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Determination of MK-0476 in human plasma by liquid chromatography

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Abstract: A simple and accurate assay for quantitating MK-0476 [sodium 1-(((1(R)-(3-(2-(7-chloro-2-quinolinyl)-(E)-ethenyl)phenyl)(3-(2-(1-hydroxy-1-methylethyl)phenyl)propyl)thio)-methyl)cyclopropane)acetate], which is a potent and selective leukotriene D_4-receptor antagonist, in human plasma has been developed. The method involves precipitation of protein and reversed-phase liquid chromatography with fluorescence detection. The assay is linear in the range of 30–3000 ng ml^{-1} of MK-0476, and the limit of detection is 5 ng ml^{-1}. The interday precision (% relative standard deviation) values of this method at 51 and 2040 ng ml^{-1} are 10 and 3%, respectively. The interday accuracy values at these concentrations are 94 and 104%, respectively. The absolute recovery of MK-0476 is 99%. The utility of this method to determine plasma concentrations of MK-0476 in humans receiving the drug orally was demonstrated.

Keywords: *MK-0476; leukotriene D_4 receptor; antagonist; LC; assay method; human plasma.*

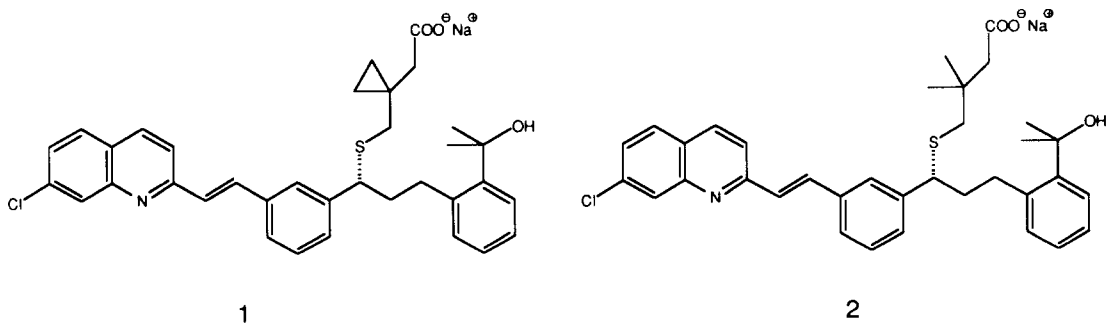
Introduction

Sodium 1-(((1(R)-(3-(2-(7-chloro-2-quinolinyl)-(E)-ethenyl)-phenyl)(3-(2-(1-hydroxy-1-methylethyl)phenyl)propyl)thio)-methyl)-cyclopropane) acetate (**1**; MK-0476; Fig. 1) is a potent and selective leukotriene D_4-receptor (LTD_4) antagonist. It is an analogue of MK-679 (1, 2) and is being investigated for the treatment of bronchial asthma. In conscious squirrel monkeys receiving LTD_4 aerosol challenges, oral treatment with MK-0476 showed dose-related improvement in airway resistance. The compound is light sensitive and thus requires protective handling (e.g. under subdued yellow light and using amber glassware) from the time of drug packaging to the assay of biological specimens. This report describes a specific and accurate method for the determination of MK-0476 in human plasma.

Experimental

Chemicals and reagents

Sodium 1-(((1(R)-(3-(2-(7-chloro-2-quinolinyl)-(E)-ethenyl)phenyl)(3-(2-(1-hydroxy-1-methylethyl)phenyl)propyl)thio)-methyl)-cyclopropane) acetate (**1**; MK-0476; lot 001M004) and sodium 1-(((1(R)-(3-(2-(7-chloro-2-quinolinyl)-(E)-ethenyl)phenyl)(3-(2-(1-hydroxy-1-methylethyl)phenyl)propyl)-thio)-methyl)isopropane) acetate (**2**; Fig. 1; lot 001P004; internal standard) were obtained



1 2

Figure 1
Chemical structures of **1** and **2**.

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from Merck Frosst Canada Inc. (Montreal, Canada). Acetonitrile (optima grade), ammonium phosphate (ACS grade), phosphoric acid (85; LC grade), and potassium hydroxide (ACS grade) were purchased from Fischer Scientific (Fairlawn, NJ, USA). Human plasma was obtained from Biological Specialty Corp. (Landsdale, PA, USA).

Chromatograph and data system

The chromatography system consisted of an LC pump and autosampler (HP 1090; Hewlett–Packard, Avondale, PA, USA), and an Apex octadecyl-3-μ column (5 cm × 4.6 mm i.d.; Jones Chromatography, Columbus, OH, USA). The temperature of column chamber was kept at 40°C. The eluant consisted of acetonitrile and 0.05 M ammonium phosphate buffer (pH 3.5) (62:38 v/v) and was monitored with a variable wavelength fluorometric detector (model 650-10S; Perkin–Elmer Co., Norwalk, CT, USA). The fluorescence excitation and emission wavelength were set at 350 and 400 nm, respectively. The fluorescence output was recorded and analysed using Turbochrom II software (PE Nelson Systems, Cupertino, CA, USA).

