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# The validation of a bioanalytical method for the determination of clopidogrel in human plasma

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**Abstract**

A fast, sensitive and specific LC–MS/MS bioanalytical method for the determination of unchanged clopidogrel in human plasma has been developed and validated over the range of 10–12,000 pg mL$^{-1}$ ($r^2$ 0.9993) by the Contract Research group at HFL. Samples (0.3 mL) were buffered (pH 6.8), extracted using diethyl ether and 10 μL of the sample extract was injected onto the LC–MS/MS system. Analysis was performed using a C8 column (temperature controlled to 50 °C) by gradient elution at a flow rate of 0.9 mL min$^{-1}$ over a 3 min run time. Retention times of 1.61 and 1.59 min were observed for clopidogrel and $^2H_3$-clopidogrel (I.S.), respectively. Detection was achieved using a Sciex API 4000, triple quadrupole mass spectrometer, in positive TurboIonspray™ (electrospray) ionisation mode. Ion transitions were monitored using MRM (multiple reaction monitoring) for clopidogrel (*m/z* 322–212) and for $^2H_3$-clopidogrel (*m/z* 327–217). This validated method was used to support a pharmacokinetic study in healthy volunteers.


*Keywords:* LC–MS/MS; Validation; Clopidogrel; Human plasma; Bioanalytical

## 1. Introduction

Clopidogrel hydrogen sulfate [methyl (+)-(S)-α-(2-chlorophenyl)-6,7-dihydrothieno[3,2-*c*]pyridin-5(4*H*)-acetate hydrogen sulfate] (Fig. 1a) is a potent anti-platelet prodrug [1]. It is a thienopyridine derivative structurally related to ticlopidine and is indicated for the reduction of artherosclerotic events in patients with atherosclerosis documented by recent stroke, recent myocardial infarction, or cardiovascular disease [2] [3]. Clopidogrel is rapidly absorbed and undergoes extensive hepatic biotransformation forming an active thiol metabolite (S configuration at C7 and Z configuration at C3–C16 double bond) [4] and an inactive carboxylic acid derivative, which is the major circulating compound [5].

In humans, very low levels of unchanged clopidogrel are present in plasma ($C_{max}$ 7921.49 ± 3921.39 pg mL$^{-1}$) [6], and as a consequence few methods have been reported for its determination.

Lagorce et al. [7] reported a GC–MS method for the analysis of the inactive carboxylic acid metabolite of clopidogrel in human plasma and serum with a lower limit of quantification (LLOQ) of 5 ng mL$^{-1}$. Caplain et al. [8] quantified the inactive metabolite in human plasma and achieved an LLOQ of 25 ng mL$^{-1}$ using HPLC-UV and an LLOQ of 1 ng mL$^{-1}$ using GC–MS. Mitakos et al. [9] proposed an LC–MS method for the determination of inactive metabolite in human plasma with an LLOQ of 100 ng mL$^{-1}$.

Taubert et al. [10] used LC–MS/MS to analyse unchanged clopidogrel and its inactive carboxylic acid metabolite in human plasma over the range of 0.5–100 ng mL$^{-1}$ and 0.5–150 μg mL$^{-1}$, respectively. Lainesse et al. [6] described an LC–MS/MS method for the measurement of unchanged clopidogrel in human plasma (EDTA, anticoagulant) over the range of 20.08–10040.00 pg mL$^{-1}$. More recently, Nirogi et al. [11] used LC–MS/MS and ticlopidine (internal standard) to analyse unchanged clopidogrel in human plasma samples (0.5 mL) over the range of 5–6000 pg mL$^{-1}$.

A fast, specific, and sensitive method for the quantitative determination of unchanged clopidogrel in human plasma (Lithium heparin, anticoagulant) was required in order to

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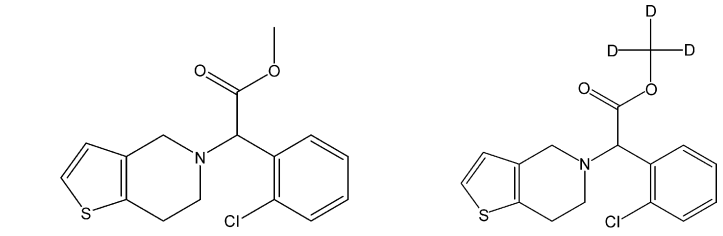


Fig. 1. Chemical structure of (a) clopidogrel [4]; (b) $^{2}H_3$-clopidogrel (I.S.).

support clinical trials. A bioanalytical method has been developed and validated over the range of 10–12,000 pg mL$^{-1}$ within the Contract Research group at HFL Ltd. The method described herein competes in sensitivity with methods published previously [6,11] and uses a greater calibration range and an isotopically labelled internal standard ($^{2}H_3$).

## 2. Experimental

### 2.1. Materials

Clopidogrel hydrogen sulphate (98.9%) was supplied by Ind-Swift Laboratories Ltd. (Punjab, India) and $^{2}H_3$-clopidogrel hydrogensulphate (98.9%) was purchased from SynFine Research (Ontario, Canada). Drug free control human plasma (lithium heparin anticoagulant) was obtained from Charterhouse Clinical Research (UK). Methanol, acetonitrile, diethyl ether (HPLC grade) and ammonium acetate (98% minimum) were obtained from Sigma–Aldrich (Poole, UK). Formic acid (GPR grade) was obtained from BDH (Poole, UK). Luna C8(2) HPLC columns (50 mm × 2.0 mm, 5 μm particle size) and guard columns (C8, 4 mm × 3.0 mm i.d.) were obtained from Phenomenex (Macclesfield, UK). Reagent grade water was supplied by an Option 5 Water Purifier purchased from Elga Ltd. (High Wycombe, UK).

### 2.2. Preparation of stock solutions, reagents, and validation samples

Clopidogrel stock solution (1 mg mL$^{-1}$) was prepared by accurately weighing the clopidogrel hydrogensulphate salt and dissolution in the appropriate volume of methanol to give a final free base concentration of 1 mg mL$^{-1}$. A clopidogrel intermediate solution (1 μg mL$^{-1}$) was prepared by diluting the clopidogrel stock solution (1 mg mL$^{-1}$) with methanol:RG water (50:50, v/v). The clopidogrel intermediate solution (1 μg mL$^{-1}$) was diluted as appropriate with methanol:RG water (50:50, v/v) to give Quality Control (QC) solutions at 95 and 50 ng mL$^{-1}$; the 50 ng mL$^{-1}$ solution was diluted as appropriate to give 0.3 and 0.1 ng mL$^{-1}$ solutions. In a similar manner, calibration solutions were created at the following concentrations: 120, 100, 45, 20, 10, 5, 1.5, 0.5, 0.2, and 0.1 ng mL$^{-1}$.

