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Rapid bioanalytical determination of dextromethorphan in canine plasma by dilute-and-shoot preparation combined with one minute per sample LC-MS/MS analysis to optimize formulations for drug delivery

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Abstract

The determination of dextromethorphan in canine plasma is used to demonstrate the high throughput bioanalytical approach of automated dilute-and-shoot (DAS) sample preparation followed by a 1 min isocratic liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis. Dilute-and-shoot preparation is commonly used for the determination of drugs in several biological matrices such as urine and saliva, but is not typically used with plasma samples because the amount of protein present in plasma can lead to a variety of problems including column failure. As a result, plasma sample preparation usually removes protein by precipitation, extraction or filtration; however, the dilute-and-shoot approach solubilizes proteins throughout the chromatographic portion of the assay. The attributes of this approach are compared with a previously validated liquid/liquid extraction procedure for determination of dextromethorphan in plasma. Accuracy and precision of both methods are similar. The lower limit of quantitation (LLOQ) of the dilute-and-shoot approach is much higher at 2 ng/ml versus 5 pg/ml with the liquid/liquid extraction; however, the sample throughput of the preparation portion of the dilute-and-shoot approach is more than 50-fold greater. The ruggedness of the dilute-and-shoot method was thoroughly investigated because of the problems traditionally associated with the direct injection of diluted plasma onto an LC-MS/MS instrument. With the optimal conditions, greater than 1000 injections of diluted plasma have been successfully performed on a single column in less than 19 h making this technique an excellent approach for the rapid preparation and high throughput of plasma samples containing drug levels in the ng/ml range or higher. Application of this methodology to measure the levels of dextromethorphan in canine plasma to evaluate drug delivery from various formulations is also presented.

Keywords: Dextromethorphan; Dilute-and-shoot; Canine plasma; Liquid chromatography; Tandem mass spectrometry; Pharmacokinetics

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1. Introduction

In the health care industry, high throughput measurement of drug levels in biological fluids is critical for minimizing time to market. One of the many reasons for measuring drug levels in biological fluids is to optimize formulation parameters that affect drug delivery to the site of action, and therefore maximize efficacy from a given dose. A wide variety of approaches for quantitation of drugs in biological matrices are available; however, the recent high throughput efforts have focused on the automated preparation of samples in the 96-well format combined with liquid chromatography tandem mass spectrometry (LC-MS/MS). Methods have been reported in the literature for solid phase extraction [1–8], acetonitrile precipitation [9,10] and liquid/liquid extraction [11,12] using 96-well technology.

The dilute-and-shoot approach for sample preparation is commonly used for drug determination in urine and saliva [13], but it is not typically used with plasma. Dilution of plasma poses a greater challenge than dilution of urine or saliva for assay ruggedness, accuracy, precision and specificity because of the greater concentration of proteins present in plasma [14,15]. In contrast to other methods of preparation that remove proteins from plasma by extraction, precipitation or filtration, the DAS approach dilutes and solubilizes the proteins throughout the chromatographic portion of the analysis. By dilution of plasma 33-fold in a compatible solvent, the present work demonstrates the successful application of the 96-well DAS approach to plasma sample preparation for the purpose of rapid quantitation of dextromethorphan (DEX, Fig. 1) in canine plasma at low ppb levels by LC-MS/MS for formulation development studies.

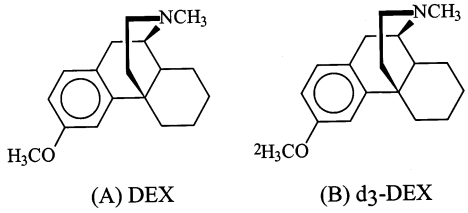

Fig. 1. Structures of (A) DEX and (B) d_3-DEX.

Dextromethorphan is the antitussive ingredient in many over-the-counter cough formulations [16–19]. A variety of methods have been employed for the analysis of DEX in plasma, urine and saliva such as HPLC with fluorescence detection [20–23], HPLC with ultraviolet (UV) detection [24], capillary electrophoresis with UV detection [25], gas chromatography (GC) with nitrogen–phosphorous detection [18] and radioimmunoassay (RIA) [26]. Typical limits of detection (LOD) for these non mass spectrometry based methods are in the range of 1 ng/ml when using 1 ml or larger sample volumes.

