

Technical note

Single quadrupole mass spectrometry for pre-clinical pharmacokinetic analysis: Quantitation of carvedilol in dog plasma

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Abstract

The pharmaceutical industry standard for bioanalysis is LC/MS/MS. There are, however, many instances where a single quadrupole detector could successfully be used to provide adequate sensitivity and selectivity for quantitation of drug substances in biological matrices. This paper presents one example of how a single quadrupole detector can be employed in a sensitive and selective analytical method for quantitation of carvedilol. A Synergi Hydro-RP (50 mm × 2 mm i.d.; 4 μm) column was used with acetonitrile:water:formic acid mobile phase (32:68:0.01, v/v) at a flow rate of 200 μL/min into a single quadrupole mass spectrometer with an electrospray interface in the positive SIM mode. Using a 300 μL plasma aliquot and a liquid–liquid extraction procedure the limit of quantitation for the assay was 1 ng/mL. The assay utility was demonstrated in the analysis of carvedilol pharmacokinetic profiles in beagle dogs following oral carvedilol administration.

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1. Introduction

The past 15 years have seen the widespread introduction of liquid chromatography–mass spectrometry (LC/MS) in pharmaceutical industry, from drug discovery through to clinical development. LC/MS analysis provides enhanced sensitivity, selectivity and speed compared to earlier analytical methodologies, and for that reason has become the method of choice for many pharmaceutical analyses [1]. The majority of analytical methods published utilize triple quadrupole mass spectrometers (LC/MS/MS). Although the cost of these instruments has declined significantly over the past several years a significant price difference remains between a triple quadrupole detector and a single quadrupole detector. For simple pharmacokinetic studies of compounds with known metabolite profiles a single quadrupole detector can generally achieve the desired level of sensitivity more cost-effectively than a triple quadrupole instrument.

There are a number of advantages to LC/MS over LC/MS/MS, most notably the cost of an LC/MS is approximately half the cost of an LC/MS/MS [2]. Additionally, the ease of use of an LC/MS instrument makes it more amenable to multiple analysts making use of MS capabilities.

When analyzing compounds using LC/MS single quadrupole techniques the chromatographic resolution and sample preparation aspects of the analysis require additional attention compared to LC/MS/MS, although LC/MS/MS benefits from good chromatography and sample cleanup also. The sensitivity of LC/MS analysis can be extended by selected ion monitoring (SIM) of a molecular ion(s) that corresponds solely to the compound(s) of interest, rather than scanning across a large mass/charge range. Establishing chromatography such that retention time is between 3–4 min and well removed from any solvent front will also benefit the signal-to-noise ratio, minimize ion suppression and improve assay sensitivity.

Two-dimensional mass spectrometry (MS/MS) analysis can also be used for quantitation in SIM mode and the additional ability to perform selected reaction monitoring (SRM) of daughter ions formed following collision induced dissociation or fragmentation of a parent ion provides a much greater capacity for information gathering on metabolite formation and trace contaminants. However, for most pharmaceutical analyses the

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formation and detection of a single molecular ion corresponding to the molecular weight of the compound is sufficient [1].

To illustrate the capabilities of LC/MS in pre-clinical pharmacokinetic studies this manuscript presents an analytical method for quantitation of carvedilol in dog plasma. Carvedilol is a non-specific β -antagonist and an α_1 -antagonist that is used in the treatment of hypertension and congestive heart failure [3,4]. There are several published methods available for quantitation of carvedilol in plasma matrices using a variety of detection methods, such as capillary electrophoresis [5], high performance liquid chromatography (HPLC) using fluorescence detection [6–10] and tandem mass spectrometry [11–13]. Some of these methods include a chiral derivatization step for analysis of the enantiomers of carvedilol [8,9,11] while others require a relatively large plasma volume (>0.5 mL) and run times generally exceeding 10 min [5–10]. The method presented in this paper requires a small plasma volume, with minimal sample preparation before injection onto a liquid chromatography system coupled with single quadrupole mass spectrometer.

2. Experimental

2.1. Materials

Carvedilol was purchased from UltraTech (India) and naftopidil, the internal standard, from Sigma (St. Louis, MO). Acetonitrile (Fisher Chemicals, NJ), formic acid and methyl *t*-butyl ether (MTBE; Aldrich, St. Louis, MO) were LC grade. All other chemicals and reagents were analytical grade. Water was obtained from a Milli-Q water purification system (Millipore, UK).

2.2. Liquid chromatographic–mass spectrometric system

The HPLC system was a modular system consisting of a Shimadzu SCL-10A system controller, SIL-10ADvp autoinjector, an LC-10ADvp pump and a DGU-14A solvent degasser. A Phenomenex Synergi Hydro-RP column (4 μ m particle size, 50 mm \times 2.0 mm i.d., Phenomenex, CA) was used in conjunction with LCMS Solutions software (Shimadzu Scientific Instruments, MD) for data acquisition and analysis. The mobile phase consisted of 32:68:0.01 (v/v) acetonitrile–water–formic acid. The flow rate was 0.2 mL/min and the column temperature was maintained at 40 °C.

