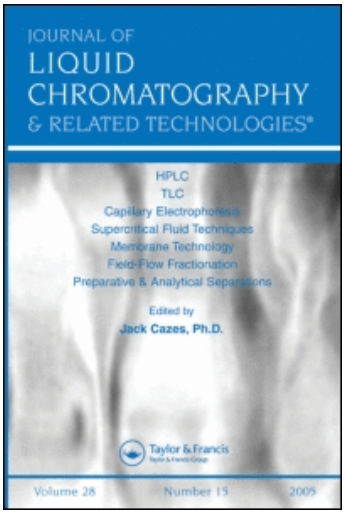

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A Sensitive Assay for the Determination of Meloxicam in Swine Plasma by Liquid Chromatography-Mass Spectrometry

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A Sensitive Assay for the Determination of Meloxicam in Swine Plasma by Liquid Chromatography-Mass Spectrometry

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Abstract: A liquid chromatographic-mass spectrometry (LC-MS) method for the determination of Meloxicam in swine plasma is described. The samples were extracted with solid phase extraction (SPE), cleaned up with water, and eluted with methanol. After they had been evaporated to dryness, diluted with acetonitrile-water, and filtered through a Spin-X micro-centrifuge tube, the samples were injected into LC-MS. The limit of quantification was 10 ng/mL.

Keywords: Meloxicam, NSAID, Plasma, Liquid chromatography-mass spectrometry, LC-MS, Anti-inflammatory

INTRODUCTION

Meloxicam (MEX) is a non-steroidal anti-inflammatory drug (NSAID) of the oxicam class. It is a potent inhibitor of prostaglandin synthesis via a preferential inhibition of cyclo-oxygenase (COX)-2.[1] The drug has anti-inflammatory, antipyretic and analgesic effects.

In veterinary medicine, MEX is marketed for use in swine for alleviation of inflammatory conditions (e.g., locomotors disorders, mastitis, metritis, and agalacti syndrome), in combination with appropriate antibiotic therapy.[1] An indication for the use of this drug in piglets is analgesic treatment after surgical procedures like castration and tail docking.

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Animal studies have shown that in addition to high anti-inflammatory efficacy, meloxicam appears to have low ulcerogenic potency.[2]

Various sample preparation techniques were employed, including protein precipitation[3] and protein precipitation combined with liquid–liquid extraction.[4] HPLC with UV detection at 364 nm has been used, and by this method one was able to detect a minimum concentration of 29 ng/mL in plasma.[5] These methods are not sensitive enough for pharmacokinetic studies where plasma concentrations have to be assayed for a period of about five half-lives. A method based on LC-tandem mass spectrometry has been reported for analysis of meloxicam with a limit of quantification of 8.96 ng/mL.[6]

The purpose of the present study was to develop a simple, specific, and sensitive LC-MS (single quadrupole) method for the determination of MEX in plasma. The sensitivity should meet the requirements for a pharmacokinetic study in piglets.

EXPERIMENTAL

Materials and Reagents

Samples of drug free swine plasma were used as control material and for spiking with MEX.

All chemicals and solvents were of analytical or HPLC grade. MEX was supplied by Sigma Co. (St. Louis, MO, USA), stock standard solutions (1 mg/mL) was prepared by dilution with acetonitrile. The working standard solutions were made by aliquots from these stock solutions diluted with water. Stock and working standard solutions were stored at $+4^{\circ}$C.

SPE columns Oasis® HLB 3 cc (60 mg) were purchased from Waters (Ireland) and Spin-X centrifuge filter units 0.22 μm, nylon type (Costar, NY, USA) were used for filtration.

Chromatographic Conditions

The LC-MS instrumentation used for the method development, consisted of a Series 200 quaternary pump and autosampler (Perkin Elmer, Norwalk, CT) and an API 100 single quadrupole mass spectrometer (Applied Biosystems, Ontario, Canada) equipped with a Turbo-Ion Spray ion source. The turbo probe of the interface was maintained at 170°C and the probe air flow rate was 6 L/min. The N_2 nebulizer and curtain gas was 5 and 10 L/min, respectively. The MS was set to collect single ion data in positive ion mode at m/z 373.9 for 10 min. The entrance electrode voltages and the position of the ion spray inlet were adjusted to provide optimum intensity for the molecular ions.