$^{2}H_3$-Clopidogrel (Fig. 1b) stock solution (250 μg mL$^{-1}$) was prepared by accurately weighing $^{2}H_3$-clopidogrel hydrogensulphate salt and dissolution in the appropriate volume of methanol to give a final concentration of 250 μg mL$^{-1}$ free base. A $^{2}H_3$-clopidogrel I.S. working solution (2.5 ng mL$^{-1}$) was prepared by diluting 5 μL of the $d_3$-clopidogrel stock solution (250 μg mL$^{-1}$) to 500 mL with methanol:R.G. water (50:50, v/v). All solutions were stored in the refrigerator (ca. 4 °C) when not in use.

Calibration and QC samples were prepared as shown in Tables 1 and 2 and then treated as described in the human plasma extraction section of this paper. Replicate 300 μL QC sample aliquots were stored frozen (ca. −20 °C) until used and calibration samples were prepared fresh for every batch.

### 2.3. Human plasma extraction

Samples were extracted in the following manner: $^{2}H_3$-clopidogrel I.S. working solution (20 μL) was added to lithium heparin anticoagulated human plasma (300 μL), 50 mM ammo-

Table 1
Preparation of calibrant samples

| Calibration standard concentration (pg mL$^{-1}$) | Concentration of spiking solution (ng mL$^{-1}$) | Volume of spiking solution (μL) | Volume of control matrix (μL) |
|---|---|---|---|
| 12000 | 120 | 30 | 270 |
| 10000 | 100 | 30 | 270 |
| 4500 | 45 | 30 | 270 |
| 2000 | 20 | 30 | 270 |
| 1000 | 10 | 30 | 270 |
| 500 | 5 | 30 | 270 |
| 150 | 1.5 | 30 | 270 |
| 50 | 0.5 | 30 | 270 |
| 20 | 0.2 | 30 | 270 |
| 10 | 0.1 | 30 | 270 |

Table 2
Preparation of QC samples

| QC concentration (pg mL$^{-1}$) | Concentration of spiking solution (ng mL$^{-1}$) | Volume of spiking solution (μL) | Volume of control matrix (μL) |
|---|---|---|---|
| 10 | 0.1 | 30 | 270 |
| 30 | 0.3 | 30 | 270 |
| 5000 | 50 | 30 | 270 |
| 9500 | 95 | 30 | 270 |

nium acetate buffer$_{(aq)}$ pH 6.8 (500 μL) was added and the solution was vortex mixed thoroughly, diethyl ether (2 mL) was added and the tubes were rotary mixed (ca. 15 min), samples were centrifuged (ca. 3500 rpm for 5 min), the organic layer transferred to a clean tube, and evaporated to dryness under nitrogen (ca. 22 °C). Samples were reconstituted in 0.1% (v/v) formic acid (acetonitrile) (50 μL). 0.1% (v/v) formic acid (R.G. water) (50 μL) was added, samples were vortex mixed. Extracts were transferred to vials prior to injection onto the LC–MS/MS system.

### 2.4. LC–MS/MS method

Extracts were analysed by LC–MS/MS using an Agilent 1100 series HPLC system (Waldbronn, Germany) comprising of a quaternary pump and an on line degasser, linked to a CTC PAL autosampler (Zwinger, Switzerland) coupled to a Sciex API 4000$^{TM}$ mass spectrometer (Toronto, Canada). Detection was achieved by positive Atmospheric Pressure Ionisation

Table 3
Gradient elution timetable

| Time (min) | %A | %B |
|---|---|---|
| 0.00 | 35 | 65 |
| 0.80 | 35 | 65 |
| 1.40 | 70 | 30 |
| 1.60 | 70 | 30 |
| 1.70 | 35 | 65 |
| 3.00 | 35 | 65 |

A = acetonitrile containing 0.1% (v/v) formic acid; B = R.G. water containing 0.1% (v/v) formic acid.

