by comparing the extracted plasma samples stored in the auto sampler at 10°C for about 3 days against the freshly prepared samples. The freeze-thaw stability was conducted by comparing stability samples (5 cycles) against the freshly spiked quality control samples. The long duration stability for a period of about 55 days was evaluated for samples stored at -20°C, -30°C and -75°C by comparing against the freshly prepared quality samples.

Matrix effect was evaluated with eight different lots of plasma containing K_2EDTA as anticoagulant including hemolysed and lipemic plasma matrices. Three post spiked samples each of LQC, MQC and HQC were prepared from different lots of plasma (in total 24 samples). Pure aqueous equivalent samples for desloratidine, desloratidine-D5, 3-OH desloratidine and 3-OH desloratidine D4 at LQC, MQC and HQC level were prepared in elution solution considering zero matrix effect. The post spiked extracted LQC, MQC and HQC samples along with six replicate injections of aqueous spiked samples of LQC, MQC and HQC were analyzed. The matrix effect was evaluated by calculating the matrix factor for desloratidine, desloratidine-D5, 3-OH desloratidine and 3-OH desloratidine D4, for analyte area, IS area and IS normalized area ratio. This was performed with the aim to evaluate the matrix effect of different lots of plasma on the %CV for mean area response and mean area ratio. It is considered there is no matrix effect if the %CV for mean area and mean response ratio is less then 15%.

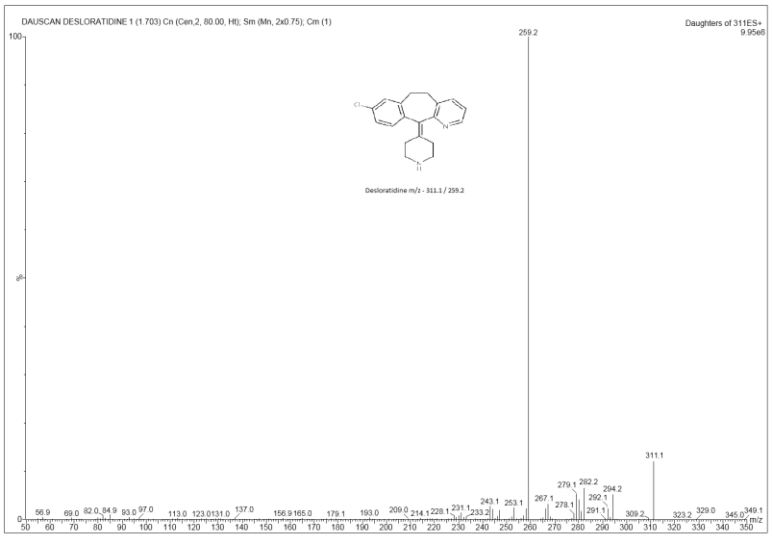
RESULTS AND DISCUSSION

Tuning and Optimization of Mass Parameters

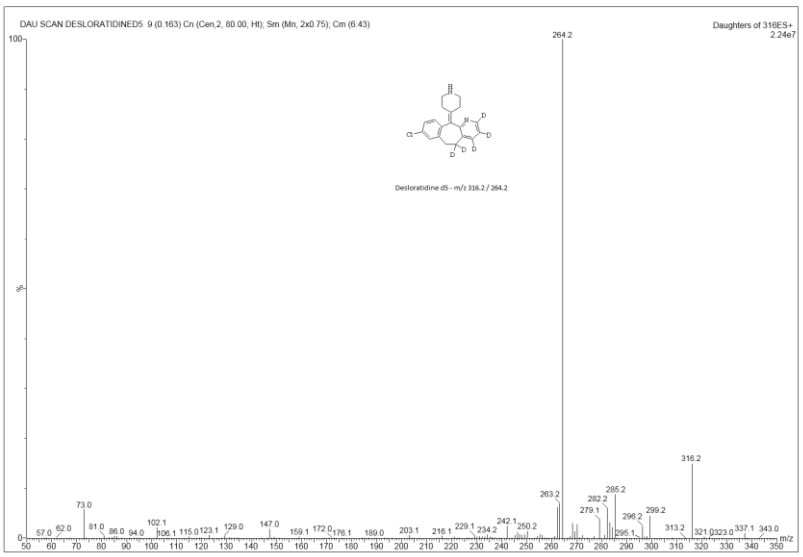
For performing the compound mass parameters optimization, a tuning stock dilutions of about 50 ng/mL concentration of each analyte desloratidine, 3-OH desloratidine and their respective internal standards were prepared and tuned in auto-tune mode and later each of these parameters have been reconfirmed manually. The positive ionization mode has shown better signal and the cone and collision energy for each of the compounds have been optimized. The optimized mass-to-charge transitions (*m/z)* were 311.1 → 259.2, 316.2 → 264.2, 327.3 → 275.2, and 331.4 → 279.2 for monitoring desloratidine, desloratidine D5, 3-OH desloratidine and 3-OH desloratidine D4 respectively. Electro spray ionization (ESI) and Atmospheric pressure chemical ionization (APCI) were evaluated to achieve better signal with consistency and low baseline noise wherein a good signal with low baseline noise was achieved with ESI positive ion mode. The selected fragments of each compound, as product ion to be monitored are indicated in (Figure-2).