A Shimadzu LCMS 2010 single quadrupole mass spectrometer with an electrospray interface in the positive mode was used for selective ion monitoring for 407.3 (carvedilol $[M+H]^+$) and 393.3 (naftopidil $[M+H]^+$) mass/charge ion peaks. The needle voltage was 4.5 Kv, the capillary voltage was 25 v and the block temperature and capillary temperature were maintained at 300 °C. The nebulizer gas flow rate was 4.5 L/min.

2.3. Extraction procedure

A 300 μ L sample of plasma in a 15 mL polypropylene centrifuge tube was vortexed for 30 s with 100 μ L of saturated ammonium sulfate solution. Three milliliters of MTBE was

added and the tube vortexed for 1 min. The organic supernatant was transferred to a clean tube and evaporated to dryness under nitrogen. The sample was then reconstituted with a 200 μ L aliquot of 0.1% formic acid and 40% acetonitrile in water. The sample was vortexed for one minute and sonicated for 3 min before being transferred into glass HPLC autosampler vials.

2.4. Carvedilol quantitation, recovery, precision and accuracy

Aliquots of blank plasma (300 μ L) were spiked with 20 μ L of standard carvedilol solutions to provide final plasma concentrations ranging between 1 and 100 ng/mL and 20 μ L of the internal standard solution was added to give a final concentration of 67 ng/mL. Spiked plasma samples were used to generate standard curves by plotting the peak area ratio of carvedilol and internal standard against the carvedilol concentration.

Recovery of carvedilol was calculated by the comparison of the peak area of the carvedilol peaks recovered from spiked plasma samples with the peak area of injected standard solvent solutions. Within-day precision ($n=6$) and the accuracy ($n=6$) of the assay were determined by replicate analyses of spiked plasma samples at three concentrations (1, 10 and 100 ng/mL). The effect of injection volume on accuracy, precision and linearity was evaluated. The accuracy of the assay is expressed as ((observed concentration)/(expected concentration) \times 100). The assay precision for each concentration is expressed as ((peak area ratio standard deviation)/(mean peak area ratio) \times 100).

Matrix suppression effects were evaluated by using a post-column infusion of 50 ng/mL of carvedilol at 20 μ L/min and making injections of blank plasma extract from four different lots of plasma. Ion suppression of carvedilol signal was examined by the presence of negative chromatographic peaks.

2.5. In vivo Studies

The study was approved and conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee and adhered to the “Principles of Laboratory Animal Care” (NIH publication #85–23, revised 1985). Dogs were fasted for at least 16 h prior to carvedilol administration, and water was available *ad libitum*. Blood samples (1 ml) were obtained via an in-dwelling catheter in the right cephalic vein or by individual venipuncture and collected into heparinized Vactainers[®]. Plasma was immediately separated by centrifugation and frozen (–20 °C) until analysis.

Four healthy male beagle dogs (12.1–13.8 kg) were administered a 25 mg Coreg[®] tablet which was followed by 30 mL of water. Blood samples were collected pre-dose (–5 min) and 10, 20, 30, 45, 60, 75, 90, 120, 150, 180, 240 and 360 min post-dose.

3. Results

The industry standard for LC/MS analysis is tandem mass spectrometry even in situations where analysis of a parent molecular ion peaks would suffice. This manuscript presents an example of a single quadrupole LC/MS assay for quantitation of

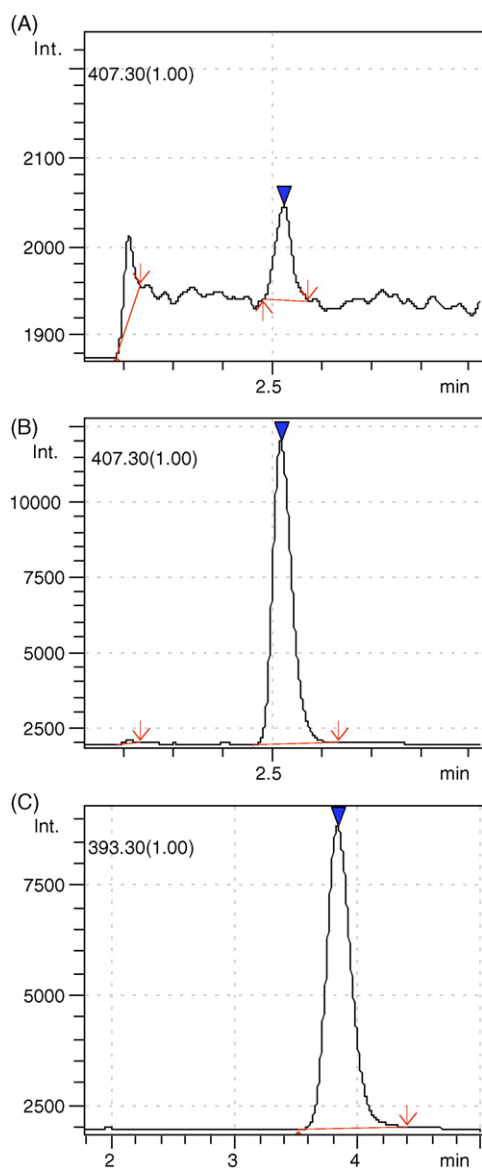


Fig. 1. Representative chromatograms of extracted dog plasma samples, following Coreg[®] administration, containing 1.54 ng/mL (panel A) and 106.3 ng/mL (panel B) carvedilol and 67 ng/mL internal standard (panel C).

carvedilol in a plasma matrix. The method demonstrates good sensitivity and accuracy, comparable to previously reported LC/MS/MS methods [12].