A previously reported LC-MS/MS method for DEX determination in plasma utilized a liquid/liquid extraction (LLE) which provides excellent sensitivity (LLOQ 5 pg/ml); however, the throughput is relatively low due to a lengthy and largely manual sample preparation [27]. For analysis of study samples to optimize drug delivery with expected DEX concentrations greater than 1 ng/ml, several methods of preparation were considered including automated acetonitrile precipitation or automation of the LLE procedure; however, the dilute-and-shoot approach was the fastest and simplest option that resulted in a rugged method with the required sensitivity in the low ng/ml range [28].

In order to gain higher throughput, the DAS approach sacrificed sensitivity that was previously shown possible with LC-MS/MS, but maintained the sensitivity required for this application and provided sensitivity comparable with that of the other published techniques listed above. Also, with the DAS approach, there is no need for column switching, on-line SPE or other complex HPLC plumbing; it is simply an off-the-shelf HPLC column used directly in-line with a Sciex API III$^+$ mass spectrometer. This method can be used to prepare more than 1000 samples in 2 h and subsequently determine DEX levels in those samples in less than 19 h of LC-MS/MS time.

Using the DAS methodology, plasma samples were prepared for analysis by automated robotic dilution with a sample diluent containing the internal standard, [$^2H_3$-*O*-methoxy]-dextromethorphan (d_3-DEX or ISTD, Fig. 1). The plasma samples were then chromatographed on a short,

high resolution reversed-phase HPLC column. Under the final chromatographic conditions, the void time was approximately 12 s and the DEX peak eluted at 30 s with a total 1.1 min cycle time for the analysis. The analyte and internal standard were selectively detected using electrospray tandem mass spectrometry. Attributes of the DAS approach are discussed along with a comparison to the previously validated LLE method for DEX quantitation and the application of the DAS methodology to large batch analysis of pharmacokinetic (PK) samples designed to optimize DEX delivery.

2. Experimental

2.1. Chemicals and reagents

DEX (USP reference standard, lot G) was obtained from the United States Pharmacopeial Convention (Rockville, MD). The stable isotope internal standard, d_3-DEX, was prepared at the Procter and Gamble Health Care Research Center (Mason, OH). Methanol (HPLC grade), diethyl ether (ACS reagent grade), sodium bicarbonate ($NaHCO_3$, reagent grade) and formic acid (reagent grade) were purchased from J.T. Baker (Phillipsburg, NJ). Blank canine plasma (sodium heparin as anticoagulant) was obtained from the Procter and Gamble animal facility (Mason, OH) according to standard animal handling procedures, or was obtained from Pel Freez (Rogers, AR).

2.2. Preparation of calibration standards and quality control samples for dilute-and-shoot

DEX stock solution was prepared at 1 μg/ml in water–methanol (50:50, v/v) and stored at 4°C. Serial dilutions of the stock solution were prepared at 2, 5, 10, 20, 50, 100, 200 and 500 ng/ml for use as standard spiking solutions and stored under the same conditions. Spiking solution aliquots (15 μl) were placed in designated wells of a 96-well plate, followed by 15 μl of blank plasma and 485 μl of sample diluent, consisting of water–methanol–formic acid (70:30:0.1, v/v/v) and 2 ng/ml d_3-DEX. Quality control (QC) samples were prepared analogously at levels of 5, 50 and 500 ng/ml.

2.3. Preparation of calibration standards and QC samples for liquid/liquid extraction

For the current studies, stock solutions containing DEX were prepared in water–methanol (50:50, v/v) at 0.002, 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20 and 50 μg/ml and stored at 4°C. DEX plasma calibration standards, covering a concentration range from 0.02 to 500 ng/ml, were prepared on the day of analysis by adding 10 μl of the appropriate DEX stock solution to 1.0 ml of plasma already spiked with 2 ng of d_3-DEX. The standards were then prepared for analysis by LLE, as described in Section 2.5. A series of control plasma samples were prepared by spiking 4 ml of blank dog plasma with an appropriate aliquot of a DEX stock solution to yield final DEX plasma concentrations of 0.05, 0.5, 5, 50, 100 and 500 ng/ml. Aliquots (1.0 ml) of the spiked QC samples, as well as control blank plasma samples, were added to Pyrex test tubes containing 2 ng of d_3-DEX and prepared for analysis by LLE, as described in Section 2.5.

