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Journal of Chromatography A, 726 (1996) 115–124

JOURNAL OF CHROMATOGRAPHY A

Quantitation of the $5HT_{1D}$ agonists MK-462 and sumatriptan in plasma by liquid chromatography–atmospheric pressure chemical ionization mass spectrometry[1]

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Received 30 March 1995; revised 1 August 1995; accepted 7 August 1995

Abstract

The $5HT_{1D}$ agonist sumatriptan is efficacious in the treatment of migraines. MK-462 is a drug of the same class which is under development in our laboratories. Bioanalytical methods of high efficiency, specificity and sensitivity were required to support the preclinical and clinical programs. These assays were based on HPLC with tandem MS–MS detection. MK-462 and sumatriptan were extracted using an automated solid-phase extraction technique on a C_2 Varian Bond-Elut cartridge. The *n*-diethyl analogues of MK-462 and sumatriptan were used as internal standards. The analytes were chromatographed using reversed-phase (nitrile) columns coupled via a heated nebulizer interface to an atmospheric pressure chemical ionization source. The chromatographic run times were less than 7 min. Both methods were precise, accurate and selective down to plasma concentrations of 0.5 ng/ml. The assay for MK-462 was adapted to separately monitor the unlabeled and ^{14}C-labeled species of the drug following intravenous administration of radiolabeled material to man.

Keywords: Liquid chromatography–mass spectrometry; Sumatriptan; $5HT_{1D}$ Agonists; MK-462

1. Introduction

Serotonin, or 5-hydroxytryptamine (5-HT), is known as a neurotransmitter which regulates circadian rhythm, temperature regulation, aggression control and sexual function [1–3]. 5-HT, which constricts cranial blood vessels, is rapidly metabolized. $5HT_{1D}$ agonists, like MK-462 {N,N-dimethyl-2-[5-(1,2,4-triazole-1-ylmethyl)-1H-indol-3-yl]ethylamine} and sumatriptan {3-[2-(dimethylamino)-ethyl]-N-methyl-1H-indole-5-methane sulfonamide}, constrict cranial blood vessels and are, accordingly, logical candidates in the treatment of migraines [1–3]. Sumatriptan was the first of this class of drugs to be approved for this treatment. Methods for the determination of sumatriptan by high-performance liquid chromatography with electrochemical detection as well as thermospray liquid chromatography–mass spectrometry reported lower limits of quantitation of 1.0 and 2.0 ng/ml, respectively [4,5]. LC–MS–MS with atmospheric pressure chemical ionization has recently proved remarkably successful for the sensitive and specific quantitation of drugs in biological fluids [6,7]. This technique again proved successful for the analysis of these $5HT_{1D}$ agonists in human plasma.

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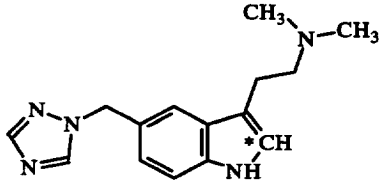
[1] Presented at the 11th Montrena Symposium on Liquid Chromatography–Mass Spectrometry, Montrena, 9–11 November 1995.

