Journal of Pharmaceutical and Biomedical Analysis 51 (2010) 210–216

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Simultaneous measurement of amiodarone and desethylamiodarone in human plasma and serum by stable isotope dilution liquid chromatography–tandem mass spectrometry assay

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ARTICLE INFO

Article history:
Received 1 April 2009
Received in revised form 29 July 2009
Accepted 3 August 2009
Available online 12 August 2009

Keywords:
Amiodarone
Desethylamiodarone
Drug monitoring
Liquid chromatography
Mass spectrometry
Stable isotope dilution liquid chromatography–tandem mass spectrometry

ABSTRACT

A stable isotope dilution liquid chromatography–electrospray ionization tandem mass spectrometry (LC–MS/MS) assay to measure amiodarone, the most frequently used agent for maintaining sinus rhythm in patients with atrial fibrillation, and its major metabolite desethylamiodarone in human plasma and serum was developed. Measurement of amiodarone and desethylamiodarone was performed during a 4.0-min run-time using amiodarone-D_4 and desethylamiodarone-D_4 as internal standards. Calibration curves covering 12 calibrators measured in four replicates each for the analysis of both amiodarone and desethylamiodarone were linear and reproducible in the range of 0.01–40.0 mg/L ($r > 0.999$). Limits of detection in plasma matrix were 2.7 μg/L for amiodarone and 1.9 μg/L for desethylamiodarone, and lower limits of quantification in plasma matrix were 7.5 μg/L for amiodarone and 2.5 μg/L for desethylamiodarone. Interassay imprecision and inaccuracy were <8% and <9% for both substances. Mean extraction yield was 99.6% (range 92.6–107.7%) for amiodarone and 90.2% (range 80.0–94.7%) for desethylamiodarone. Agreement was moderate for amiodarone ($n = 162$) and desethylamiodarone ($n = 117$), respectively, between the present method and a HPLC method with UV detection using a commercially available reagent set for the HPLC analysis of these drugs. The Passing–Bablok regression line was HPLC = 0.98 (LC–MS/MS) + 0.10 [mg/L]; $r = 0.94$ for amiodarone and HPLC = 1.05 (LC–MS/MS) + 0.02 [mg/L]; $r = 0.90$ for desethylamiodarone. This sensitive and interference-free LC–MS/MS assay permits rapid and accurate determination of amiodarone and desethylamiodarone in human plasma and other body fluids.

1. Introduction

Amiodarone (2-butyl-3-benzofuranyl){4-[2-(diethylamino) ethoxy]-3,5-diiodophenyl}methanone, is a Class III antiarrhythmic agent which is often used to treat arrhythmias, especially when other antiarrhythmic drugs are inefficient. It is the most effective drug for maintaining sinus rhythm in patients with atrial fibrillation [1]. In addition, amiodarone controls a broad spectrum of cardiac arrhythmias, ranging from simple symptomatic premature ventricular contractions to continuous ventricular tachycardia and fibrillation and similarly those of supraventricular arrhythmias [2]. Its main mechanism of action is to block myocardial potassium channels, but it also possesses beta-blocking properties [3]. Amiodarone has a long duration of action and a very long serum elimination half-life time, ranging from 30 to 120 days [4]. The typical therapeutic plasma concentration of amiodarone ranges from 0.5 to 2.5 mg/L [5]. Although there is a linear relationship between oral doses of amiodarone and its plasma and myocardial tissue concentrations, the concentrations in patients administrated with the same dose vary markedly. The effectiveness of amiodarone for maintaining sinus rhythm remains unrivalled, but the drug has many side effects, such as hyper- and hypothyroidism, pulmonary fibrosis, peripheral neuropathy, dermatological changes, as well as a lot of further adverse healthy effects which necessitate use of the lowest effective dose of amiodarone, as well as careful monitoring of the blood concentration of the drug [6]. Several methods, such as HPLC assays [7–15], capillary electrophoresis method [16] and LC–MS/MS method [17–19], have been described for the measurement of amiodarone and desethylamiodarone in human plasma. High specificity was reached with the MS/MS technology, but the retention behavior of amiodarone and tamoxifen [17,18] or ethopropazine [19], which were used as the internal standard in the assays were different. Thus different ion suppressions are able to affect amiodarone ions and internal standard ions, resulting in an inexact measurement of the drug. Isotope

Abbreviations: LC–MS/MS, liquid chromatography–tandem mass spectrometry; LOD, limit of detection; LLOQ, lower limit of quantification; PBS, phosphate buffered saline; SPE, solid phase extraction.

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doi:10.1016/j.jpba.2009.08.004

dilution mass spectrometry eliminates this problem because the analyte and its corresponding isotope have the same retention time. Here, we develop for the first time an isotope dilution liquid chromatography–tandem mass spectrometry (LC–MS/MS) method to measure amiodarone and its metabolite desethylamiodarone in serum and plasma with both high precision and specificity.