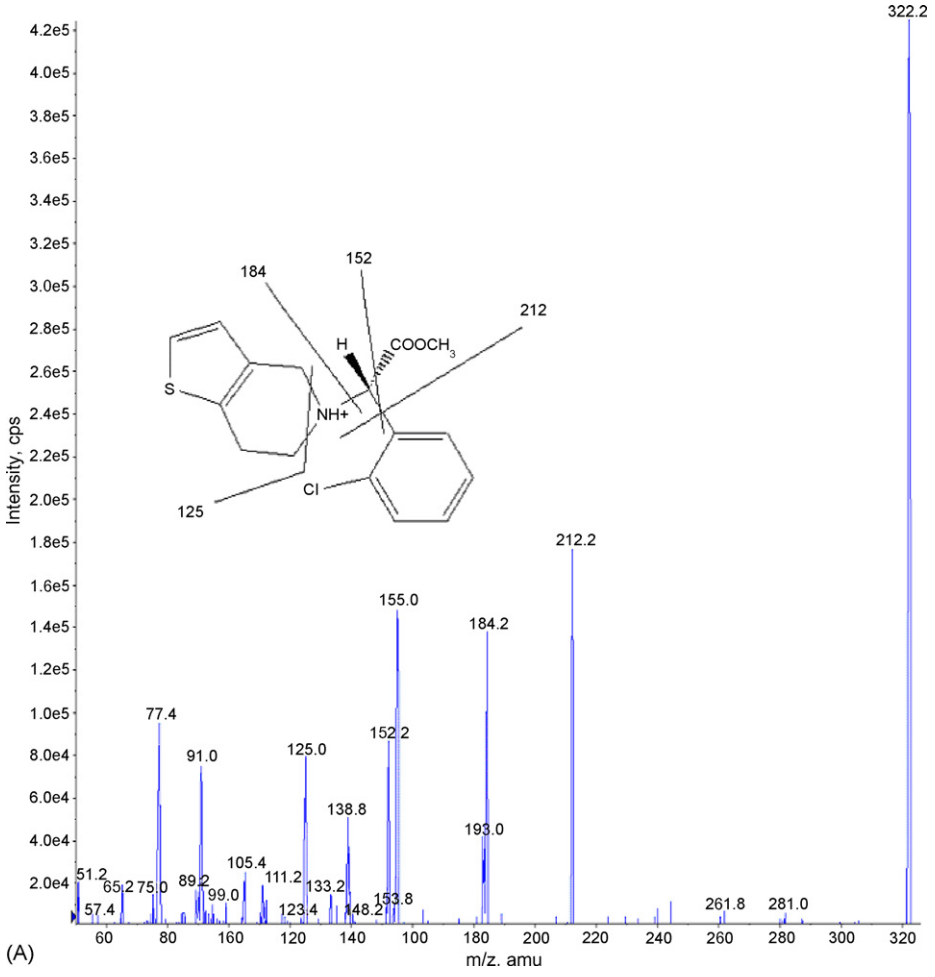


Fig. 2. Full-scan positive ion TurboIonspray$^{TM}$ product ion mass spectra for clopidogrel (A) and $^{2}H_3$-clopidogrel (B).

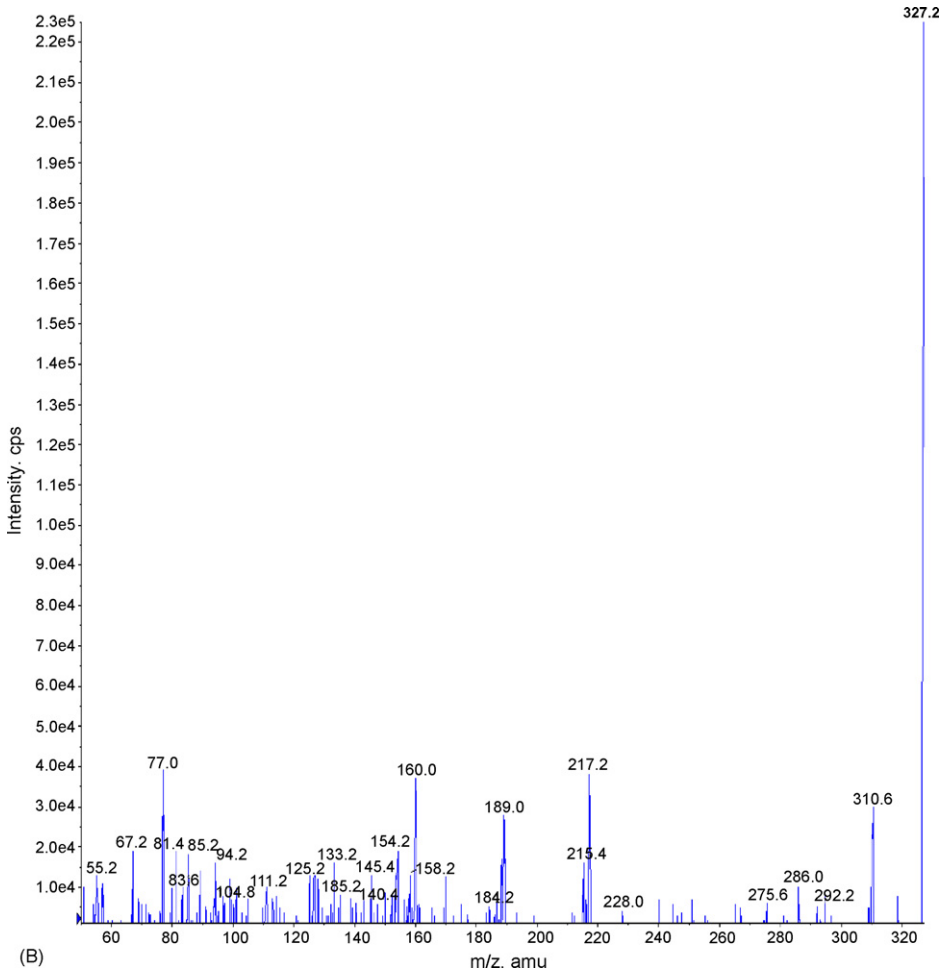


Fig. 2. (*Continued*).

(API) (TurboIonspray$^{TM}$) in Multiple Reaction Monitoring (MRM) mode. A 10 μL aliquot of the extract was injected onto a Luna C8(2) HPLC column (50 mm × 2.0 mm, 5 μm particle size). A fast gradient was used at a flow rate of 0.9 mL min$^{-1}$ for a 3 min run time (Table 3 for gradient timetable). The column was thermostatically controlled to a temperature of 50 °C. The post column eluent was not split prior to introduction to the mass spectrometer. The tandem mass spectrometer settings

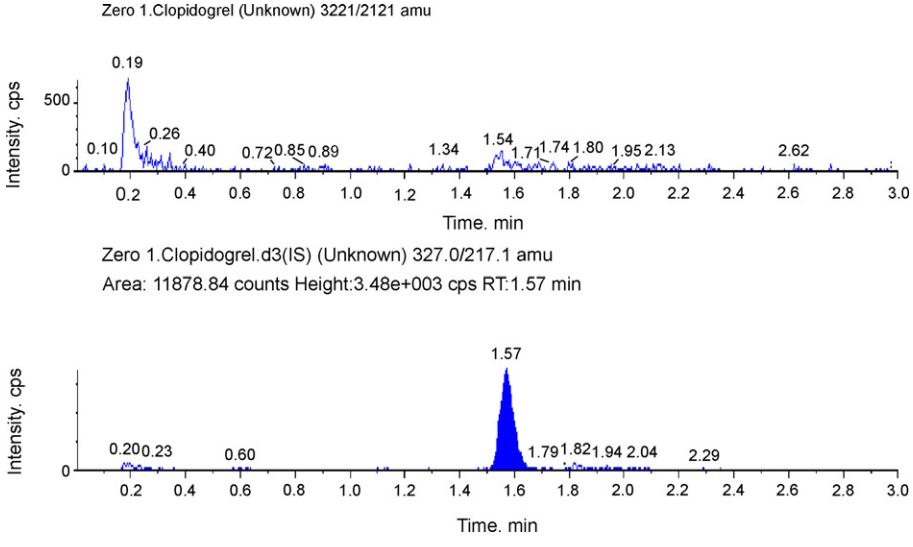


Fig. 3. Representative MRM chromatograms of an extracted zero plasma sample.