Further Liquid chromatography conditions were initiated with isocratic separation but the retention of all the compounds was found to be same, hence to have a good resolution between the desloratidine and its metabolite, linear gradient elution methods have been used but a proper resolution could not be achieved. The columns of C_8, C_{18} and sub-2µm narrow bore columns have been used wherein the peak shape, Signal-to-Noise (S/N) and low baseline noise could be achieved but there was no resolution between the desloratidine and 3-OH desloratidine. Later the use of simultaneous time and flow gradient flow was tried wherein a well resolved chromatographic separation could be achieved.

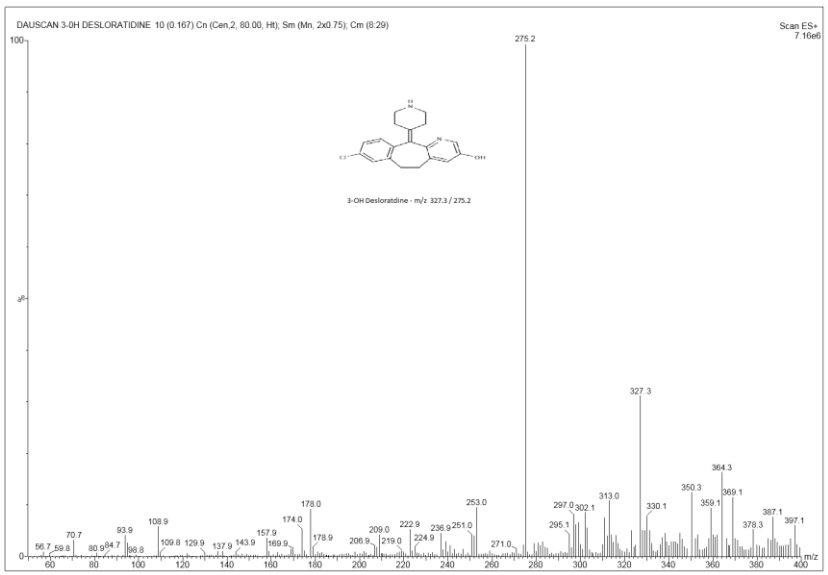
Autosampler Carryover was removed by including a weak wash solvent equivalent to the first gradient of the inlet gradient of the liquid chromatography program. It is observed that in neutral buffer pH resulted with a mixture of methanol and acetonitrile has improved response and peak symmetry. The use of Hypruity C_{18} (50 x 4.6 mm id, 5 µ) column enabled use of high flow rate, which resulted in run time as low as 3 minutes with better peak symmetry and signal of analytes.