Preparation of standards

Stock solutions of MK-0476 and **2** were prepared by dissolving the compounds separately in methanol–water (7:3, v/v) to a concentration of 1 and 0.2 mg ml^{-1}, respectively. A working standard of **2** (5 μg ml^{-1}) was obtained by diluting the stock solution with methanol–water (7:3, v/v). Plasma standards of MK-0476 were prepared in duplicate by diluting the stock solution with control human plasma to obtain concentrations of 30, 50, 100, 200, 500, 1000, 2000 and 3000 ng ml^{-1}. These standards and samples were analysed using the procedure described below.

Analytical procedure

To each standard and unknown sample (200 μl) was added 40 μl of the working standard solution of internal standard, followed by the addition of 400 μl of acetonitrile to precipitate protein. The contents were immediately mixed and centrifuged at 3000 rpm for 10 min. The supernatant was transferred to an amber glass autosampler vial, and 50 μl was injected onto the LC. The ratios of peak heights for the standards of MK-0476 to the internal standard peak heights were determined. The standard line for the weighted (1/concentration) peak height ratio vs concentration of MK-0476 was calculated by linear regression analysis, and the concentration of MK-0476 in each unknown sample was calculated from the standard line by interpolation.

Validation procedure

Individual plasma samples from ten normal subjects were analysed for possible chromatographic interference with MK-0476 or **2**. Possible interference from endogenous substances in plasma was also evaluated by analysing predose samples from several clinical studies.

The absolute recovery of MK-0476 was determined by comparing the slope of a standard line of MK-0476 in water to that of a standard line obtained by preparing and analysing standards of MK-0476 in the plasma supernatant after protein precipitation.

The limit of detection of the method was determined as follows: 200 μl of heparinized control plasma from each of 10 normal subjects was transferred into 10 separate culture tubes (blanks). To another 10 tubes, 200 μl of the corresponding control plasma containing 5 ng ml^{-1} of MK-0476 was added separately (spiked samples). All of the samples were then analysed by the described assay method. The measured peak height at the retention time of MK-0476 for each sample was recorded and the difference in response for spiked vs blank sample pairs was evaluated with Student's *t* test for paired samples.

The stability of MK-0476 in human plasma at −70°C was evaluated by preparing and freezing a large set of 200-μl stability samples containing 50 or 2000 ng ml^{-1} of MK-0476. At various times, groups of five samples were thawed and analysed to determine the stability.

The intraday precision and accuracy of the method were assessed by analysing six replicates of human plasma samples spiked at eight different concentrations of MK-0476 (30, 50, 100, 200, 490, 980, 1960 and 2940 ng ml^{-1}). The observed mean, percentage theoretical recovery and percentage relative standard deviations (% RSD) were calculated.

Interday precision and accuracy were determined by analysing five replicates of human plasma samples spiked at 51 and 2040 ng ml^{-1}, on three different days. Again, the observed mean, percentage theoretical recovery, and % RSD were calculated.

Results and Discussion

The method is relatively simple; it involves only two steps: protein precipitation by acetonitrile and direct injection of the sample into the LC system. Typical chromatograms of a plasma pre-dose sample and a plasma sample are shown in Fig. 2. The retention times of MK-0476 and **2** are 4.0 and 4.9 min, respectively. Thus, satisfactory peak resolution and reasonable retention times were obtained using the short octadecylsilane column and eluant containing acetonitrile and ammonium phosphate buffer.

No interfering peaks were present in chromatograms of blanks at the retention times for MK-0476 and **2**. Pre-dose plasma samples from either healthy volunteers or asthmatic patients also showed no interferences.

Analysis of eight plasma standards in the concentration range of 30–3000 ng ml^{-1}, in duplicate, showed excellent linearity. Linear regression analysis gave a correlation coefficient close to unity (>0.99) on each day of analysis (Table 1). The slope for standard lines was reproducible from day to day with an RSD of 3.4% (n = 12). The mean intercept was −0.0043 (range: from −0.0017 to −0.0064), which is equivalent to 3 ng of MK-0476 per millilitre of plasma.