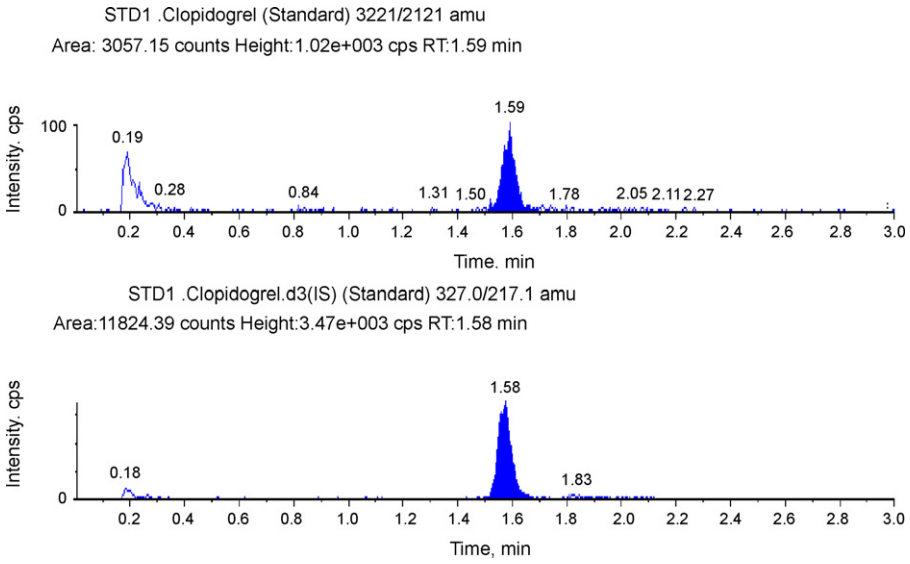


Fig. 4. Representative MRM chromatograms of an extracted 10 pg mL$^{-1}$ (LLOQ) plasma sample.

Table 4
Mass spectrometer settings for the clopidogrel method

| | |
|---|---|
| Effluent split ratio | Splitless |
| Ionisation/interface | TurboIonSpray™ |
| Source temperature (°C) | 550 |
| Curtain gas | 12 |
| GS1 | 40 |
| GS2 | 50 |
| Ionspray voltage (V) | 5000 |

are detailed in Table 4. Clopidogrel and $^{2}H_3$-clopidogrel (I.S.) were monitored at *m/z* transitions of 322.07 → 212.15, and 327.00 → 217.10, respectively, with dwell times of 150 ms for each (Fig. 2). I.S. transition was chosen for the $Cl^{37}$ isotopic structure to prevent interfering signal breakthrough from clopidogrel.

LC–MS/MS peaks were observed at approximately 1.61 and 1.59 min for clopidogrel and $^{2}H_3$-clopidogrel, respectively. Integration was performed using the Analyst™ software associated with the mass spectrometer, version 1.3.1 (Applied Biosystems,

Table 5
Summary of the inter-batch quality control data

| | QC LLOQ (10.00 pg mL$^{-1}$) | QC LOW (30.00 pg mL$^{-1}$) | QC MED (5000 pg mL$^{-1}$) | QC HIGH (9500 pg mL$^{-1}$) |
|---|---|---|---|---|
| Mean | 9.932 | 30.40 | 5149 | 9645 |
| S.D. | 0.7150 | 1.660 | 141.9 | 215.7 |
| CV (%) | 7.2 | 5.5 | 2.8 | 2.2 |
| RE (%) | −0.7 | 1.3 | 3.0 | 1.5 |
| *n* | 18 | 17 | 18 | 18 |

CV = coefficient of variation, RE = relative error.

Table 6
Stability results for clopidogrel in human plasma

| | Concentration measured (pg mL$^{-1}$) | | |
|---|---|---|---|
| | 4 h (RT) | 1 month (−20 °C) | Freeze–thaw |
| QC LOW (30.00 pg mL$^{-1}$) | | | |
| Mean | 31.49 | 29.36 | 29.02 |
| S.D. | 1.2 | 1.3 | 2.1 |
| CV (%) | 3.7 | 4.4 | 7.4 |
| Stability (%) | 106.0 | 98.9 | 97.7 |
| *n* | 6 | 6 | 6 |
| QC HIGH (9500 pg mL$^{-1}$) | | | |
| Mean | 9414.50 | 9367.83 | 9622.83 |
| S.D. | 164.6 | 199.3 | 321.5 |
| CV (%) | 1.7 | 2.1 | 3.3 |
| Stability (%) | 97.1 | 96.7 | 99.3 |
| *n* | 6 | 6 | 6 |

USA). Data was imported into Watson™ LIMS, version 7.0 (Innaphase, Buckinghamshire, UK), for regression and quantification. The calibration curve was constructed over the range of 10–12,000 pg mL$^{-1}$ by plotting the nominal concentration of each calibrant against the peak area ratio with reference to the internal standard. The regression type used was quadratic with a weighting factor of 1/*x* applied. The concentration of clopidogrel in unknown, validation and QC samples was back-calculated from the calibration curve.

### 2.5. Clinical trial

A test formulation of Clopidogrel was compared to a reference formulation (Plavix®, Sanofi-Synthelabo, France) in a relative bioavailability study in 36 healthy volunteers. Subjects received a single dose of 150 mg (2 × 75 mg) of test or reference formulation in a standard two-period randomised crossover design, with blood samples taken at intervals up to 36 h after dosing. Study periods were separated by a washout of at least 7 days. Pharmacokinetic parameters including maximum plasma concentration following dosing ($C_{max}$) and area under the plasma concentration curve (AUC) were calculated from the relevant plasma concentration data using Kinetica 2000® Version 4.2 (Innaphase Clinical Information Engineering). Data were to be presented with 90% confidence intervals (CI) calculated from mean ratios of test and reference formulations and intra-individual coefficients of variation (CV). Treatment comparisons (ANOVA) were made on log-transformed data for $C_{max}$ and AUC; statistical analyses were performed using SAS® Version 9.1.3 (SAS® Institute, Cary, NC, USA).