DAUSCAN DESLORATIDINE 1 (1.703) Cn (Cen,2, 80.00, Ht); Sm (Mn, 2x0.75); Cm (1)
Daughters of 311ES+
9.95e6
259.2
Desloratidine m/z - 311.1 / 259.2
311.1
%
m/z

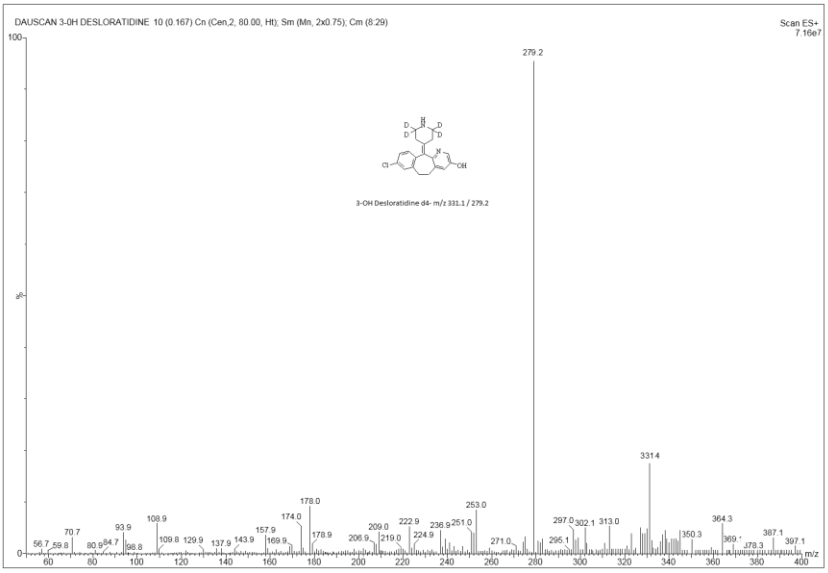
(A)


DAU SCAN DESLORATIDINED5 9 (0.163) Cn (Cen,2, 80.00, Ht); Sm (Mn, 2x0.75); Cm (6:43)
Daughters of 316ES+
2.24e7
264.2
Desloratidine d5 - m/z 316.2 / 264.2
316.2
%
m/z

(B)


DAUSCAN 3-0H DESLORATIDINE 10 (0.167) Cn (Cen,2, 80.00, Ht); Sm (Mn, 2x0.75); Cm (8:29)
Scan ES+
7.16e6
275.2
3-OH Desloratidine - m/z 327.3 / 275.2
327.3
%
m/z

(C)



(D)

Fig. 2: Parent ion scan of Desloratidine (A), Deslortidine-D5 (B), 3-OH Desloratidine (C), 3-OH Desloratidine-D5 (D)

Sample extraction was initially evaluated with the protein precipitation and LLE technique, but the sample extraction recovery was very low. The use of SPE extraction was found to better in sample cleanup, with a better sample extraction recovery. The optimization of elution solution was critical phase in the SPE extraction optimization and the use of mixture of 5 mM ammonium acetate(pH~3.5), acetonitrile and methanol as elution solvent has ensured sample extraction from matrix of about 99% for all analytes. The time consuming and error-prone solvent evaporation and reconstitution steps were avoided and a direct elution procedure has shown a clean sample with low sample extraction process duration ready for analysis. The optimized detection, sample extraction chromatography enabled to reduce processing and analysis time without compromising the sensitivity.

Specificity

Specificity was demonstrated by the absence of interfering peaks at the retention times of desloratidine, desloratidine D5, 3-OH desloratidine and 3-OH desloratidine D4 in all the eight different lots of extracted blank plasma [Figure-3]. Representative chromatograms of extracted blank plasma, extracted plasma sample containing 50pg/mL each of desloratidine and 3-OH desloratidine (low standard) are presented [Figure-4].

Linearity

The peak area ratio (Area of drug /Area of internal standard) of calibration standards were proportional to the concentration of analytes in each assay over the nominal concentration range of 50.618-10048.161 pg/mL for desloratidine and 50.672-10058.788 pg/mL for 3-OH desloratidine. The calibration curves appeared linear and were well described by least squares lines. A linear regression weighing factor of 1/concentration^2 was chosen to achieve homogeneity of variance. The correlation coefficients were ≥0.99 (n=6) for both desloratidine and 3-OH desloratidine. The mean (± SD) slopes of the calibration curves (n=6) were 0.000302885(± 0.000005674) and 0.000234247(± 0.000005013) for desloratidine and 3-OH desloratidine respectively.

Sensitivity (lower limit of quantification)

Sensitivity was determined at LOQ concentration by evaluating accuracy (%bias) and precision (%CV). The LOQ for desloratidine and 3-OH desloratidine were 50.618 and 50.672pg/ml respectively. The precision and accuracy at the LOQ concentration (n=6) samples for desloratidine and 3-OH desloratidine were 4.44% & -10.27% and 6.24 & -15.27% respectively.

