Analytical Chemistry Research 3 (2015) 63–69

Contents lists available at ScienceDirect

Analytical Chemistry Research

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

Micellar high performance liquid chromatographic determination of flunixin meglumine in bulk, pharmaceutical dosage forms, bovine liver and kidney

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ARTICLE INFO

Keywords:
Micellar liquid chromatography
Flunixin meglumine
Bovine liver
Bovine kidney

ABSTRACT

A simple, sensitive and rapid liquid chromatographic method was developed and validated for the analysis of flunixin meglumine (flunixin-M) in bulk, pharmaceutical dosage forms, bovine liver and kidney. Analytical separation was performed in less than 4 min using a C_{18} column with UV detection at 284 nm. A micellar solution composed of 0.15 M sodium dodecyl sulphate, 8% *n*-butanol and 0.3% triethylamine in 0.02 M phosphoric acid buffered at pH 7.0 was used as the mobile phase. The method was fully validated in accordance with the International Conference on Harmonization (ICH) guidelines. The limit of detection and the limit of quantitation were 0.02 and 0.06 μg mL^{-1}, respectively. The recoveries obtained were in range of 95.58–106.94% for bovine liver and kidney. High extraction efficiency was obtained without matrix interference in the extraction process and in the subsequent chromatographic determination. The method showed good repeatability, linearity and sensitivity according to the evaluation of the validation parameters.

1. Introduction

Flunixin, 2-[[2-methyl-3-(trifluoro-methyl) phenyl] amino]-3-pyridine carboxylic acid (Fig. 1) [1], is usually found as its meglumine salt. Its actions are related to its ability to inhibit cyclooxygenase. It is used in horses for the alleviation of inflammation, pain associated with musculoskeletal disorders and visceral pain associated with colic. In cattle, it is indicated for the control of pyrexia associated with bovine respiratory diseases, endotoxemia and acute bovine mastitis [2]. Flunixin was the second leading violative residue reported in 2007, so the FDA Center for Veterinary Medicine (FDA-CVM) warned veterinarians to use flunixin in the proper and labeled manner. The FDA-CVM states that using a different route of administration for convenience is not adequate reason for extra label use, making most intramuscular or subcutaneous use of flunixin illegal. Given intravenous, the label withdrawal time is 4 days and the milk withdrawal is 36 h. Given intramuscular or subcutaneous the withdrawal time may be more in order of 40 days [3]. Flunixin has no Codex maximum residual limits (MRLs) approved for use at national level for food animals and definitive MRLs have been established for use in veterinary medicinal products in the EC (European Commission) (Annex I of Regulation, No. 2377/90), in bovine liver 300 μg/kg and in the kidney 100 μg/kg [4].

Various methods have been reported for the determination of flunixin-M including electrochemical [5], gas chromatography [6–8], thin layer chromatography [9], spectrophotometric [10]. Few liquid chromatographic (LC) methods have been reported for its determination. It was determined in swine muscles and processed food using tandem mass spectrometric detection [11,12], also in horse urine, mutton muscle, pharmaceutical dosage forms and bovine plasma using UV detection [9,13–15].

Micellar liquid chromatography (MLC) allows complex matrices to be analyzed without the need of extraction and with direct injection of the samples [16]. Micelles tend to bind proteins competitively, thereby releasing protein-bound drugs and proteins, rather than precipitating into the column. Proteins are solubilized and washed harmlessly away, eluting with the solvent front. This means that costs and analysis times are cut considerably [17]. Micellar mobile phases usually need less quantity of organic modifier and generate less amount of toxic waste in comparison to aqueous–organic solvents, so that they are less toxic, non-inflammable, biodegradable and relatively inexpensive [18]. MLC has proved to be a useful technique in the determination of diverse

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http://dx.doi.org/10.1016/j.ancr.2014.12.003
2214-1812/

groups of compounds in several matrices [19–24], including food samples [25–28].

The aim of the present study was to develop simple, rapid, sensitive, selective and relatively inexpensive LC method for analysis of flunixin-M in bulk, dosage forms and in bovine liver and kidney with a simple and rapid sample preparation especially for the routine analysis.

2. Experimental

2.1. Materials

Pure flunixin-M sample was kindly supplied by Delta Pharma, Cairo, Egypt. Its purity was of 99.9% as stated by the supplier. Flunidyne injections, B.N. 0846/11, each mL is labeled to contain 83 mg flunixin-M equivalent to 50 mg flunixin, a product of Arab company for medical products, Egypt, purchased from local market. Bovine liver and kidney were purchased from the local market.

2.2. Reagents and chemicals

All reagents and solvents used were of HPLC grade. High purity water was used throughout the study.