## 3. Results

Chromatograms for extracted zero and lower limit of quantification (LLOQ) calibration standard extracts are presented in Figs. 3 and 4. A signal-to-noise ratio greater than 10 was achieved at the LLOQ.

Three consecutive precision and accuracy batches were completed. Each batch included calibration standards freshly prepared and extracted from human plasma, a blank and a zero, and replicate ($n = 6$) QC samples at LLOQ (10 pg mL$^{-1}$), LOW (30 pg mL$^{-1}$), MEDIUM (5000 pg mL$^{-1}$), and HIGH (9500 pg mL$^{-1}$) levels. The percentage relative error (%RE) for the calibration standards ranged from −2.2% to 1.6% over the three plasma curves. The three curves had slopes with a percentage coefficient of variation (%CV) of 0.1%, negligible intercepts and mean coefficient of determination ($r^2$) of 0.9993.

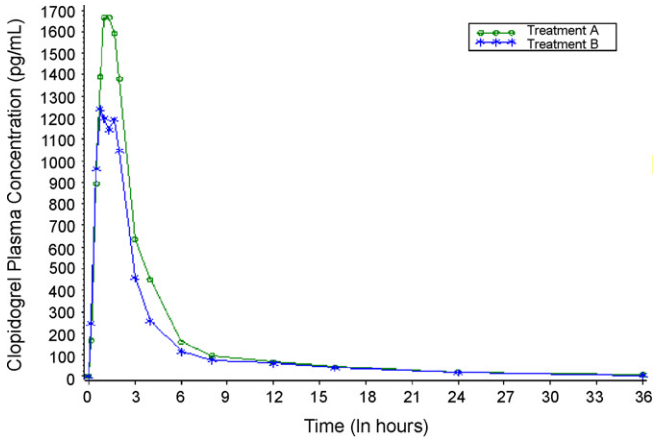


Fig. 5. Mean clopidogrel plasma concentration graphs by treatment, $n = 36$. Treatment A = 2 × 75 mg clopidogrel (test formulation). Treatment B = 2 × 75 mg clopidogrel (Plavix®, Sanofi-Synthelabo, France).

Inter- and intra-batch precision and accuracy were calculated using all the replicate QC determinations over the three precision and accuracy batches. The accuracy, defined as the percentage difference between the back-calculated concentrations of QCs and the theoretical prepared concentrations was expressed as %RE. The precision of the replicate data was expressed as %CV. The intra-batch accuracy (%RE) ranged from −5.6% to 5.9% at QC LLOQ, −1.1% to 5.7% at QC LOW, 2.2% to 4.0% at QC MEDIUM, and 1.3% to 2.0% at the QC HIGH level. Intra-batch precision was 8.2% (%CV) at every QC level over each of the three batches. The inter-batch accuracy ($n = 18$) over the three batches was 3.0% (%RE) at each QC level. The inter-batch precision ($n = 18$) was 7.2% (%CV) at each of the QC levels. A summary of the inter-batch precision and accuracy data is presented in Table 5.

Table 7
Matrix effect results for clopidogrel in human plasma

| Replicate no. | Peak area | | | |
|---|---|---|---|---|
| | [Analyte(plasma)] P1 | [Analyte(aqueous)] P2 | [I.S.(plasma)] P3 | [I.S.(aqueous)] P4 |
| 1 | 2015179.23 | 2535014.16 | 16120.41 | 20313.02 |
| 2 | 2182519.35 | 2630355.12 | 17149.30 | 21559.57 |
| 3 | 2246993.74 | 2688069.80 | 18791.37 | 21186.99 |
| 4 | 2122919.82 | 2913865.13 | 17784.93 | 23539.56 |
| 5 | 2233716.55 | 2998162.31 | 17686.96 | 24246.76 |
| 6 | 2098692.54 | 3132886.80 | 16401.83 | 26137.94 |
| Mean | 2150003.5 | 2816392.2 | 17322.5 | 22830.6 |
| S.D. | 88335.9 | 233649.4 | 982.5 | 2195.5 |
| CV (%) | 4.1 | 8.3 | 5.7 | 9.6 |

Matrix effect (analyte): 100 − (Mean P1/Mean P2 × 100) = 23.7%. Matrix effect (I.S.): 100 − (Mean P3/Mean P4 × 100) = 24.1%.

Table 8
Recovery results for clopidogrel in human plasma

| Extracted peak area | Unextracted peak area | Mean unextracted peak area | Recovery (%) | Mean recovery (%) |
|---|---|---|---|---|
| LOW (30 pg mL$^{-1}$) | | | | |
| 5676.26 | 8959.94 | 8696.40 | 65.3 | 68.6 |
| 5307.58 | 8404.95 | | 61.0 | |
| 5963.51 | 8665.61 | | 68.6 | |
| 5745.17 | 8347.64 | | 66.1 | |
| 6612.76 | 8750.69 | | 76.0 | |
| 6486.63 | 9049.58 | | 74.6 | |
| MED (5000 pg mL$^{-1}$) | | | | |
| 826199.37 | 1412989.32 | 1417914.00 | 58.3 | 61.5 |
| 778212.64 | 1424083.47 | | 54.9 | |
| 859863.48 | 1414528.32 | | 60.6 | |
| 895620.16 | 1391997.56 | | 63.2 | |
| 907320.62 | 1434912.18 | | 64.0 | |
| 966208.15 | 1428973.17 | | 68.1 | |
| HIGH (9500 pg mL$^{-1}$) | | | | |
| 1755187.91 | 2580964.07 | 2683273.20 | 65.4 | 62.8 |
| 1610674.77 | 2754658.24 | | 60.0 | |
| 1595622.74 | 2752499.3 | | 59.5 | |
| 1728199.34 | 2795971.01 | | 64.4 | |
| 1648208.73 | 2649330.22 | | 61.4 | |
| 1777612.06 | 2566216.38 | | 66.2 | |

Table 9
Summary pharmacokinetic and statistical results of clopidogrel bioavailability study

| | $C_{max}$ (pg mL$^{-1}$) | $AUC_{0-t}$ (pg mL$^{-1}$ h) | $AUC_{0-\infty}$ (pg mL$^{-1}$ h) |
|---|---|---|---|
| Treatment A, mean (S.D.) | 2584.74 (2542.86) | 5771.82 (4736.86) | 5968.49 (4774.99) |
| Treatment B, mean (S.D.) | 2128.51 (2435.27) | 4409.07 (3307.62) | 4626.73 (3350.72) |
| Point estimate (%) | 121.54 | 124.61 | 122.79 |
| 90% Confidence interval (%) | 94.59–156.16 | 101.29–153.31 | 100.71–149.71 |
| Intra-individual CVs (%) | 69.66 | 55.71 | 52.98 |

Mean, arithmetic mean; S.D., standard deviation; Treatment A, 2 × 75 mg Clopidogrel (test formulation); Treatment B, 2 × 75 mg Clopidogrel (Plavix®, Sanofi-Synthelabo, France); CVs, coefficients of variation.