2. Material and methods

2.1. Reagents, internal standards, calibrators and quality-control samples

HPLC-grade water and methanol were purchased from Fisher Scientific GmbH (Schwerte, Germany). Amiodarone, ammonium acetate, ammonium hydroxide, orthophosphoric acid and formic acid were obtained from Sigma–Aldrich (Deisenhofen, Germany). Desethylamiodarone and desethylamiodarone-D_4 (purity > 99%) were purchased from Synfine Research Inc. (Newkirk, Canada) and amiodarone-D_4 (purity > 98%) was obtained from Medical Isotopes (Pelham, NH).

Stock solution of amiodarone, amiodarone-D_4, desethylamiodarone and desethylamiodarone-D_4, each at a concentration of 1 mg/ml, were prepared separately in methanol and stored at −80 °C. Using drug-free serum, we prepared several calibrators (0.02, 0.04, 0.08, 0.16, 0.31, 0.63, 1.25, 2.50, 5.00, 10.0, 20.0 and 40.0 mg/L of both amiodarone and desethylamiodarone) and in-house quality-control samples (1.00, 3.00, 15.0 mg/L of both amiodarone and desethylamiodarone) for the assay. In addition, commercially available calibrators and quality-control samples for amiodarone and desethylamiodarone purchased from Chromsystems (Munich, Germany), as well as from Recipe (Munich, Germany), were used.

2.2. Plasma and serum samples

Venous blood samples from blood donors and patients were collected in EDTA sample tubes, as well as in serum monovettes from KABE Labortechnik GmbH (Nümbrecht-Elsenroth, Germany). All samples were anonymized prior to inclusion in the study. The study was conducted according to the guidelines of the Declaration of Helsinki and approval of the institutional review board was obtained.

2.3. Sample preparation for LC–MS/MS

Sample preparation was performed in a 1.5-ml polypropylene microcentrifuge tube. To 100 μl each of EDTA plasma, serum, calibrator or quality-control sample were added 20 μl orthophosphoric acid (85%), followed by 20 μl internal standard solution containing 10 mg/L amiodarone-D_4 and 10 mg/L desethylamiodarone-D_4 in methanol. The mixture was vortex-mixed for 5 s and samples were allowed to equilibrate at RT without further mixing for at least 30 min. Solid phase extraction (SPE) was performed using Oasis MCX cartridges (1 ml/30 mg). SPE cartridges were conditioned and equilibrated with 1 ml methanol and 1 ml of 0.1 mol/L hydrochloric acid at RT. Each spiked and acidified specimen was applied to the SPE cartridges and passed to the bed by centrifugation at 200 × *g* for 1 min. Cartridges were sequentially washed with 1 ml 0.1 mol/L hydrochloric acid and 1 ml methanol. Analytes were eluted with 1 ml methanol containing 1% ammonia at RT, collected into appropriately labeled tubes and evaporated at 40 °C in a vacuum concentrator (run-time: 1 h, vacuum concentrator: BA-VC-300, Bachhofer, Germany). The solid residues were solved by vortex-mixing for 10 s in 100 μl HPLC-grade water containing 0.1% formic acid and 2 mmol/L ammonium acetate, transferring to the autosampler vessel and injecting a 20-μl volume into the LC–MS/MS system.

Table 1
Analytical imprecision of LC–MS/MS assay.

Concentration	Intraassay (n = 10)		Interassay (n = 10)	
	Mean ± SD (mg/L)	CV (%)	Mean ± SD (mg/L)	CV (%)
Amiodarone				
Low	0.43 ± 0.02	4.9	0.36 ± 0.01	3.6
Medium	1.92 ± 0.04	1.9	1.76 ± 0.04	2.1
High	6.70 ± 0.16	2.3	5.98 ± 0.14	2.4
Desethylamiodarone				
Low	0.23 ± 0.01	2.6	0.24 ± 0.01	5.8
Medium	0.53 ± 0.01	2.4	0.43 ± 0.03	7.0
High	2.12 ± 0.06	2.6	2.19 ± 0.07	3.1