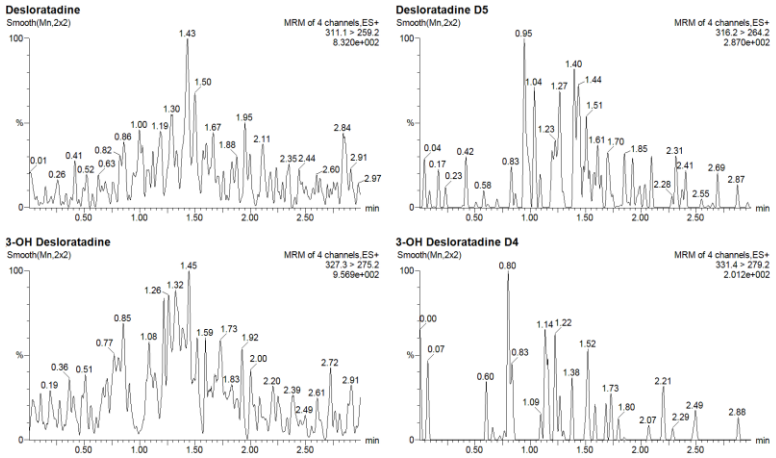


Fig. 3: A representative chromatogram of extracted blank plasma

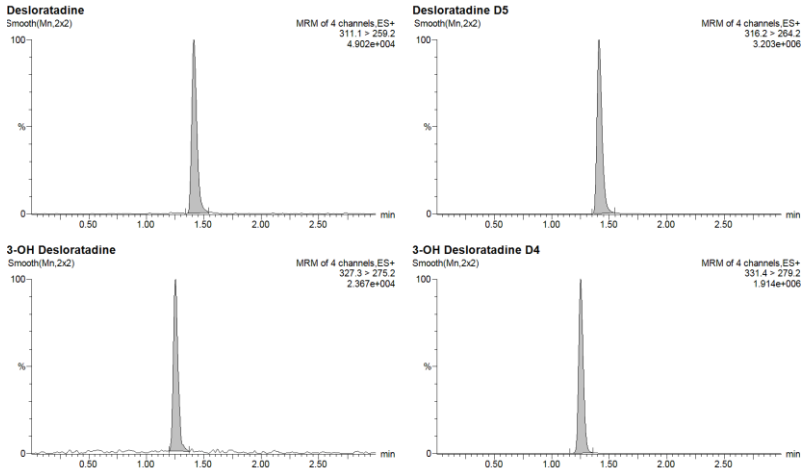


Fig. 4: A representative chromatogram of extracted LLOQ standard

Precision and Accuracy

Intra-run precision (%CV) at HQC, MQC and LQC level ranged from 2.05% to 5.72% and at LLOQQC level it was 5.46% for desloratidine. The intra-run precision (%CV) at HQC, MQC and LQC levels ranged from 2.33% to 7.82% and at LLOQQC it was 3.82% for 3-OH desloratidine.

The intra-run accuracy (%bias) at HQC, MQC and LQC levels ranged from -6.56% to 2.03% and at LLOQC level it was -2.15% for desloratidine. The intra-run accuracy (%bias) at HQC, MQC and LQC levels ranged from 0.67% to -9.86% and at LLOQC level it was -9.037% for 3-OH desloratidine (Table 3a & 3b).

The inter-run precision (%CV) at all QC levels ranged from 1.62% to 4.90% and inter-run accuracy (%bias) for all QC samples ranged from -5.54% to 2.55% for desloratidine.

The inter-run precision (%CV) at all QC levels ranged from 2.05% to 5.93% and inter-run accuracy (%bias) for all QC samples ranged from -8.04% to -0.48% for 3-OH desloratidine (Table-4a & 4b).