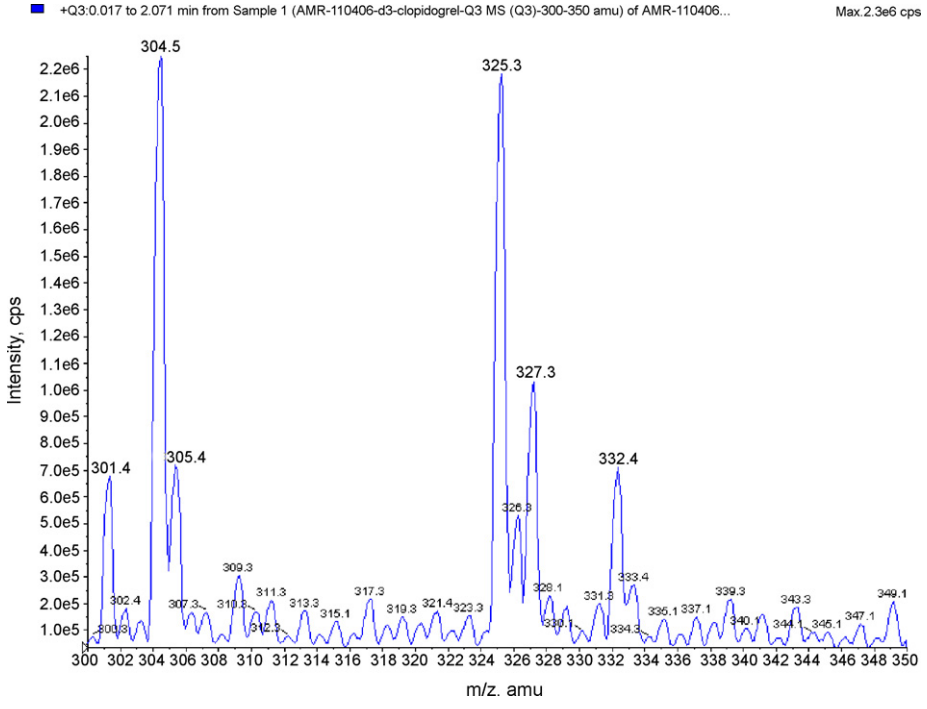


Fig. 6. MS scan of an infused $^{2}H_3$-clopidogrel standard solution.

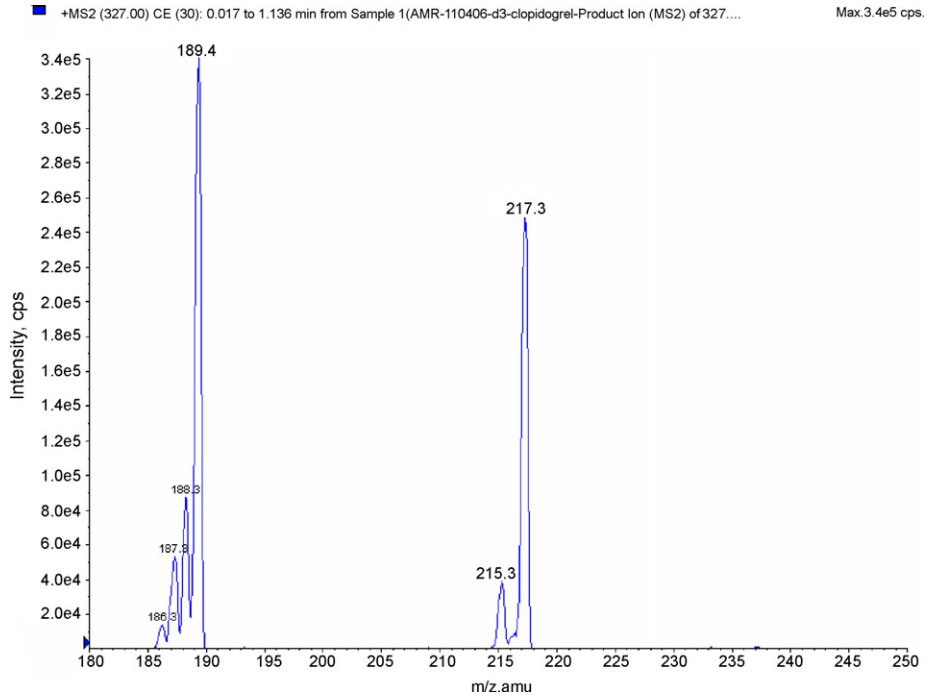


Fig. 7. Product ion (327) scan of an infused $^{2}H_{3}$-clopidogrel standard solution.

Clopidogrel was stable (±15% change in measured concentration compared to concentration in stability samples at 0 h) in human plasma for at least 4 h at room temperature (ca. 20 °C), for at least 1 month when frozen (ca. −20 °C), and following three freeze/thaw cycles (Table 6).

Clopidogrel was stable in extracts for at least 96 h when stored in the fridge (ca. 4 °C) and for at least 96 h at room temperature (ca. 20 °C).

Additionally, clopidogrel was also stable for up to at least 2 months in methanol stored in the fridge (ca. 4 °C).

An evaluation of sample-matrix interference was performed by extracting six independent sources of blank matrix and reconstituting in a standard solution of clopidogrel and internal standard. Peak areas of the extracted blank matrix samples were compared with peak areas of replicate injections ($n = 6$) of the non-extracted standard solution. No significant matrix effects were observed (Table 7).