2.4. LC–MS/MS analysis

For measurement of amiodarone and desethylamiodarone, a 2 mm × 20 mm LC/MS cartridge (Phenomenx, 2.5 μm Hydro-RP phase, Part No: 00M-4 387-B0-CE) maintained at 50 °C was used for separation by an HPLC system (Waters Alliance 2 795XE) directly coupled to a Quattro LC tandem mass spectrometer fitted with Z Spray ion source (Waters, model: VB-Qmicro Quattro Micro Tandem MS/MS). A 20-μl sample was injected at a flow rate of 0.8 ml/min. The gradient program was 97.5%/2.5% water/methanol containing 0.1% formic acid and 2 mmol/L ammonium acetate for 1.0 min, followed by a step gradient of 100% methanol containing 0.1% formic acid and 2 mmol/L ammonium acetate. After additional 1.5 min, the mobile phase was reverted to the initial state, and the run was terminated at 4.0 min. The mass spectrometer was operated in electrospray positive ionization mode, and the system control and data acquisition were performed using MassLynx NT 4.0 software, with automated data processing by the MassLynx QuanLynx program provided with the instrument. Nitrogen was used as the nebulizing gas and argon as the collision gas. Instrument settings were as follows: capillary voltage, 0.5 kV; source temperature, 120 °C; desolvation temperature, 450 °C; sample cone energy for amiodarone, amiodarone-D_4, desethylamiodarone, and desethylamiodarone-D_4, 62, 62, 46, and 46 eV, respectively; and collision energy for amiodarone, amiodarone-D_4, desethylamiodarone, and desethylamiodarone-D_4, 42, 40, 26 and 26 eV, respectively. The collision gas pressure was 3.3×10^{-3} mbar. Sample analysis was performed in the multiple reaction monitoring (MRM) mode of the instrument, with a dwell time of 0.1 s for all compounds and the mass transitions for amiodarone, amiodarone-D_4, desethylamiodarone, and desethylamiodarone-D_4 m/z 646.0 → 201.2, m/z 650.0 → 201.2, m/z 618.0 → 547.0, and m/z 622.0 → 546.9, respectively.

2.5. Validation

The checklist of STARD (Standard for Reporting of Diagnostic Accuracy) [20,21] and the validation report [22] were used as the basis for validating the LC–MS/MS assay for amiodarone and desethylamiodarone to determine the most important test characteristics like linearity, LOD, LLOQ, imprecision, and extraction yield.

2.5.1. Linearity studies

A matrix-based calibration curve for both amiodarone and desethylamiodarone was constructed using drug-free serum. 80 μl of a 1 mg/ml amiodarone stock solution, as well as 80 μl of a 1 mg/ml desethylamiodarone stock solution were mixed and diluted with 1840 μl serum. The solution was mixed and used as calibrator 20. 1.0 ml of calibrator 20 was further diluted with 1.0 ml

serum, mixed and used as calibrator 19. 1.0 ml of calibrator 19 was used to prepared calibrator 18 as described above, continuing this procedure until calibrator 1 was prepared.

2.5.2. Limit of detection (LOD) and lower limit of quantification (LLOQ)

The limit of detection (LOD) which is the lowest value that significantly exceeds the measurement of a blank sample was estimated on the basis of repeated measurements of a blank sample and reported as the mean plus $3SD_0$ of the blank measurements. Therefore, 25 replicate measurements in a single LC–MS/MS assay with drug-free EDTA plasma as a sample were performed and used for the analysis of the LOD. The lower limit of quantification (LLOQ) which specifies the lower limit at which the assay is able to provide quantitative results of a stated analytical quantity was defined as the lowest concentration of the analyte at which the coefficient of variation (CV) was <20%.

2.5.3. Imprecision and inaccuracy

The intraassay imprecision was determined by analyzing 10 replicates of low (0.43/0.23 mg/L), medium (1.92/0.53 mg/L), and high (6.70/2.12 mg/L) amiodarone and desethylamiodarone samples on the same day. The interassay imprecision was also obtained by measurement of low (0.36/0.24 mg/L), medium (1.76/0.43 mg/L), and high (5.98/2.19 mg/L) amiodarone and desethylamiodarone samples on 10 different days over 1 month (Table 1). Inaccuracy was assessed by performing replicate analysis of quality-control samples at four levels (0.74/0.82, 1.00/1.04, 2.48/2.12, and 4.51/4.69 mg/L, for amiodarone and desethylamiodarone). The procedure was repeated on three different days for the serum, as well as the plasma matrix to determine interassay inaccuracy.

2.5.4. Extraction yield

The extraction yield of the assay was established by measuring the amiodarone, as well as the desethylamiodarone concentration in serum before and after addition of different amounts of amiodarone and desethylamiodarone, respectively. Analytical extraction yields were calculated as the measured concentrations divided by the expected concentrations and expressed as a percentage.

2.5.5. Stability

The stability of amiodarone, as well as desethylamiodarone, in plasma and serum was investigated by measurement of these compounds in freshly collected patient samples and measurement of the same samples at 4 °C, RT, and 37 °C after 1 day, 1 week, 2 weeks, 3 weeks and 1 month. In addition, the stability of the samples after preparation for LC–MS/MS was determined after 1 day, 1 week and 1 month stored at 4 °C, RT, and 37 °C.

2.5.6. Ion suppression

Ion suppression was investigated using a 0, 10, 20, 30, 40, and 50 μl/min continuous post column infusion of a 0.1 M NaCl solution. During the infusion, sample containing 9.6 mg/L amiodarone and desethylamiodarone were subjected to LC–MS/MS analysis, and the MS/MS responses of the MRM transitions for the four compounds were monitored.

2.6. Comparison of amiodarone and desethylamiodarone concentration in serum and plasma

We used 43 serum and 43 EDTA plasma samples, each from the same patient from routine amiodarone monitoring, to compare the amiodarone and desethylamiodarone concentrations in the different material.