Ortho-phosphoric acid (85%, w:v), 1-propanol and *n*-butanol were obtained from Sigma–Aldrich (Germany). Methanol and acetonitrile were obtained from Fisher Scientific (UK). Sodium dodecyl sulphate (SDS) was obtained from Oxford Laboratory, Mumbai (India). Triethylamine was obtained from SD-Fine-Chem. limited (India). Nylon filters and syringe filters were from Sartorius–Stedium (Goettingen, Germany).

2.3. Instrumentation

Chromatographic analysis was carried out using a Shimadzu Prominence HPLC system, (Shimadzu, Japan) with a LC-20 AD pump, DGU-20 A5 degasser, CBM-20A interface, and SPD-20A UV–Vis detector with 20 μL injection loop. Centrifugation was carried out using a TDL-60 B Centrifuge (Anke, Taiwan). Ultrasonic bath used was BHA-180 T (Abbotta, USA) was used. Tissue homogenization was made using Tissue Master-125 with 7-mm stainless steel generator probe (Omni International, GA, USA). The pH was measured with Jenway pH meter, 4510, (Essex-UK). The mobile phase was filtered through Charles Austen Pumps Ltd. Filter, model-B100 SE (England, UK) using 0.45 μm milli-pore filters (Gelman, Germany).

2.4. Chromatographic conditions

MLC was performed on Shim-Pack VP-ODS column (150 mm × 4.6 mm i.d., 5 μm particle size) Shimadzu, Japan using micellar mobile phase consisting of 0.15 M sodium dodecyl sulphate, 8% *n*-butanol and 0.3% triethylamine in 0.02 M ortho-phosphoric acid buffered at pH 7.0. The mobile phase was filtered and sonicated for 30 min before use. The flow rate was 1.0 mL/min and sample injection volumes were 20 μL at room temperature (25 °C). The UV detector was operated at 284 nm.

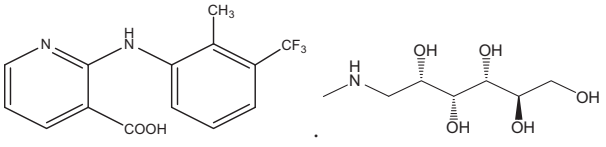

Fig. 1. Chemical structure of flunixin-M.

2.5. Standard solutions

Stock solution of 0.2 mg mL^{-1} of flunixin-M was prepared by dissolving 10 mg flunixin-M in 50 mL of water then the solution was sonicated in an ultrasonic bath for 5 min. Working solutions were prepared by diluting the stock solution with the mobile phase. Stock solution was found to be stable for 5 days if stored in the refrigerator.

2.6. Preparation of calibration curves

Working solutions containing (0.1–2.0 μg mL^{-1}) and (2.0–20 μg mL^{-1}) of flunixin-M were prepared by serial dilutions of aliquots of the stock solution. Then, 20 μL aliquots were injected (triplicate) and eluted with the mobile phase under the reported chromatographic conditions. The average peak areas were plotted versus the concentrations of the drug in μg/mL. Alternatively, the corresponding regression equations were derived.

2.7. Application to injection

Five Flunidyne® injections were mixed and an aliquot of the mixed solution equivalent to 100 mg was transferred to a 100-mL volumetric flask and completed to volume with water to obtain a solution claimed to contain 1.0 mg mL^{-1} flunixin-M. 10-mL of the above solution was diluted to 50 mL with water to obtain a drug solution claimed to contain 0.2 mg mL^{-1}. Solutions were analyzed following the details under "Preparation of calibration curves".

2.8. Bovine liver and kidney samples preparation

2.5 g of the bovine liver or kidney was accurately weighed and spiked with aliquots of flunixin-M solution. The spiked samples were homogenized and completed to 25 mL of 0.15 M SDS solution of pH 7.0. The samples were homogenized at 5000 rpm for 5 min; then, the homogenate was sonicated for 15 min and then centrifuged at 3000 rpm for 5 min. The supernatant of the samples was filtered through 0.45-μm membrane filters using vacuum pump. The filtrate was diluted with the mobile phase, filtered through syringe filter. Aliquots of 20 μL were injected (triplicate) and eluted with the mobile phase under the above chromatographic conditions. The average peak area was plotted versus the concentration of flunixin-M in μg mL^{-1} to get the calibration curve.

3. Results and discussion

The proposed method permits the quantitation of flunixin-M in bulk, pharmaceutical dosage forms, bovine liver and kidney. The proposed method offers high sensitivity as low as 0.0196 μg mL^{-1} of flunixin-M could be detected accurately.

Different parameters affecting the chromatographic performance of flunixin-M were carefully studied in order to achieve the most suitable chromatographic system. The results of the optimization study can be summarized as follows:

3.1. Choice of appropriate detection wavelength

UV detection was set at different wavelengths depending on the absorbance properties of the drug (Fig. 2). It was found that, 284 nm is the optimal wavelength to maximize the sensitivity of determination of the drug.