Table 3a: Intra-run(within-batch) precision and accuracy of Desloratidine in human plasma (n=6)

QC Level	Spiked concentration (pg/mL)	Mean (+SD) Calculated concentration (pg/mL)	%CV	%bias
LLOQQC	51.621	50.513 (2.756)	5.46	-2.15
LQC	150.807	140.913 (8.054)	5.72	-6.16
MQC	4075.859	4031.395 (82.806)	2.05	-1.09
HQC	7838.189	7997.099 (452.455)	5.66	2.03

Table 3b: Intra-run(within-batch) precision and accuracy of 3-OH Desloratidine in human plasma (n=6)

QC Level	Spiked concentration (pg/mL)	Mean (+SD) Calculated concentration (pg/mL)	%CV	%bias
LLOQQC	51.684	47.016 (1.798)	3.82	-9.03
LQC	150.990	136.096 (10.641)	7.82	-9.86
MQC	4080.818	3959.168 (92.129)	2.33	-2.98
HQC	7847.726	7795.426 (408.317)	5.24	-0.67

Table 4a: Inter-run(between-batch) precision and accuracy of Desloratidine in human plasma (n=24)

QC Level	Spiked concentration (pg/mL)	Mean (+SD) Calculated concentration (pg/mL)	%CV	%bias
LLOQQC	51.621	49.419 (2.424)	4.90	-4.27
LQC	150.807	142.454 (5.003)	3.51	-5.54
MQC	4075.859	4019.873 (65.123)	1.62	-1.37
HQC	7838.189	8037.983 (259.163)	3.22	2.55

Table 4b: Inter-run(between-batch) precision and accuracy of 3-OH Desloratidine in human plasma (n=24)

QC Level	Spiked concentration (pg/mL)	Mean (+SD) Calculated concentration (pg/mL)	%CV	%bias
LLOQQC	51.684	48.081 (2.851)	5.93	-6.97
LQC	150.990	139.851 (6.315)	2.92	-8.04
MQC	4080.818	3922.832 (80.337)	2.05	-3.87
HQC	7847.726	7809.864 (283.574)	3.63	-0.48

The inter- and intra-run precision and accuracies were determined by pooling all individual assay results of replicate (n=24) QC samples over the four individual analytical runs.

Recovery

Six replicates at LQC, MQC, HQC concentrations for desloratidine and 3-OH desloratidine alongwith their internal standards were prepared for recovery determination. The mean recoveries were 98.72% and 98.35% with precision (%CV) of 0.22% & 0.44% for desloratidine and 3-OH desloratidine. The mean recoveries were 101.13% & 100.64% for desloratidine D5 and 3-OH desloratidine D4.

Stability

The results of the stability studies are enumerated in Table 5a, 5b. The bench top stability, process stability, freeze-thaw stability and long-term stability of desloratidine and 3-OH desloratidine in plasma were

investigated by analyzing quality control samples in replicates (n=6) at LQC, MQC and HQC levels for each stability experiment. Bench top stability results allowed us to conclude that desloratidine and 3-OH desloratidine are stable for 6 hours at room temperature in plasma samples. Process stability results indicated that the processed samples stored at 10 °C in autosampler are stable for period of about 75 hours. Freeze and thaw stability results indicated that the repeated freezing and thawing (five cycles) did not affect the stability of desloratidine and 3-OH desloratidine stored in -30 °C and -75 °C. Long-term stability of desloratidine and 3-OH desloratidine in plasma at 20 °C, -30 °C and -75 °C were performed at LQC, MQC and HQC levels; it was found to be stable for at least 55 days.