2.7. Method comparison

The LC–MS/MS method proposed here was compared with a HPLC method with UV detection using a commercially available reagent set for the HPLC analysis of amiodarone and desethylamiodarone in plasma (Chromsystems, Munich, Germany) by measurement of the same patient samples from routine amiodarone monitoring.

2.8. HPLC method with UV detection using a commercially available reagent

HPLC analysis of amiodarone and desethylamiodarone in plasma was performed on an isocratic system with UV detection (Waters, Lambda-Max LC Spectrophotometer, Model: 481) using a commercially available reagent kit containing an equilibrated 4.6 mm × 100 mm HPLC column (Chromsystems, Order No: 25100), mobile phase (Chromsystems, Order No: 25011), precipitation reagent (Chromsystems, Order No: 25003), internal standard (Chromsystems, Order No: 25044), calibration standard (Chromsystems, Order No: 25005), light protected reaction vials (Chromsystems, Order No: 33005) and control level I and II (Chromsystems, Order No: 0067 and 0068). Sample preparation was performed by mixing of 100 μl plasma sample with 50 μl precipitation reagent for 20 s at RT. 20 μl of the supernatant was injected into the HPLC system after centrifugation of the mixture at RT (9000 × *g*) for 10 min. The elution was performed isocratically with mobile phase at RT with a flow-rate of 1.0 ml/min. The UV wavelength was fixed at 242 nm and the run time was approximately 11 min. LLOQ was 0.20 mg/L for amiodarone and 0.15 mg/L for desethylamiodarone and the assay was linear up to 25 mg/L.

3. Results

3.1. General characterization of the LC–MS/MS assay

Maximum sensitivity for amiodarone and desethylamiodarone, as well as its corresponding D_4 isotopes was achieved by monitoring the fragmentation of single-charged molecule ions [amiodarone + H^+], [amiodarone-D_4 + H^+], [desethylamiodarone + H^+], and [desethylamiodarone-D_4 + H^+] with *m*/*z* transitions of 646.0 → 201.2, 650.0 → 201.2, 618.0 → 547.0, and 622.0 → 546.9, respectively (Fig. 1). Sample preparation by solid phase extraction, evaporation and dissolving produced a clear, colorless solution that gave an interference-free chromatogram for amiodarone, amiodarone-D_4, desethylamiodarone and desethylamiodarone-D_4 (Fig. 2). All compounds were clearly separated from the void volume (0.1 min) and elute in <3 min, permitting an injection-to-injection cycle time of <4 min. The retention time of amiodarone, as well as amiodarone-D_4, was 1.99 min, whereas desethylamiodarone and desethylamiodarone-D_4 both eluted at 1.98 min (Fig. 2). Therefore, quantitative errors resulting from potential ion suppression are compensated via the internal standard (D_4 isotope) which is structurally identical to the corresponding analyte. As shown in Table 2 the peak area decrease resulting from NaCl infusion is analog for amiodarone and its internal standard amiodarone-D_4. Therefore, different ion suppression generated by different NaCl infusion has no effect on the measured amiodarone concentration. The same result is shown for desethylamiodarone and its internal standard desethylamiodarone-D_4 in Table 3. Furthermore, desethylamiodarone was not detected as an in-source fragmentation product of amiodarone, as shown in Fig. 2D.