2.4. Preparation of plasma samples by DAS

For the dilute-and-shoot sample preparation, dog plasma samples were collected in a 96-well format and subsequently processed using a MultiMek 96 automated pipettor (Beckman Coulter, Incorporated, Fullerton, CA). Samples were centrifuged and the 96-well plate was loaded into the sampling position of the MultiMek pipettor. Because the delivery volume of the MultiMek is limited to 200 μl, 350 μl of the sample diluent described in Section 2.2 was first transferred to autosampler vials in the 96-well format using two 175 μl aliquots. An additional 135 μl of diluent was then aspirated followed by a 25 μl air gap. A 15 μl aliquot of plasma sample was then aspirated into the tips containing diluent, and tips were then emptied into the autosampler vials. Vials were capped and the rack was mixed prior to sample analysis. The dilution resulted in a slightly viscous solution with no observed precipitation.

2.5. Preparation of plasma samples by LLE

For LLE, samples were collected in 2 ml polypropylene cryovials. Aliquots (1.0 ml) of the plasma samples were transferred to separate screw top test tubes containing internal standard and 1 ml of sodium bicarbonate buffer (pH 10.5; 0.1 M). Tubes were vortexed and 2 ml of diethyl ether was added to each test tube. Test tubes were capped with a teflon-lined cap and the samples extracted by gentle mixing for 5 min. Test tubes were centrifuged for 5 min and the ether layer of each sample was transferred to separate test tubes containing 0.2 ml of 1% aqueous formic acid. Tubes were then gently mixed 5 min to back-extract to the formic acid layer. Samples were again centrifuged and the aqueous layer was transferred to small volume autosampler vials and injected directly.

2.6. LC-MS/MS conditions

A Gilson 234 autosampler, Gilson 305/308 HPLC, (Middletown, WI), and PE-Sciex API III$^+$ triple quadrupole mass spectrometer (Thornhill, Ont., Canada) were used with a Waters Symmetry (Milford, MA) C8 column (2.1 × 50 mm, 3.5 μm) for LC-MS/MS analysis. The mobile phase for both DEX methods consisted of water–methanol–formic acid. For the DAS method the solvent ratio was 60/40/0.1 (v/v/v) while for the LLE method it was 67/33/0.1 (v/v/v). The mobile phase flow rate was 450 μl/min for the analysis of DAS samples and 300 μl/min for the analysis of LLE samples. The injection volume for all DAS standards, QCs and study samples was 5 μl. The LLE calibration standards, QC samples and study samples ranging from 0.02 to 10 ng/ml were analyzed as a batch using a 20 μl injection volume while the calibration standards, QCs and study samples ranging from 10 to 500 ng/ml were analyzed as a second batch using an injection volume of 2 μl. The entire chromatographic effluent from both methods was passed into the mass spectrometer interface for subsequent detection.

The mass spectrometer was operated in the TurboIonSpray configuration, consisting of the articulated IonSpray inlet used in conjunction with the heated TurboProbe desolvation unit.

The TurboProbe temperature and nitrogen gas flow rate were 450°C and 8 l/min, respectively, and the nebulizer gas pressure was 60 psi (nitrogen). Protonated analyte ions were generated using ESI and orifice potentials of 4000 and 70 V, respectively. Collisional activation was achieved using argon as the collision gas, at a thickness of 300×10^{13} molecules/cm^2 and a collision energy of 30 eV. The selected reaction monitoring (SRM) transitions, m/z 272–147 and m/z 275–150, were sequentially monitored for detection of DEX and d_3-DEX, respectively. The dwell time for each transition was 200 ms.

2.7. Quantitation of DEX

Peak area ratios for the SRM chromatographic peaks were determined using the PE-Sciex software package, MacQuan, version 1.4. Calibration curves were constructed by plotting peak area ratios (DEX/d_3-DEX) for standards versus DEX concentrations and fitting these data to a weighted ($1/x^2$) linear regression line, within the MacQuan software package. Drug concentrations in study samples were interpolated from this line.

2.8. Dog dosing protocol

Prior to each study, male beagles weighing between 9 and 17 kg were fasted overnight. For each study leg, one of nine different formulations containing DEX was administered to five dogs in a cross-over study design. Blood samples were collected in sodium heparin containing tubes prior to dosing and at 0.03, 0.1, 0.25, 0.5, 1 and 2 h after dosing. After centrifugation, plasma was harvested, placed in 96-well plates and stored at −70°C until the time of analysis.