The effect of diluting plasma was assessed by preparing QC samples ($n = 6$) with a clopidogrel concentration of 60,000 pg mL$^{-1}$. A ten-fold dilution with control human plasma

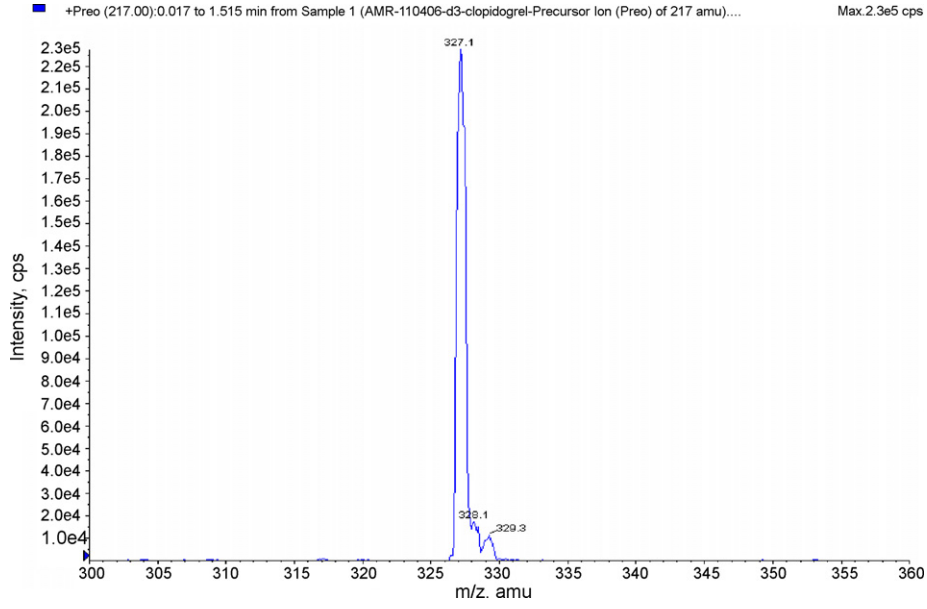


Fig. 8. Precursor ion (217) scan of an infused $^{2}H_{3}$-clopidogrel standard solution.

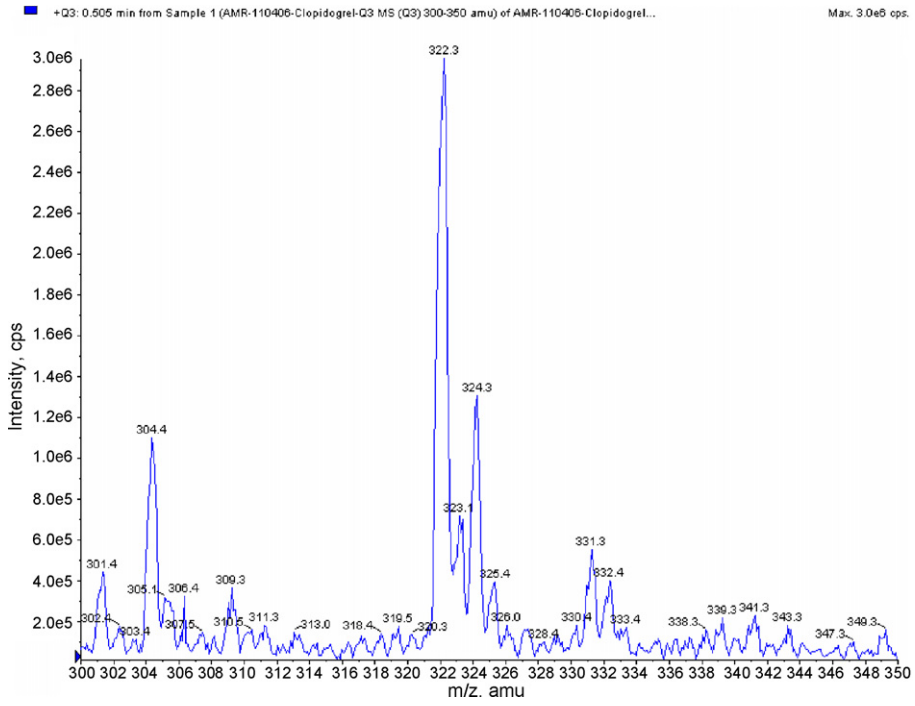


Fig. 9. MS scan of an infused clopidogrel standard solution.

was performed prior to extraction to give a nominal sample concentration of 6000 pg $mL^{-1}$. The back-calculated concentrations for dilution controls were in agreement with the theoretical (prepared) concentrations; the relative error was 6.9% after 10-fold dilution with plasma with a %CV of 3.6%.

The absolute recovery of clopidogrel was evaluated by adding known amounts of clopidogrel (at QC LOW, MEDIUM, and HIGH) to control human plasma before extraction ($n = 6 \times 3$) and comparing peak areas from these extracted standards with peak areas from standards prepared by adding clopidogrel to control human plasma after extraction ($n = 6 \times 3$). The recovery of clopidogrel was 68.6% at 30 pg $mL^{-1}$, 61.5% at 5000 pg $mL^{-1}$ and 62.8% at 9500 pg $mL^{-1}$ (Table 8).

Mean plasma concentration time curves for the test and reference formulations from 36 volunteers are shown in Fig. 5. Summary statistics for $C_{max}$, $AUC_{0-t}$ last and $AUC_{0-\infty}$ are reported in Table 9. The test formulation was found to be suprabioavailable compared to the reference formulation; the