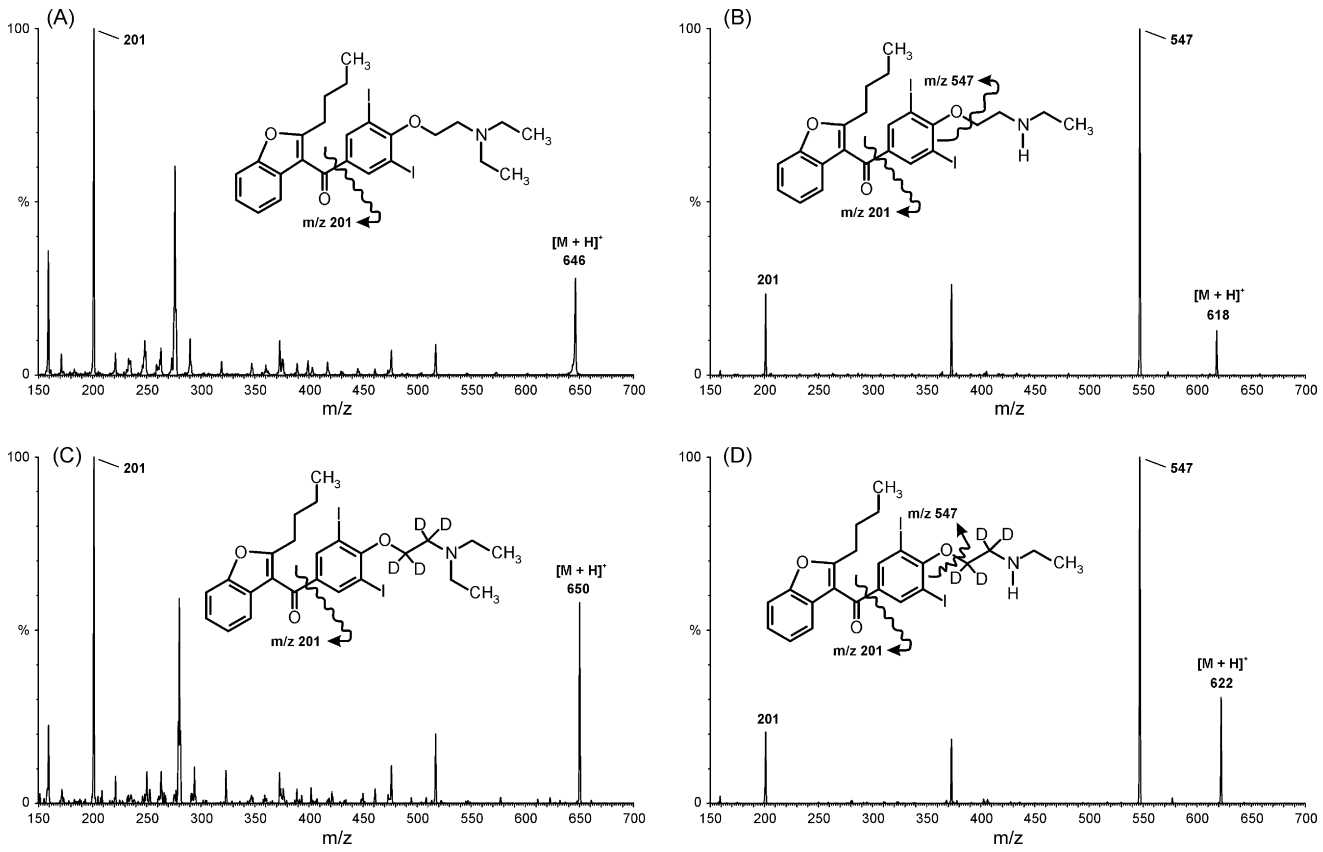

Fig. 1. Product ion spectra of amiodarone (A) and desethylamiodarone (B), as well as its internal standards amiodarone-D_4 (C) and desethylamiodarone-D_4 (D). The MH^+ precursor ions and the fragment ions are shown. The chemical structures of the molecules are depicted. In addition, the positions of the D atoms of the internal standards amiodarone-D_4 (C) and desethylamiodarone-D_4 (D) are shown.

Table 2
Ion suppression investigations of LC–MS/MS assay.

NaCl solution flow (μl/min)	Peak area amiodarone[a] Mean ± SD (units)	Peak area amiodarone-D_4[a] Mean ± SD (units)	Amiodarone concentration[a] Mean ± SD (mg/L)
0	3554 ± 105	2424 ± 72	9.61 ± 0.10
10	1900 ± 56	1285 ± 34	9.69 ± 0.20
20	1146 ± 80	776 ± 56	9.69 ± 0.20
30	884 ± 74	605 ± 42	9.58 ± 0.28
40	526 ± 75	365 ± 48	9.43 ± 0.25
50	239 ± 27	163 ± 16	9.60 ± 0.30

[a] Mean of four determinations.

3.2. Validation

Carryover from the 40 mg/L calibrator to the drug-free serum or plasma sample was less than 0.1% for both amiodarone and desethylamiodarone. Both the amiodarone and desethylamiodarone calibration curves were linear over the working range between 0.01 and 40.0 mg/L ($r > 0.999$). The LODs in plasma matrix were 2.7 μg/L for amiodarone and 1.9 μg/L for desethylamiodarone, with an injection volume of 20 μl. The LLOQ in plasma matrix were 7.5 μg/L for amiodarone and 2.5 μg/L for desethylamiodarone. The within-run and between-run CVs which were measured for several concentrations of amiodarone, as well as desethylamiodarone, were all <8% (Table 1). Interassay inaccuracy (% error) for each of the four quality-control levels ranged from 1% to 9%. The mean

Table 3
Ion suppression investigations of LC–MS/MS assay.

NaCl solution flow (μl/min)	Peak area desethylamiodarone[a] Mean ± SD (units)	Peak area desethylamiodarone-D_4[a] Mean ± SD (units)	Desethylamiodarone concentration[a] Mean ± SD (mg/L)
0	29685 ± 922	10183 ± 216	9.70 ± 0.13
10	8428 ± 371	2822 ± 89	9.70 ± 0.14
20	4791 ± 430	1648 ± 127	9.66 ± 0.14
30	3690 ± 370	1265 ± 130	9.70 ± 0.07
40	2170 ± 305	754 ± 112	9.58 ± 0.11
50	917 ± 94	322 ± 37	9.48 ± 0.27

[a] Mean of four determinations.