3. Results and discussion

3.1. ESI mass spectra

The single stage mass spectra obtained for DEX and d_3-DEX were dominated by intense protonated molecular ions at m/z 272 and 275, respectively. The spectra for the analyte and inter-

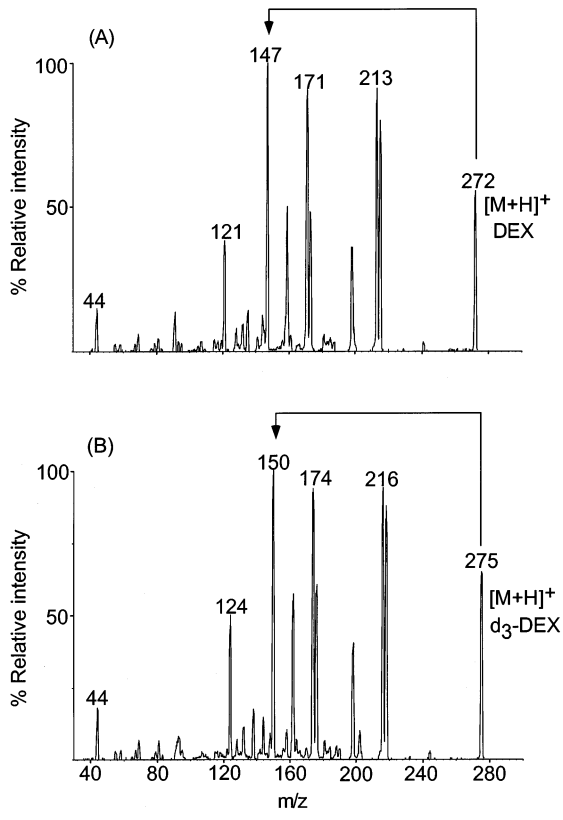

Fig. 2. Product ion spectra of (A) DEX and (B) d_3-DEX. The arrows indicate the transitions used for quantitation.

nal standard were identical except for the m/z shifts due to the incorporation of the isotopic label. The product ion spectra obtained for DEX and d_3-DEX following collisional activation of their respective protonated molecular ions are shown in Fig. 2. Fragmentation of the precursor ions resulted in an abundance of products ions presenting a variety of options for method development. Several of the SRM transitions were evaluated for sensitivity and specificity. The transition from m/z 272 to 147 for DEX and m/z 275 to 150 for d_3-DEX resulted in the best combination of sensitivity and specificity for samples prepared by both clean-up procedures and were used for quantitative applications.

3.2. SRM chromatograms of DEX in plasma after DAS and LLE preparation

A typical plasma profile obtained from a 2 ng/ml DEX spike in blank dog plasma with the DAS clean-up is shown in Fig. 3. No response is observed for the blank. A peak with a signal-to-noise ratio of approximately 5:1 is shown for a 2 ng/ml sample which was generated with 300 femtograms of DEX injected on-column. This sample was prepared by dilution of 15 μl of plasma to a total volume of 500 μl with diluent, of which 5 μl was injected on column for an equivalent of 150 nl of plasma on-column (0.015% of a 1 ml sample). For comparison, Fig. 4(B) shows a chromatogram generated by processing a full 1 ml of plasma containing 2 ng of DEX by LLE. The resulting chromatogram shows no interferences and an excellent signal-to-noise of approximately

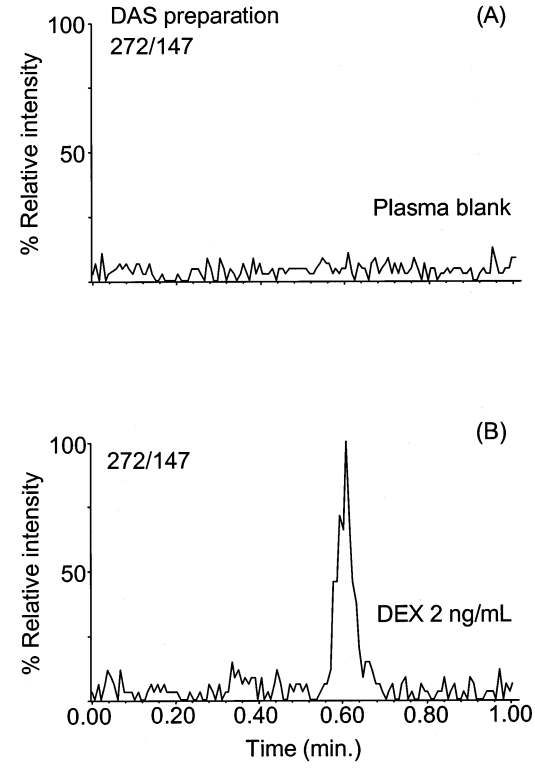

Fig. 3. SRM chromatograms of a (A) plasma blank and (B) 2 ng/ml DEX spike in plasma using dilute-and-shoot sample preparation (300 fg on column).