Table 5a: Stability experiment results for Desloratidine (n=6) for each QC level

Stability	QC level	Mean(±SD) obtained concentration		Percentage Stability* (%)
		Comparison samples (pg/mL)	Stability samples (pg/mL)	
Bench Top [a]	HQC	8073.342 (± 54.207)	8055.681(± 62.808)	99.78
	MQC	3959.484 (± 80.126)	4004.205(± 85.385)	101.12
	LQC	139.170 (± 2.134)	138.476(± 2.705)	99.50
Process stability [b]	HQC	8706.663 (± 111.508)	7898.955(± 434.043)	90.72
	MQC	4248.220 (± 53.986)	3985.122(± 59.513)	93.81
	LQC	157.263 (± 1.276)	147.673(± 6.821)	93.90
Freeze / Thaw [c1]	HQC	7740.505 (± 78.043)	7734.598(± 110.20)	99.92
	MQC	3959.444 (± 42.012)	3925.528(± 68.024)	99.14
	LQC	155.138 (± 1.216)	155.216(± 3.440)	100.05
Freeze / Thaw [c2]	HQC	7740.505 (± 78.043)	7751.624(± 62.712)	100.14
	MQC	3959.444 (± 42.012)	3920.336(± 93.093)	99.01
	LQC	155.138 (± 1.216)	153.821(± 4.561)	99.15
Long term [d1]	HQC	8538.359 (± 57.097)	8586.016(± 20.022)	100.67
	MQC	4257.185 (± 27.183)	4243.712(± 28.923)	99.79
	LQC	155.404 (± 2.371)	153.407(± 2.583)	98.82
Long term [d2]	HQC	8538.359 (± 57.097)	8531.271(± 41.313)	100.03
	MQC	4257.185 (± 27.183)	4281.832(± 28.587)	100.69
	LQC	155.404 (± 2.371)	152.150(± 2.543)	98.01
Long term [d3]	HQC	8538.359 (± 57.097)	8551.350(± 44.408)	100.26
	MQC	4257.185 (± 27.183)	4284.741(± 20.035)	100.76
	LQC	155.404 (± 2.371)	153.235(± 2.273)	98.71

** - %Stability – Determined by calculating the percentage of mean stability QC concentration against mean comparison QC concentrations*.

[a] After 6 hours at room temperature

[b] After 75 hours in autosampler at 10 ºC

[c1] After five freeze/thaw cycles at -30 ºC

[c2] After five freeze/thaw cycles at -75 ºC

[d1] -30ºC for 55 days

[d2] -75ºC for 55 days

[d3] -20ºC for 55 days

Table 5b: Stability experiment results for 3-OH Desloratidine (n=6) for each QC level

Stability	QC level	Mean(±SD) obtained concentration		Percentage Stability* (%)
		Comparison samples (pg/mL)	Stability samples (pg/mL)	
Bench Top [a]	HQC	8160.022 (± 163.266)	8103.850(± 79.511)	99.31
	MQC	3992.529 (± 74.694)	3968.537(± 72.039)	99.40
	LQC	143.383 (± 5.592)	141.939(± 5.560)	98.99
Process stability [b]	HQC	8591.248 (± 93.803)	7909.804(±435.518)	92.07
	MQC	4152.887 (± 70.696)	3991.371(± 55.798)	96.11
	LQC	144.998 (± 2.549)	138.575(± 12.105)	95.57
Freeze / Thaw [c1]	HQC	7657.213 (± 129.547)	7622.081(± 68.190)	99.54
	MQC	3862.021 (± 67.029)	3940.623(± 69.983)	102.04
	LQC	151.483 (± 5.203)	154.357(± 3.593)	101.9
Freeze / Thaw [c2]	HQC	7657.213 (± 129.547)	7656.861(± 60.171)	100.00
	MQC	3862.021 (± 67.029)	3884.098(± 74.200)	100.57
	LQC	151.483 (± 5.203)	152.501(± 5.414)	100.67
Long term [d1]	HQC	8664.331 (± 44.188)	8634.420(± 56.637)	99.34
	MQC	4454.248 (± 33.823)	4447.450(± 18.373)	99.53
	LQC	161.813 (± 1.634)	160.789(± 0.884)	99.05
Long term [d2]	HQC	8664.331 (± 44.188)	8657.036(± 79.618)	99.60
	MQC	4454.248 (± 33.823)	4445.151(± 15.652)	99.48
	LQC	161.813 (± 1.634)	158.926(± 3.467)	97.90
Long term [d3]	HQC	8664.331 (± 44.188)	8652.125(± 47.895)	99.54
	MQC	4454.248 (± 33.823)	4441.711(± 22.576)	99.40
	LQC	161.813 (± 1.634)	159.412(± 3.469)	98.20

** - %Stability – Determined by calculating the percentage of mean stability QC concentration against mean comparison QC concentrations*.