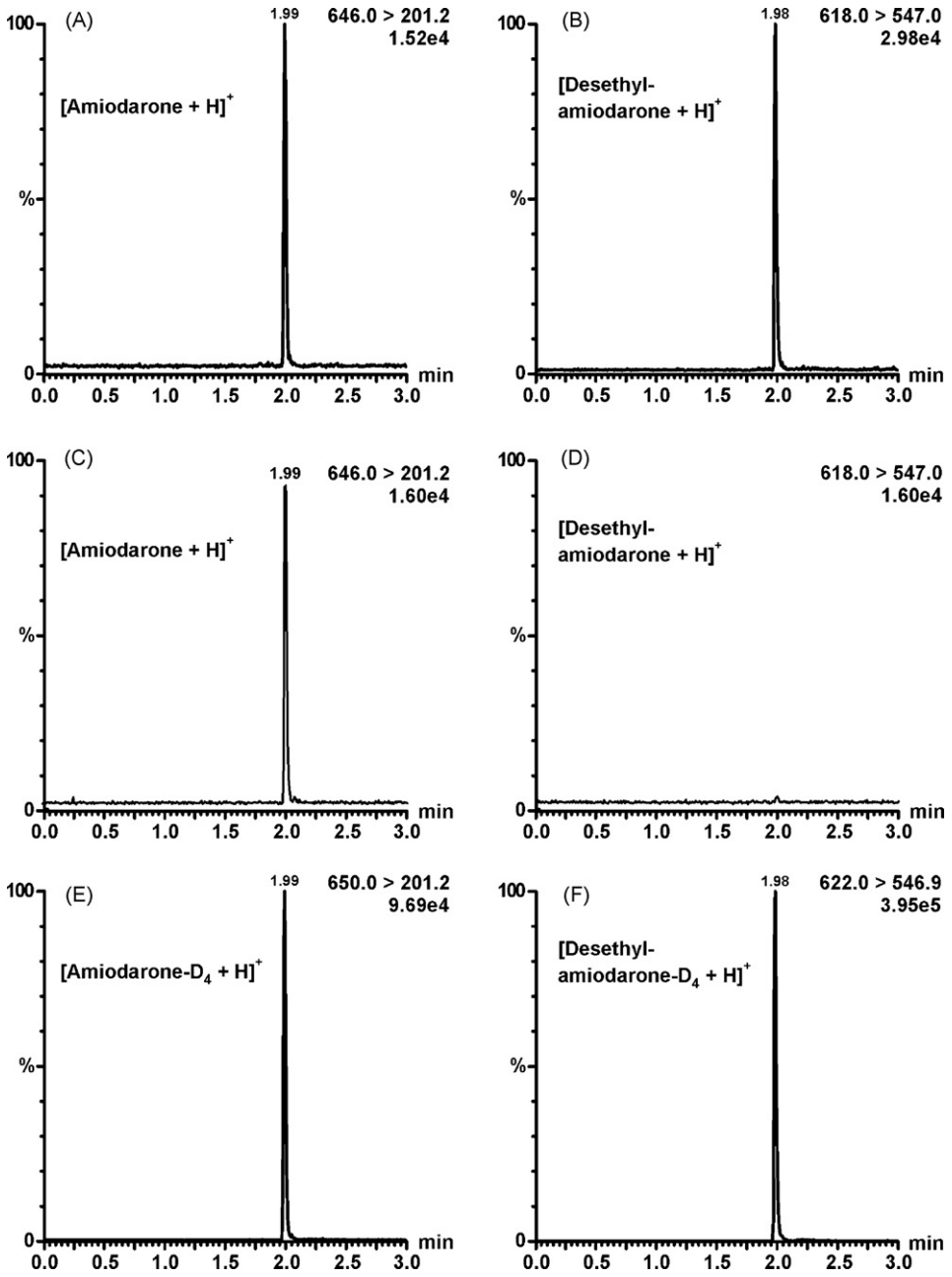

Fig. 2. LC–MS/MS extracted ion chromatograms for a dissolved solid phase extract for amiodarone (A) and desethylamiodarone (B) from a serum containing 0.5 mg/L amiodarone, as well as 0.5 mg/L desethylamiodarone (upper chromatograms). The chromatograms in the middle ((C) for amiodarone and (D) for desethylamiodarone) resulted from a drug-free serum which was only spiked with amiodarone to a concentration of 0.5 mg/L. The lower chromatograms show the corresponding internal standards amiodarone-D_4 (E) and desethylamiodarone-D_4 (F), respectively. Multiple reaction monitoring (MRM) analysis was 4 min. The *y*-axes are scaled to 100% for the largest peak for the specified mass transitions. The retention time is the number above the peak, and full-scale signal intensity is listed on the upper right, below the MRM *m*/*z* values.

extraction yield for amiodarone at concentrations of 0.5–10.0 mg/L was 99.6% (range 92.6–107.7%) and for desethylamiodarone at the same concentrations 90.2% (range 80.0–94.7%). No change of the amiodarone and desethylamiodarone concentrations in serum and plasma for at least 2 weeks at 4 °C, RT, and 37 °C was observed by systematic testing of chemical stability of the drugs.