The analytical column (Chromolith Performance RP-18e, 100 × 4.6 mm i.d.) and the guard column (5 × 4.6 mm i.d.) (Merck, Germany) were operated at a constant temperature of 23°C. The guard column was connected to an A-318 precolumn filter on line, with an A-102X frits (Upchurch Scientific, USA). The mobile phase consisted of a mixture of three solutions. Solution A consisted of 999 mL water added to 1 mL formic acid, solution, B was methanol, and solution C was acetonitrile. The mobile phase operating conditions are shown in Table 1. After separation, the LC fluent was connected to a two position micro electric valve actuator (Vici, Valco Instruments Co. Inc. Texas, USA) and programmed in mode two by our Norwegian provider. Thereafter, the LC fluent was split approximately 1:20 before entering the MS interface.

Sample Pretreatment

A volume of 0.5 mL water, 0.2 mL 0.2% formic acid in water was added to 1 mL plasma. The mixture was homogenized and loaded on a conditioned Oasis column.

Clean-Up SPE Column

The column was conditioned with 3 mL methanol, followed by 3 × 3 mL water (column volumes) before the extract was applied. Conditioning and application of the sample took place under gravity flow (dropwise rate). The column was washed with 3 × 3 mL water in a vacuum of − 5 in. Hg, and sucked to dryness for 10 sec in a vacuum of −10 in Hg. The elution of the analytes was achieved with 2 × 2.25 mL methanol at a vacuum of −3 in Hg. The organic layer was evaporated to dryness under a stream of air at 40°C. To the dry residue, 0.2 mL acetonitrile was added before it was whirl mixed for 10 sec and left in an ultrasonic bath for 2 min. After addition of 0.3 mL water, the sample was mixed again to reconstitute the

Table 1. Mobile phase operating conditions

Step	Total time (min)	Flow (μL/min)	Solution A (%)	Solution B (%)	Solution C (%)	TE#1
0	0.0	1200	38	62		Open
1	2.8	1200	38	62		Close
2	5.0	1200	19	47	34	Close
3	9.0	2000	38	62		Open
4	10.0	1200	38	62		Open

TE#1 = events.

residue. The sample was then filtered through a Spin-X centrifuge filter. Aliquots of 80 μL were injected into the LC-MS at intervals of 10 min for the determination of MEX.

Calibration Curves and Recovery Studies

The precision, recovery, and linearity for MEX were determined by spiking swine plasma samples with standard solutions to yield 10, 20, 50, 100, 200, 500, and 1000 ng/mL. The recovery was determined by comparing the analyses of spiked plasma samples with those of standard solutions of MEX. The linearity of the standard curves for MEX in swine plasma was calculated using peak height measurements.

RESULTS AND DISCUSSION

The standard curves were linear in the investigated areas from 10 to 1000 ng/mL for MEX. The linear correlation coefficient was $r = 0.9997$ for MEX in plasma. The recovery varied from 96 to 99%, and the standard deviation from 0.54 to 1.08% in swine plasma. The recovery and repeatability values for MEX from swine plasma are shown in Table 2.

Chromatograms obtained from swine drug free plasma samples, and from the corresponding sample spiked with MEX are shown in Figure 1.

Different solutions were tested for optimum ionization of the analytes and it was found that formic acid gave the best result.

Protein precipitation and direct injection is the most cost effective method compared with solid-phase and liquid–liquid extraction methods, but a more powerful instrument (LC-MS-MS) is necessary.

The limit of detection for MEX was calculated as three times the peak-to-peak baseline noise ($S/N = 3$) from drug-free swine plasma. It was 5 ng/mL. The limit of quantification was 10 ng/mL.