Fig. 1 shows representative chromatograms for carvedilol in plasma obtained during the pharmacokinetic evaluation of Coreg[®] in dogs using the extraction procedure described. Panel A shows a time point where the carvedilol concentration was 1.54 ng/mL, panel B shows a chromatogram where the carvedilol concentration is 106.3 ng/mL and panel C shows the internal standard peak. The retention time for carvedilol under these conditions was 2.5 min, and a 5.0 min run time was utilized.

The limit of quantitation for the assay was 1 ng/mL, where the signal-to-noise ratio was approximately 5:1 and the assay precision was 10.4% (Table 1).

Across the concentration range studied the extraction efficiency for carvedilol from plasma was between 93 and 99% and

Table 1

The precision, accuracy and recovery of the assay procedure for carvedilol from spiked beagle plasma samples ($n=6$) with a 5 μ L injection

Carvedilol concentration (ng/mL)	Precision (CV, %)	Accuracy (%)	Recovery (%)
1	10.4	95.0	93.4
10	10.2	102.1	98.1
100	4.8	103.7	98.7

the internal standard extraction efficiency was 76%. The assay was linear between the concentration range of 1–100 ng/mL using a 5 μ L injection volume, with correlation coefficients greater than 0.998. Evaluation of the potential for matrix related ion suppression found no evidence of significant ion suppression.

The assay was then applied to a pharmacokinetic evaluation of Coreg[®] 25 mg tablets when administered orally to four male beagle dogs. Fig. 2 shows a representative plasma versus time profile.

4. Discussion

Previously reported LC/MS/MS methods for analysis of carvedilol demonstrate limits of quantitation ranging from 0.1 to 200 ng/mL, retention times from less than 1 min up to 20 min and flow rates as high as 1.8 mL/min [10–14]. A recently published LC/MS/MS method for quantitation of carvedilol enantiomers in human plasma using a 1.5 min run time showed a high level of matrix suppression with some plasma sources [13]. The method reported in this paper has a LOQ of 1 ng/mL and a retention time of 2.5 min with a flow rate of 200 μ L/min. The assay precision and accuracy indicate that the assay performs as well as any of the LC/MS/MS and HPLC methods published on carvedilol analysis from plasma matrices, and there was no evidence of matrix related ion suppression. The linear range of the assay can be improved by reducing the injection volume.

The method described demonstrates the suitability of liquid chromatography coupled with single quadrupole mass spectrometers for simple pre-clinical pharmacokinetic evaluations of pharmaceutical compounds. The reduced selectivity of a single quadrupole instrument compared to a triple quadrupole instru-

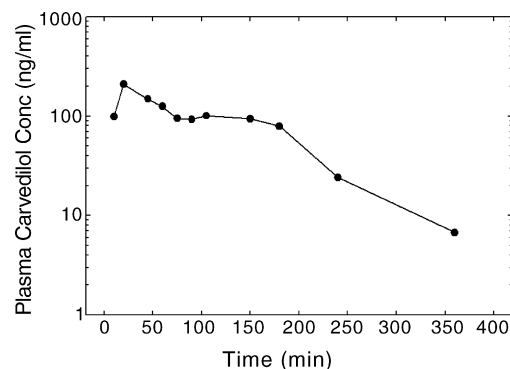


Fig. 2. A representative plasma versus time profile of carvedilol plasma concentrations in a dog following oral administration of a 25 mg Coreg[®] tablet.

ment is generally not a major limitation in pre-clinical studies where concomitant drug administration is not an issue. For clinical studies where patients may be taking several drugs, a single quadrupole mass spectrometer would still provide improved lower limits of quantitation over more traditional methods of detection in HPLC analysis, such as UV or fluorescence and even in instances of interference from a co-administered drug at the same molecular weight manipulation of the chromatography could be used to shift the interfering peak. A single quadrupole instrument is significantly less expensive to purchase and maintain than a triple quadrupole detector and still provides many advantages over more traditional methods of detection in HPLC analysis, such as UV or fluorescence. LC/MS analysis allows for increased analytical sensitivity and therefore a reduced sample volume, in addition to smaller injection volumes, which contributes to longer column life. Lower mobile phase flow rates and shorter run times also contribute to economic advantages in LC/MS analysis compared to HPLC methods.

5. Conclusions

The methods described here for the analysis of carvedilol in plasma allows for rapid sample preparation and LC/MS analysis of carvedilol concentrations between the range of 1–100 ng/mL, with an accuracy and sensitivity level equal to previously reported methodologies. The plasma volume required for sample

analysis is sufficiently small that carvedilol pharmacokinetics could be studied in laboratory animals.

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