To estimate the influence of sample matrix, we compared the amiodarone and desethylamiodarone concentrations of serum and plasma samples ($n = 43$), each from the same patient, measured with the LC–MS/MS assay on the same day and performed our analysis by least-square linear regression. The resulting regression line was: y (plasma) = 0.98x (serum) − 0.01 [mg/L]; $r = 0.96$ for amiodarone and y (plasma) = 0.76x (serum) + 0.22 [mg/L]; $r = 0.92$ for desethylamiodarone.

3.3. Method comparison

Method comparison analysis was performed using samples from 162 patients who used amiodarone as an antiarrhythmic drug. As shown in Fig. 3, the correlation between a HPLC method with UV detection using a commercially available reagent set for the HPLC analysis of amiodarone and desethylamiodarone in plasma and the LC–MS/MS method was moderate for both drugs.

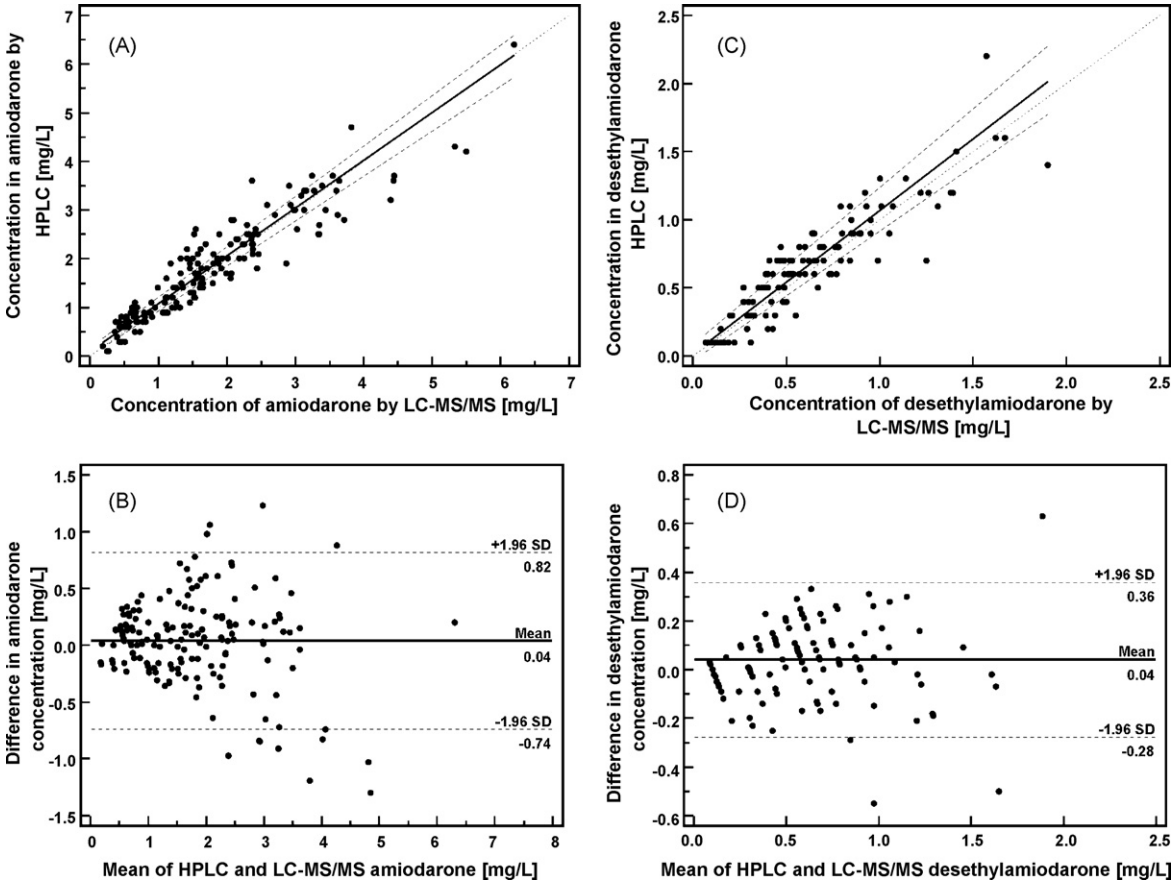

Fig. 3. (A) Comparison of amiodarone results obtained by the LC–MS/MS assay and a HPLC method with UV detection using a commercially available reagent set for the HPLC analysis of amiodarone and desethylamiodarone by Passing–Bablok regression: HPLC = 0.98 (LC–MS/MS) + 0.10 [mg/L] (r = 0.94; n = 162; 95% CI for slope, 0.92–1.03; 95% for intercept, 0.01–0.17). (B) Bland–Altman plot for the comparison of LC–MS/MS assay vs. HPLC assay with UV detection. The mean value (n = 162) of the two methods is plotted against the difference between the two values (HPLC assay with UV detection – LC–MS/MS assay). The mean difference between the two methods was 0.04 mg/L. The mean (—) and ±2 SD lines (- - -) are plotted for reference. (C) Comparison of desethylamiodarone results obtained by the LC–MS/MS assay and the HPLC assay with UV detection by Passing–Bablok regression: HPLC = 1.05 (LC–MS/MS) + 0.02 [mg/L]; (r = 0.90; n = 117; 95% CI for slope, 0.95–1.15; 95% for intercept, −0.03–0.08). (D) Bland–Altman plot for the comparison of LC–MS/MS assay vs. HPLC assay with UV detection. The mean value (n = 117) of the two methods is plotted against the difference between the two values (HPLC assay with UV detection – LC–MS/MS assay). The mean difference between the two methods was 0.04 mg/L. The mean (—) and ±2 SD lines (- - -) are plotted for reference.

4. Discussion

Liquid chromatography–mass spectrometry is becoming increasingly important as a routine technique in clinical laboratories owing to the broader range of biological molecules that can be analyzed [23–27]. The use of tandem mass spectrometry and stable isotope internal standards permits highly sensitive and specific assays to be developed [28].

Here, we report validation of the first high-performance stable isotope dilution liquid chromatography–tandem mass spectrometry method (LC–MS/MS) to measure amiodarone and its main metabolite desethylamiodarone in both human serum and plasma. The use of isotopically labeled analogs as the internal standard has many advantages, such as compensation of the alteration of the analyte in complex matrices during sample preparation or the compensation of ion suppression during electrospray ionization during the mass spectrometric measurement. As shown in other applications, ion suppression is one of the most undesirable processes that can occur during electrospray mass spectrometric analysis, as a result of a nonlinear decrease in ionization by sample or mobile phase [25,29,30]. Amiodarone-D_4 and desethylamiodarone-D_4, which were used in our method as the internal standard for amiodarone and desethylamiodarone, eliminate this undesirable effect. Furthermore, in-source fragmentation of drug or drug metabolites, another undesirable process which can occur during electrospray ionisation mass spectrometry, was not detected in our assay (Fig. 2D).