The mean detector response for 5 ng of MK-0476 per millilitre of spiked plasma samples

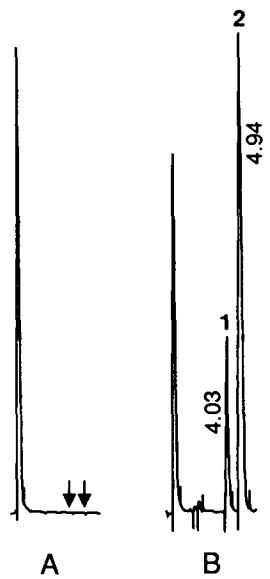

Figure 2
Typical chromatograms of (A) a pre-dose plasma sample from a healthy volunteer (the retention times of MK-0476 (**1**) and internal standard, **2**, are denoted by arrows); and (B) a plasma sample from the same volunteer. The concentration of MK-0476 in this sample was 0.21 μg ml^{-1}.

Table 1
Standard line data

Day	Correlation coefficient	Slope	Intercept
1	0.9998	1.4129	−0.0039
2	0.9999	1.3723	−0.0034
3	0.9997	1.4907	−0.0064
4	0.9999	1.3660	−0.0049
5	0.9999	1.3836	−0.0046
6	0.9998	1.4241	−0.0042
7	0.9999	1.4729	−0.0055
8	0.9999	1.4193	−0.0057
9	0.9998	1.4653	−0.0058
10	0.9998	1.4998	−0.0015
11	0.9998	1.5006	−0.0043
12	0.9999	1.4258	−0.0017
Mean	0.9998	1.4361	−0.0043
RSD (%)	0.01	3.4	35.8

was significantly greater than the corresponding blank samples ($P < 0.001$). Thus, the 5-ng ml^{-1} concentration was established as the detection limit of the method. Since both MK-0476 and **2** are sensitive to light, this detection limit and the lower limit of quantitation (30 ng ml^{-1}) could not be achieved without protecting the samples from exposure to light during sample preparation and injection. Precautions should also be taken to protect samples from exposure to light during sample collection and storage.

MK-0476 was found to be stable for at least 19 months in human plasma at −70°C. In addition, repeated freezing and thawing of the plasma samples three times had no apparent adverse effects on stability. The absolute recovery of MK-0476 was 99%. Similarly, the absolute recovery of **2** was also quantitative.

The intraday precision values (% RSD) were in the range of 0–5.7%, while the accuracy values at similar concentrations were in the range of 95–104%. The effect of dilution of the samples with control plasma on the precision and accuracy of the method was also studied. Study results indicate that dilution of the unknown samples do not appreciably affect the precision and accuracy of the method.

As shown in Table 2, the interday precision

Table 2
Interday precision and accuracy

Concentration (ng ml^{-1})		
Nominal	Found*	Mean recovery ± RSD (%)
51	48 ± 5	94 ± 10
2040	2120 ± 70	104 ± 3

*Mean ± SD; n = 3.

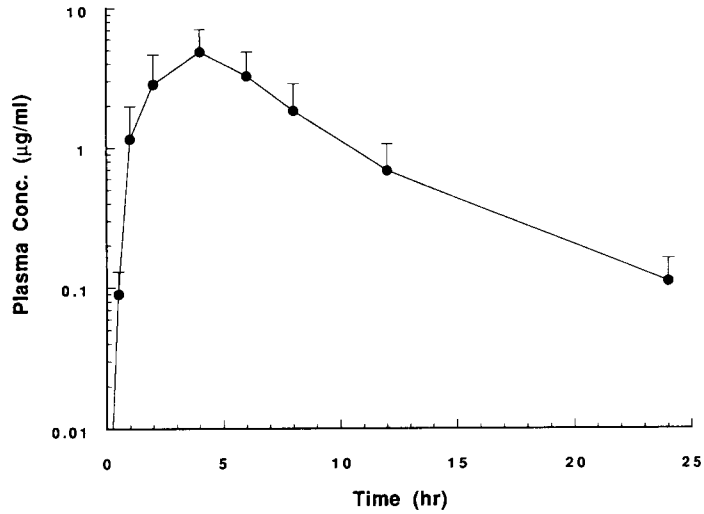

Figure 3
Mean (±SD; n = 6) plasma concentration–time profile of **1** in healthy volunteers receiving 200 mg of **1** orally.

values (% RSD) at concentrations of 51 and 2040 ng ml^{-1} are 10 and 3%, respectively. The interday accuracy values are in the range of 94–104%. Thus, this method yielded precise and accurate measurements over the concentration range studied.

The method has been utilized in pharmacokinetic studies of MK-0476 in humans. In one study, following a 200-mg oral dose to six fasted healthy volunteers, peak plasma concentrations of MK-0476 averaged 4.89 ± 2.18 μg ml^{-1} (Fig. 3). These concentrations were achieved at 3.7 ± 0.8 h post-dose. These applications also demonstrated that the method was suitable for monitoring a wide range of plasma concentrations in a large number of clinical samples from subjects given multiple doses of MK-0476.

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