Table 2. Recovery and repeatability for MEX from spiked of swine plasma

Number of samples	Amount of drug (ng/mL)	MEX (%) SD	MEX (%) RC
5	10	1.08	96
5	50	0.54	96
5	200	0.96	98
5	1000	0.60	99

SD = Standard deviation.
RC = Recovery.

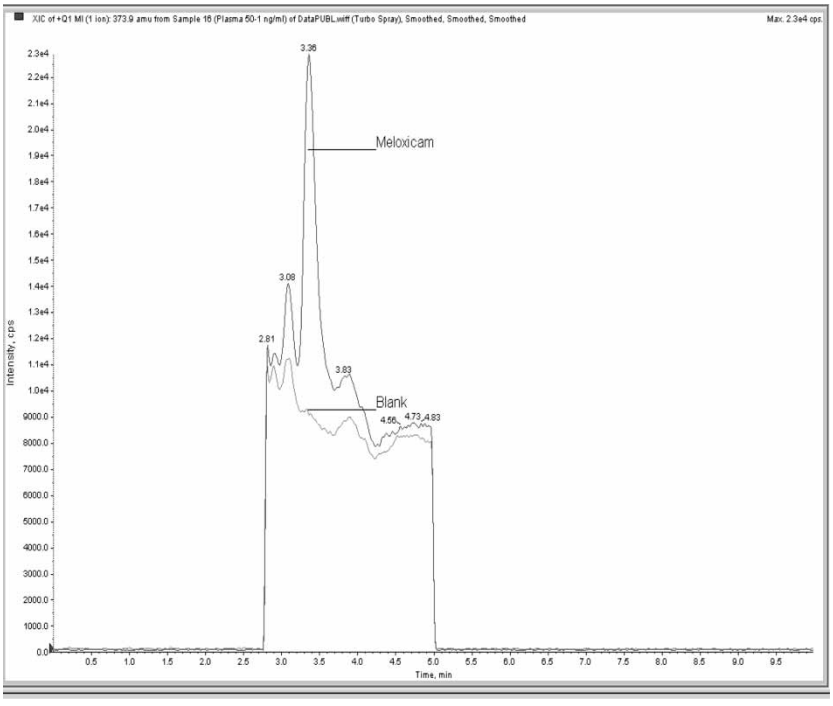

Figure 1. Chromatograms of extracts from swine plasma. Drug free plasma (blank) and plasma spiked with 50 ng/mL Meloxicam.

In many laboratories, a stream of nitrogen is used to evaporate samples to dryness. In this study, air produced from a central air compressor was used for evaporation. The use of air is a practical and economically favorable alternative compared with nitrogen, when the analytes of interest are not easily oxidized.

The use of a two position micro electric valve actuator avoids use of unnecessary mobile phase and, thereby, possible contamination from sample extract streaming into the MS. The use of the micro electric valve actuator appears favorable in all MS analyzing. It is essential that the actuator is programmed in mode two. The micro electric valve actuator was guided from data software under LC pump (events). When the event is open, the mobile phase flows to waste. When the event is closed, the mobile phase flows to the analytical column.

The LC separation is done with a Chromolith Performance column. The Chromolith (Merck) analytical column is a relatively new generation analytical column, developed on the basis of a new gel process for the preparation of monolithic porous silica rods using highly pure metal free alkoxysilanes. The silica rod possesses a biporous structure, typically consisting of macropores

and mesopores in the skeleton, providing a higher porosity compared with particulate columns. Consequently, Chromolith^{TM} columns can be operated at higher flow rates without loss of performance and limitations, due to the column back pressure.

The LC-MS (single quadrupole) method presented is selective, robust, and the sensitivity satisfies the requirements for a pharmacokinetic study in piglets.

The advantage of the LC-MS technique lies in the combination of the separation capabilities of HPLC and the power of MS as an identification and confirmation method with high sensitivity, selectivity, and quantitative capability. While conventional HPLC methods may require long complex separations, the LC-MS method generally requires only a simple clean up procedure and no derivatization.

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