The correlation between our LC–MS/MS assay and the commercially available HPLC assay with UV detection was moderate for both amiodarone and desethylamiodarone (Fig. 3). In comparison to the HPLC assay with UV detection (LLOQ for amioadarone and desethylamiodarone: 0.20 and 0.15 mg/L), the LC–MS/MS assay (LLOQ for amioadarone and desethylamiodarone: 0.075 and 0.025 mg/L) was not only three-fold more sensitive for both substances, but also measurement of amiodarone and desethylamiodarone was performed within 4 min, whereas the run-time of the HPLC assay was approximately 11 min. The advantage of amiodarone and desethylamiodarone analyzing by HPLC combined with UV detection is commonly the simple sample preparation as similarly described in the literature [7–12,14,18] whereas the advantage of LC–MS/MS methods is the more sensitivity [18]. In addition, LC–MS/MS methods are often faster because of the shorter retention time which where possible in the chromatographic step [17,18].

Indications for the described LC–MS/MS method are a suitable sample preparation, a selective detection of analyte of interest, and the use of isotopically labeled analogs as internal standards, resulting in a highly precise measurement of amiodarone and desethylamiodarone in complex matrices. Intra- and interassay imprecision was, at less than 8% for both amiodarone and desethy-

lamiodarone, low in comparison to other LC–MS/MS assays which show CV values of higher than 10% for both substances [17–19]. The high precision of our LC–MS/MS assay is undoubtedly due to the appropriate mass chromatogram without any significant interfering peaks from the serum or plasma matrix, and not least because of the isotopically labeled internal standards which eliminate undesirable ion suppression effects.

5. Conclusion

A stable isotope dilution LC–MS/MS assay has been created and evaluated for the simultaneous analysis of amiodarone and its major metabolite desethylamiodarone in human serum and plasma. Sample preparation which was performed by solid phase extraction, evaporation and dissolving produced a clear colorless solution for a fast chromatographic separation on a 2 mm × 20 mm cartridge. Amiodarone-D_4 and desethylamiodarone-D_4 were used as internal standards. The results of ion suppression investigations clearly show that the use of these deuterium isotopes as internal standards eliminate interfering ion suppression effects during the LC–MS/MS measurement of amiodarone and desethylamiodarone. In summary, the current method is not only very fast and highly specific for the drug and its major metabolite, but it also shows high precision, accuracy and sensitivity, making it potentially suitable as a candidate reference method for both substances.

Acknowledgements

We thank Annika Wolf for her excellent technical assistance and Sarah Kirkby for her linguistic advice.

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