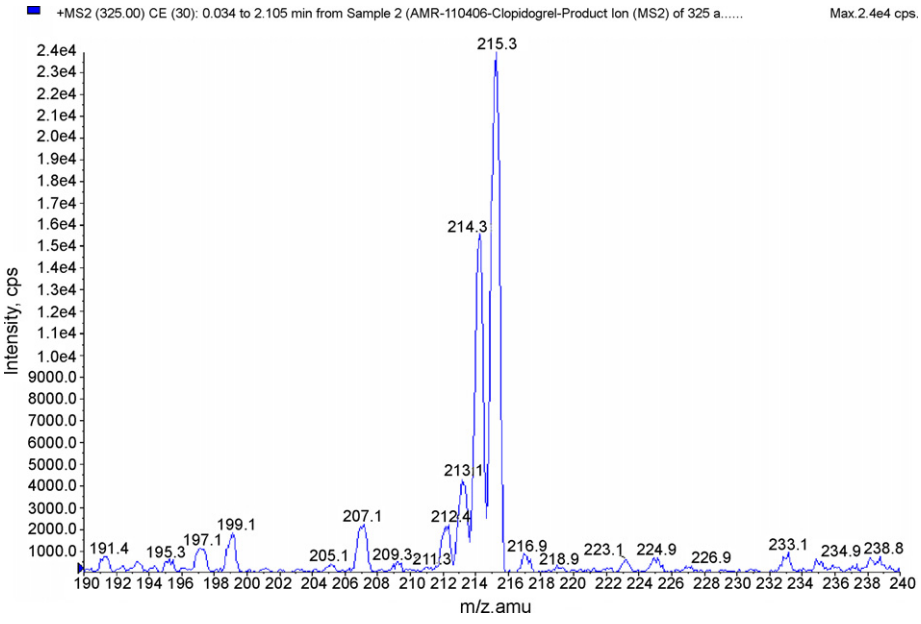


Fig. 10. Product ion (325) scan of an infused clopidogrel standard solution.

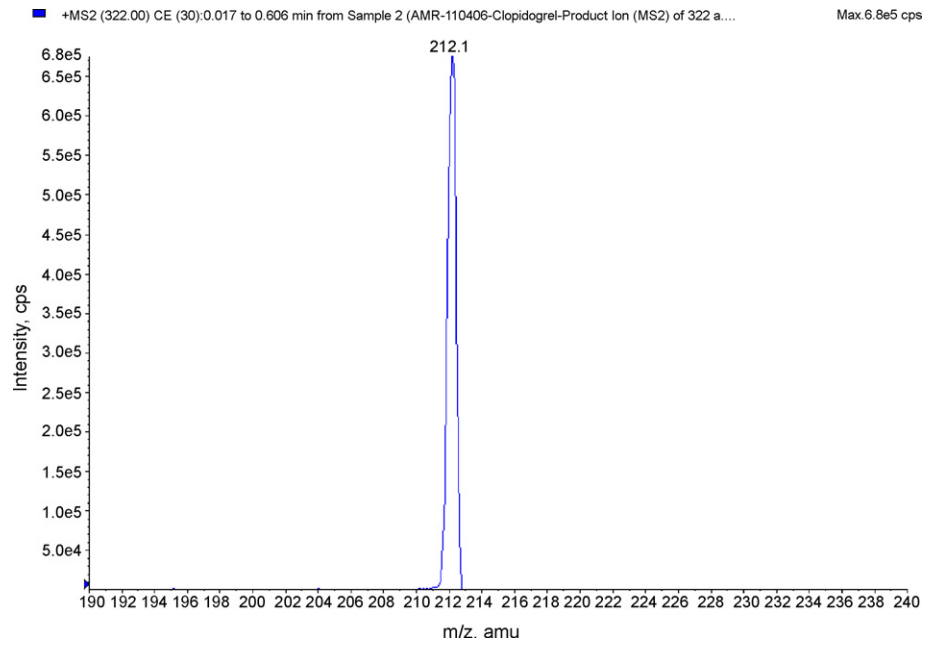


Fig. 11. Product ion (322) scan of an infused clopidogrel standard solution.

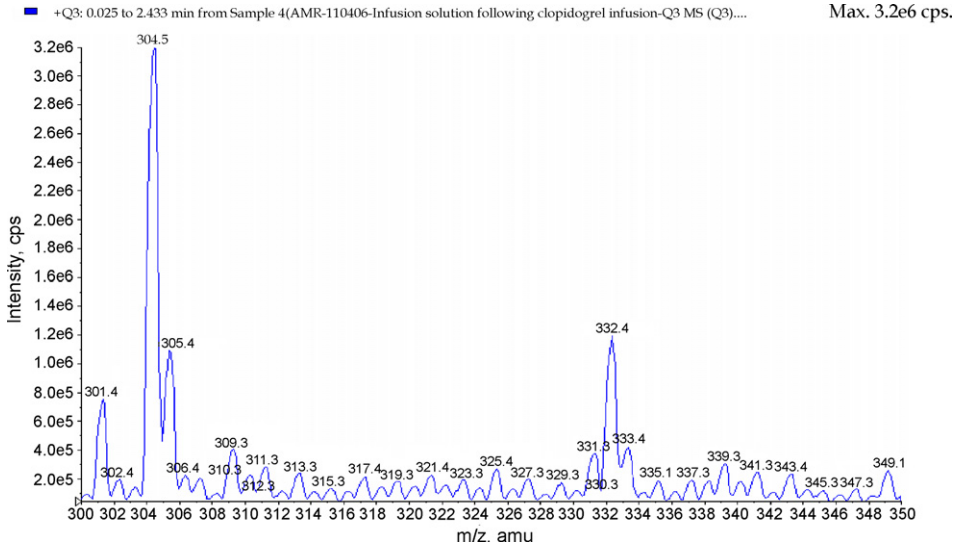


Fig. 12. MS scan of infusion solution (following infusion of clopidogrel standard solution).

intra-subject variability seen for the primary parameters was consistent with that of a drug showing highly variable pharmacokinetics.

## 4. Discussion and conclusions

An LC–MS/MS bioanalytical method for the determination of unchanged clopidogrel from human plasma has been successfully validated over the range of 10–12,000 pg $mL^{-1}$. This method competes in sensitivity with methods published previously [6,11] and uses a greater calibration range.

This rapid, sensitive and specific method was achieved using a triple quadrapole mass spectrometer and an isotopically labelled internal standard ($^2H_3$). $^2H_3$-Clopidogrel was monitored using the MRM ion transition (*m/z* 327–217, Figs. 6–8) corresponding to its $Cl_{37}$ isotopic structure to prevent interference from clopidogrel (*m/z* 325–215, Figs. 9 and 10). Fig. 11 shows a product ion (*m/z* 322–212) scan of an infused clopidogrel standard solution and Fig. 12 shows an MS scan of the infusion solution (following infusion of clopidogrel standard solution) showing that the interference was inherent of the clopidogrel stock.

The precision and accuracy of the method met and exceeded the criteria laid down in Guidance for Industry, Bioanalytical Methods Validation, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM), May, 2001, BP. Sufficient stability was shown to allow for the completion of sample analysis in clinical trials. The

method that has been described in this paper was applied in support of a pharmacokinetic study.

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