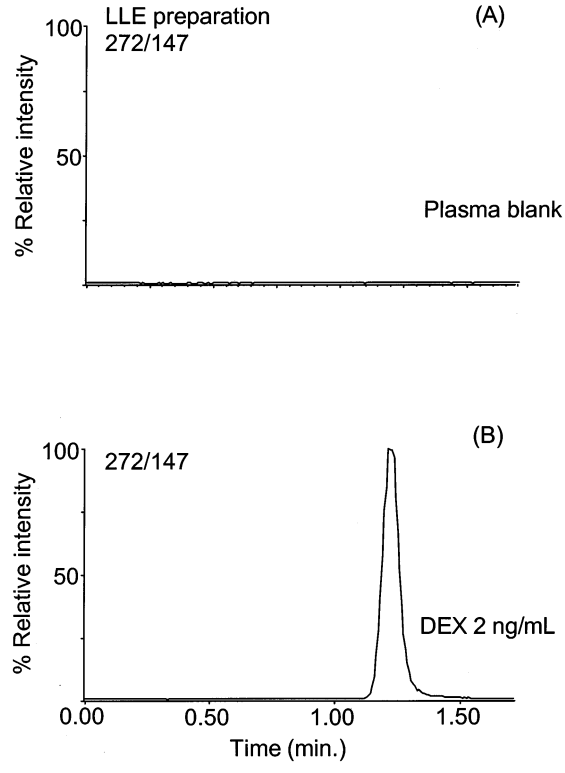

Fig. 4. SRM chromatograms of a (A) plasma blank and (B) 2 ng/ml DEX spike in plasma using liquid/liquid extraction sample preparation (170 pg on column).

2000:1. In this case, 170 pg of DEX (10% of a 1 ml sample with 84% recovery of DEX) was injected on column. Fig. 5 shows the SRM chromatograms of DEX and d_3-DEX generated upon analysis of a typical study sample prepared by DAS; this sample contained 31.6 ng/ml of DEX. No significant signal suppression was observed from samples prepared by either method.

3.3. Calibration, accuracy and precision of DAS LC-MS/MS methodology

Calibration of the mass spectrometer from 2 to 500 ng/ml of DEX was typically performed for analysis of the formulation study samples using the DAS method. Calibration with this range of standards resulted in linear curves (weighted with $1/x^2$) with correlation coefficients of greater than 0.995. The accuracy and precision data for LC-MS/MS QC samples at 5, 50 and 500 ng/ml for representative PK studies are presented in Table 1. For all sets of QC samples, the average accuracy across the entire range of spiked DEX concentrations was within 7% of the spiked value. The precision, as assessed by the relative standard deviation (RSD), for the replicate sample preparations was 8% or better. For multiple plate batches, calibration standards were placed on one plate and a minimum of three QC samples were placed on every plate to assess possible plate-to-plate variation. For this method, no significant bias was observed from plate-to-plate.

3.4. Ruggedness of DAS methodology with plasma samples

Ruggedness of the dilute-and-shoot approach was extensively investigated because of the possibility of column failure, loss of MS sensitivity, reduction of accuracy and/or loss of precision when directly injecting diluted plasma onto an LC-MS/MS instrument. To maximize ruggedness, the volume of plasma injected per analysis was reduced as far as possible to still meet the sensitiv-