[a] After 6 hours at room temperature

[b] After 75 hours in autosampler at 10 ºC

[c1] After five freeze/thaw cycles at -30 ºC

[c2] After five freeze/thaw cycles at -75 ºC

[d1] -30ºC for 55 days

[d2] -75ºC for 55 days

[d3] -20ºC for 55 days

Matrix effect

The matrix factor for desloratidine, 3-OH desloratidine, desloratidine-D5 and 3-OH desloratidine-D4 were calculated by comparing the area response observed in post spiked samples with that of unextracted samples at LQC, MQC and HQC level. The matrix effect was evaluated from the %CV of matrix factor at each level. Three quality control samples at each level were analyzed and the mean of %CV of the samples analyzed was found less than 15% for each QC level for desloratidine, 3-OH desloratidine, desloratidine D5 and 3-OH desloratidine D4 [Table 6a, 6b].

Application of Method

The validated analytical method was applied to clinical samples for the determination of desloratidine and 3-OH desloratidine in plasma samples from two formulations of desloratidine in normal, adult, healthy male subjects under fasting condition. Plasma samples were periodically collected up to 144 hours after an oral dose administration of a 5 mg tablet. A representative chromatogram of the healthy subject has been attached for reference (Figure-5).

The set calibration curve range from 50-10000pg/mL for desloratidine and 3-OH desloratidine, were sufficient covering the concentrations of the unknown sample concentrations. The bioequivalence between two formulations was assessed by pharmacokinetic evaluation of the treatment wise ratios of test/reference for the parameters which include maximum concentration (C_{max}), area under the curve ($AUC_{(0-t)}$ and $AUC_{(0-inf)}$. The present method has sufficed to obtain complete pharmacokinetic profile for desloratidine and its 3-OH desloratidine, which indicate the suitability of the analytical method for pharmacokinetic study.

Table 6a: Matrix effect (n=8) for Desloratidine and Desloratidine D5

S. No.		HQC			MQC			LQC		
	Parameters	**Mean Matrix Factor**	**SD**	**%CV**	**Mean Matrix Factor**	**SD**	**%CV**	**Mean Matrix Factor**	**SD**	**%CV**
1	Analyte Area	1.056	0.027	2.56	1.084	0.032	2.95	1.073	0.038	3.54
2	IS Area	1.050	0.047	4.48	1.080	0.056	5.19	1.069	0.049	4.58
3	IS Normalized	1.057	0.029	2.74	1.083	0.036	3.32	1.074	0.033	3.07

Table 6b: Matrix effect (n=8) for 3-OH Desloratidine and 3-OH Desloratidine D4

S. No.	**Parameters**	HQC			MQC			LQC		
		Mean Matrix Factor	**SD**	**%CV**	**Mean Matrix Factor**	**SD**	**%CV**	**Mean Matrix Factor**	**SD**	**%CV**
1	Analyte Area	1.052	0.030	2.85	0.999	0.050	5.01	1.000	0.067	6.70
2	IS Area	1.032	0.060	5.81	0.998	0.030	3.01	1.057	0.065	6.15
3	IS Normalized	1.022	0.046	4.50	1.001	0.040	4.00	0.947	0.036	3.80

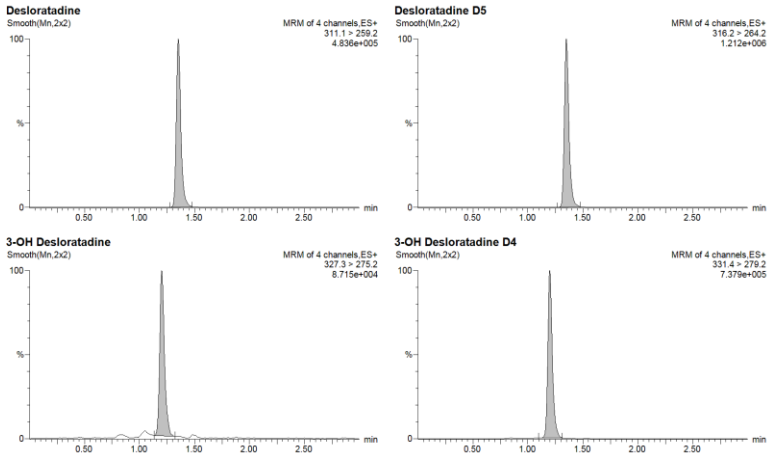


Fig. 5: A representative chromatogram of extracted subject sample

CONCLUSIONS

A simple, specific, rapid and sensitive LC-MS/MS method has been developed for the determination of desloratidine and 3-OH desloratidine in human plasma. The use of simultaneous time and flow gradient application using the ultra performance liquid chromatography as front end, the use of dual gradient separation technique can be applied to resolve the peaks which may be chemically similar peaks but not resolved with normal gradient separation. The validated method provided excellent specificity and reproducibility with a limit of quantification of 50pg/mL for desloratidine and 3-OH desloratidine which was sufficient for the Pharmacokinetic evaluation of both the compounds.