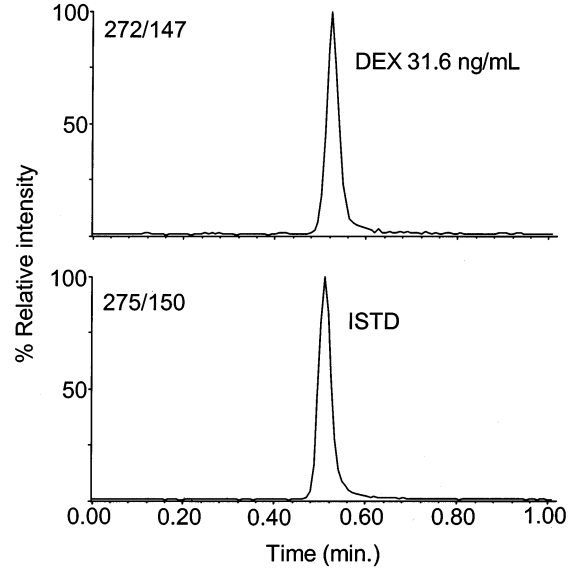

Fig. 5. SRM chromatograms of DEX and d_3-DEX generated during analysis of a PK study sample prepared by DAS and containing 31.6 ng/ml of DEX.

Table 1
Average recoveries and relative standard deviations for QC samples using DAS preparation combined with LC-MS/MS

Day	*n*	DEX spike (ng/ml)	Mean recovery (%)	RSD (%)
1	3	5.0	93.0	3.9
	3	50.0	103.2	2.6
	3	500.0	97.7	4.4
2	3	5.0	96.6	3.6
	3	50.0	94.4	1.9
	3	500.0	97.7	4.9
3	15	5.0	93.6	5.8
	15	50.0	98.5	8.0
	15	500.0	95.7	7.8

ity requirements of these studies. The final conditions were a 1:33 dilution of plasma with a 5 μl injection; these conditions resulted in an equivalent volume of 150 nl of plasma injected for each sample analysis or 15 μl of plasma on-column per 100 injections.

To demonstrate the method ruggedness, calibration standards, plasma blanks and QCs were prepared using plasma collected from fasted dogs. A shot-to-shot run time of slightly more than 1 min was used and 1031 injections were performed on one column in less than 19 h. Plots of d_3-DEX peak height, peak area and percent recovery versus injection number are shown in Fig. 6. The d_3-DEX peak height stayed essentially constant while the peak area dropped by only 30% over the entire run. Recoveries of calibration standards and QC samples with spiked DEX levels of greater than 5 ng/ml were included in Fig. 6(C) so that the variability would be minimized to observe slight trends in the data. The accuracy of the QC samples was consistent throughout the run and the average percentage recovery for the study was 99.3% with a 5.7% RSD. The column back pressure was not a problem and actually dropped from 3.3 to 3.1 kpsi from the first to last injection.

The successful application of methods using the DAS approach is dependent on several factors including the analyte, dilution factor, diluent, organic content of the mobile phase and injection volume. There is a direct trade-off between ruggedness and LLOQ depending on the needs of a given assay. This method was applied for ultra high throughput at the ppb level and all variables were balanced so that there were no issues with ruggedness. Lowering the LLOQ of this method can be achieved by increasing the injection volume to increase the amount of DEX on-column. However, this does reduce the number of injections that can be made on a particular column before failure occurs.

One further variable investigated was the use of plasma from non-fasted versus the fasted dogs used for these studies. Cholesterol, triglyceride, high density lipoprotein (HDL), low density lipoprotem (LDL), very low density lipoprotein (VLDL) and total protein were determined in plasma of a single dog after fasting overnight, and 4 h after eating 240 g of Purina high protein dog chow. The primary plasma difference observed between the fasted and non-fasted states was total triglyceride level. The level of triglycerides in the fasted dog samples was determined to be 23 mg/dl, while the animal in a non-fasted state had plasma triglycerides of 97 mg/dl. VLDL was also higher in the non-fasted animals (19 mg/dl non-fasted, vs 5 mg/dl fasted), while LDL was determined to be slightly higher in the fasted animals (5 mg/dl non-fasted, vs 10 mg/dl fasted). Plasma cholesterol, HDL and protein levels were roughly equal for the two types of plasma. To examine the effect of these differences on the methodology, plasma from both lots were used to prepare blanks, calibration standards and QC samples for analysis using the DAS procedure. The results from the fasted animal are summarized above. For the non-fasted samples, over a 600 injection run, the peak area and height of the internal

standard dropped about 2-fold. The average accuracy was 93.8 with a 9.5% RSD. These values were not as good as those observed when using the fasted plasma. During the course of 600 injections, there were no back pressure problems and