It is concluded that this sensitive and specific method is applicable for the quantitative determination of desloratidine and 3-OH desloratidine in human plasma in pharmacokinetic and bioavailability studies of desloratidine.

ACKNOWLEDGEMENTS

Authors would like to thank Lupin Limited, India for the technical support provided. This paper is part of research work for the grant of doctorate in philosophy in science from Pacific University, Rajasthan, India.

REFERENCES

1. Glass D, Harper A. Assessing patient satisfaction with desloratadine after conversion from loratadine, fexofenadine, or cetirizine. Manag Care Interface. 2004 Feb;17(2):29-34.
2. Schroeder JT, Schleimer RP, Lichtenstein LM, Kreutner W. Inhibition of cytokine generation and mediator release by

human basophils treated with desloratadine. Clin Exp Allergy. 2001 Sep;31(9):1369-77.

3. Molimard M, Diquet B, Benedetti MS. Comparison of pharmacokinetics and metabolism of desloratadine, fexofenadine, levocetirizine and mizolastine in humans. Fundamental & Clinical Pharmacology, Vol. 18 (2004) 399.
4. El-Enany N, El-Sherbiny D, Belal F. Spectrophotometric, spectrofluorometric and HPLC determination of desloratadine in dosage forms and human plasma. Chem Pharm Bull (Tokyo). (2007) 1662.
5. Zheng J, Rustum AM. Rapid separation of desloratadine and related compounds in solid pharmaceutical formulation using gradient ion-pair chromatography. J Pharm Biomed Anal. (2010) 146.
6. Shen JX, Wang H, Tadros S, Hayes RN. Orthogonal extraction/chromatography and UPLC, two powerful new techniques for bioanalytical quantitation of desloratadine and 3-hydroxydesloratadine at 25 pg/mL. Pharm Biomed Anal. (2006) 689.
7. Lu XY, Sheng-Tu JZ, Chen ZG, Huang MZ, Zhou HL, Zheng GG.(translated version). [Study on determination of desloratadine in human serum and its pharmacokinetics by HPLC/MS]. Zhejiang Da Xue Xue Bao Yi Xue Ban. (2005) 372.
8. Hasnain MS, Rao S, Singh MK, Vig N, Singh MK, Budakoti SK et al. Development and validation of an improved LC-MS/MS method for the quantification of desloratadine and its metabolite in human plasma using deutrated desloratadine as internal standard. J Pharm Bioallied Sci. (2013) 74.
9. Craig Aurand and An Trinh. Selective Depletion of Phospholipids in Bioanalysis using Hybrid SPE-PPT Technology. Chromatography Today,(2009) 38.
10. Yang L, Clement RP, Kantesaria B, Reyderman L, Beaudry F, Grandmaison C, et al. Validation of a sensitive and automated 96-well solid-phase extraction liquid chromatography-tandem mass spectrometry method for the determination of desloratadine and 3-hydroxydesloratadine in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci. (2003) 229.
11. Xu, H.R., Li, X.N., Chen, W.L., Chu, N.N. Simultaneous determination of desloratadine and its active metabolite 3-hydroxydesloratadine in human plasma by LC/MS/MS and its application to pharmacokinetics and bioequivalence. Journal of Pharmaceutical and Biomedical Analysis, Vol. 45, No. 4, pages 659-666 (2007).
12. Venkata Suresh Ponnuru, B.R. Challa, Ramarao Nadendla. Quantification of desloratadine in human plasma by LC-ESI-MS/MS and application to a Pharmacokinetic study. Journal of Pharmaceutical Analysis (2012) 180.
13. Guidance for industry: bioanalytical method validation, US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), May 2001.
14. Guideline on bioanalytical method validation, EMEA/ CHMP/EWP/192217/2009, Committee for Medicinal Products for Human Use (CHMP) (2011).