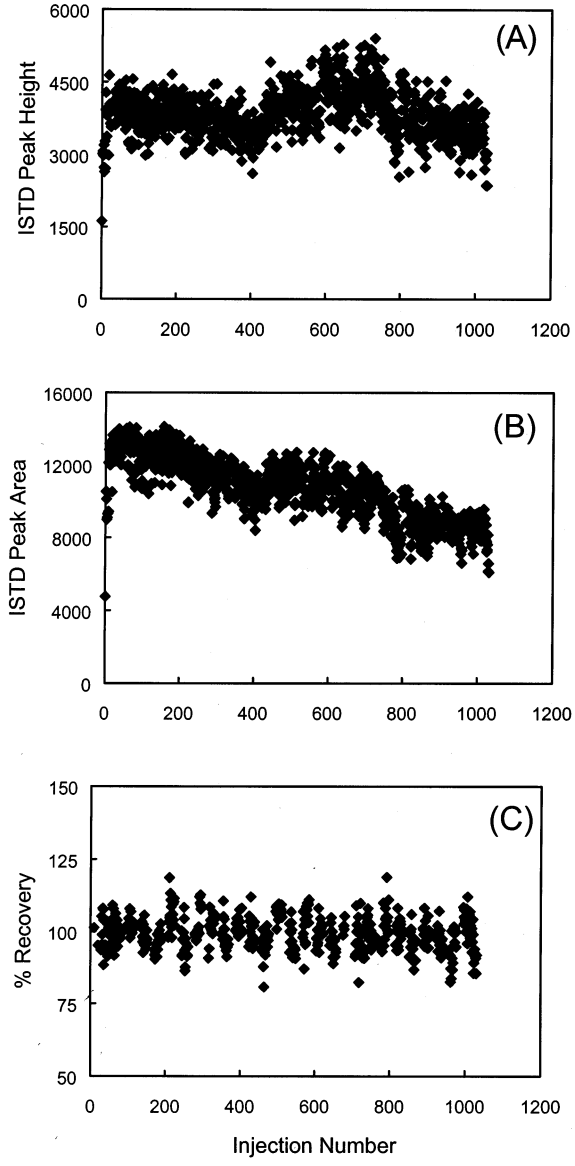

Fig. 6. Plots of (A) internal standard peak height; (B) internal standard peak area and (C) percentage recovery versus injection number to demonstrate the ruggedness of the plasma dilute-and-shoot methodology for a single, 19 h, 1031 injection run.

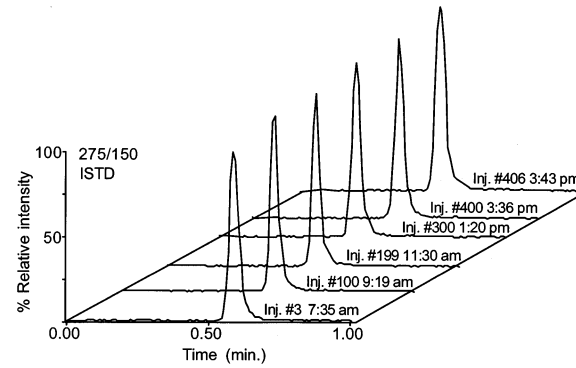

Fig. 7. SRM chromatograms of the internal standard generated throughout the analysis of a batch of PK study samples using dilute-and-shoot sample preparation.

no significant loss of peak shape. Generally, the method did not perform quite as well with non-fasted plasma as with fasted plasma; however, it was acceptable.

Similar ruggedness was observed when running PK samples collected from several different fasted animals in one batch. The peak shape of the ISTD throughout a 406 sample PK batch is shown in Fig. 7 at the beginning, every 100th injection throughout the run (or as near to every 100th injection as possible due to blank injections) and at the end of the run. Slight peak broadening is observed, but overall the peak shape is consistent throughout the batch run.

3.5. Comparison of DAS and LLE methodologies

Several samples from a PK study were prepared by both the DAS and LLE methods to assure that comparable results would be obtained to address concerns that the dilute-and-shoot preparation might not efficiently extract DEX from canine plasma. Table 2 displays the comparison of drug levels determined in study samples by both preparations showing that the results for all samples analyzed are within 10% for both methodologies with an average 2% bias toward recovery from the LLE.

Linearity of both methods is adequate for their intended purposes. The LLE method has been shown to be linear over four orders of magnitude while the DAS method has been shown to be

Table 2
Comparison of quantitative results produced by liquid/liquid extraction and dilute-and-shoot sample preparation of plasma samples followed by LC-MS/MS determination of DEX levels

Sample	Liquid/liquid extraction DEX (ng/ml)	Dilute-and-shoot DEX (ng/ml)	Deviation (%)
33-0	0.0	0.0	–
33-1	241.9	254.0	5.0
33-2	118.0	118.1	0.1
33-3	130.3	127.2	−2.4
33-4	74.1	66.6	−10.1
33-5	52.5	51.4	−2.1

linear over more than two orders of magnitude. A more extensive linear range was not investigated for the DAS methodology. Accuracy and precision of both methods is comparable as defined by recoveries of spiked plasma samples and analysis of study samples by both methodologies. The specificity of the transitions used for detection eliminated chemical noise for both preparations; this is an important factor in successfully applying the DAS methodology. The ruggedness of both methods is adequate, whereby bioanalytical batches of several hundred plasma samples can be prepared and analyzed with a single HPLC column with very little chance of HPLC or MS failure. The ruggedness of the LLE method was not dimensioned as extensively for single batch runs, but typically a single column can be used for a minimum of 500 injections prior to replacing the column provided that there is a good separation between the organic and aqueous phases prior to the back extract step. Stability of DEX in plasma samples prepared by either clean-up was not an issue.

The practical differences in these two preparation methodologies is observed most strikingly in sensitivity and throughput. The LLE preparation has an LLOQ of 5 pg/ml in plasma while that of the DAS method is 2 ng/ml. This 400-fold difference is accounted for in the amount of DEX that is injected on the column. For throughput, the manual LLE method can be used to prepare an average of ten samples per analyst per hour while a single analyst can use the DAS method to process approximately 576 samples (six plates) per hour when considering the preparation step alone, and excluding preparation of diluent, documentation and other activities usually associated with sample preparation. This results in an improvement in preparation throughput of more than 50-fold. Recently, the LLE method has been automated in the 96-well format using a MicroLab AT plus 2 (Hamilton Company, Reno, NV) to increase throughput from ten samples per hour to 96 samples per hour (one plate) which places the throughput of this methodology somewhere between that of automated DAS and manual LLE. Another difference in the DAS and LLE methods is that the DAS approach has been successfully applied using very small volumes of plasma (15 μl). This attribute is especially important when working with small animals.

3.6. Pharmacokinetic study

The results of a typical formulation study using

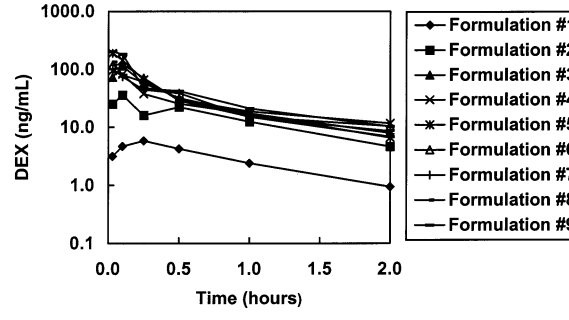

Fig. 8. Plots of average DEX levels measured in canine plasma by DAS preparation followed by LC-MS/MS analysis. Each curve shows the average DEX plasma levels in five dogs after dosing with a given formulation over the 2 h collection period.

the DAS methodology are graphically displayed in Fig. 8. These plots are the results of dosing nine different exploratory formulations to five dogs in a cross-over study design. Significant effects of various formulation parameters on drug delivery are readily obtained from these data which is then used for further optimization that eventually results in selection of an optimal dose form.

4. Conclusions

By leveraging the sensitivity and specificity of MS/MS, it is possible to use simple dilution as the only preparation step for LC-MS/MS quantitative analysis of drugs at the low ng/ml level in canine plasma. Accuracy, precision and linearity of the dilute-and-shoot approach are very good, and are comparable with results obtained with liquid/liquid extraction. Although the LLOQ (pg DEX/ml of plasma) of the liquid/liquid extraction method is 400 times lower than the LLOQ of the DAS method, the throughput of the sample preparation step for the dilute-and-shoot method is approximately 50 times higher. In addition, the dilute-and-shoot approach only requires a very small sample volume of 15 μl. Ruggedness of the DAS method of plasma preparation was demonstrated for large sample batches (>1000), and application of the automated dilute-and-shoot LC-MS/MS approach to drug quantitation in plasma allows high sample throughput and rapid feedback to formulators for iterative optimization of drug delivery.

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