0021-9673/96/$15.00
SSDI 0021-9673(95)00022-4

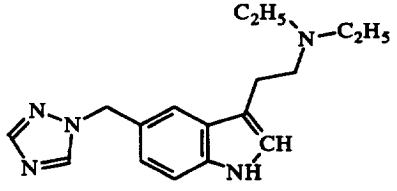
2. Experimental

2.1. Materials

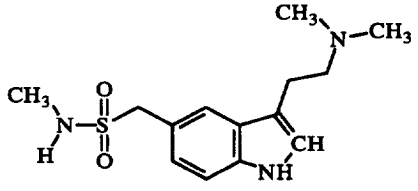
MK-462, ^{14}C-labeled MK-462 (both as their benzoate salts), L-743,214, sumatriptan (L-690,583) and L-737,404 (Fig. 1) were obtained from Merck Research Laboratories (Terlings Park, UK and Rahway, NJ, USA). The radiolabeled MK-462 had a specific activity of 25.2 μCi/mg, resulting in 11% of the molecules carrying the ^{14}C-label. A solution containing 1 mg/ml of the radiolabeled drug, ca. 0.89 mg/ml of ^{12}C- and 0.11 mg/ml of ^{14}C-MK-462, was used as an analytical standard when analysis of radiolabeled samples was required. Ammonium acetate and trifluoroacetic acid (TFA) were obtained from Sigma (St. Louis, MO, USA). Methanol and acetonitrile (HPLC grade) were obtained from Fisher (Fair Lawn, NJ, USA). Acetic acid was analytical grade and was purchased from Mallinckrodt (Paris, KT, USA). Bond-elut C_2 cartridges (1 ml × 100 mg) were obtained from Varian (Harbor City, CA, USA). Liquid nitrogen, nitrogen (99.999%) and argon (99.999%) were purchased from West Point Supply (West Point, PA, USA). Disposable vials, tapered inserts and press-on caps were purchased from Scientific Resources (Somerset, NJ, USA).



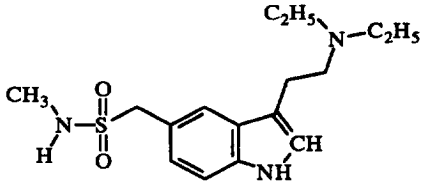
MK-462



L-743,214



Sumatriptan



L-737,404

Fig. 1. Structures of MK-462, sumatriptan and their respective internal standards L-743,214 and L-737,404. (* indicates position of ^{14}C label).

2.2. Standard and sample preparation

Standard stock solutions of MK-462, sumatriptan and their respective internal standards, L-743,214 and L-737,404 were prepared as 1-mg/ml solutions (of the free base) in water except for L-743,214 which was prepared in methanol. All subsequent dilutions were made in water.

Analytical standards and quality control samples, prepared from independent weighings, were prepared by the addition of known quantities of standard solutions (0.1 ml) to 1.0 ml aliquot of control human plasma. The concentrations of MK-462 and sumatriptan were 0.5, 1, 2, 5, 10, 20 and 50 ng/ml for the analysis of clinical samples. Quality control samples were prepared at low, mid and high concentrations and were processed in duplicate.

2.3. Extraction procedure

A plasma sample (1.0 ml) was placed in a 10×75 mm glass tube. The appropriate internal standard (L-743,214 for MK-462 or L-737,404 for sumatriptan) was diluted to a working concentration of 100 ng/ml. Approximately 100 μl of the appropriate internal standard was added to each sample. The samples were briefly vortexed and loaded onto a Gilson Aspec XL (Westwood, NJ, USA) for automated sample preparation. The following manual

procedure was easily adapted to the Gilson Aspec XL. Each sample was processed individually. The Bond-Elut C_2 cartridges were preconditioned by washing with methanol (2×1 ml), followed by distilled water (2×1 ml). The samples were loaded onto the cartridges and pushed through at a flow-rate of 2 ml/min. The column was washed with 1 ml of distilled water followed by two 1 ml washes of methanol–water (30:70). MK-462, sumatriptan and their respective internal standards were eluted using 1 ml of methanol–10 m*M* ammonium acetate pH 5.0 (60:40). The samples were evaporated to dryness at 50°C in a Turbo Vap (Zymark, Hopkinton, MA, USA) and reconstituted in 200 μl of the mobile phase appropriate for the drug being assayed (see below). The samples were then agitated in an ultrasonic bath for 10 min, vortex mixed and transferred to microinjection vials. Aliquots of 25 μl were injected.

The absolute recoveries of MK-462, L-743,214, sumatriptan and L-737,404 from plasma were determined in the following manner. Aliquots (1.0 ml) of control human plasma were spiked with analyte or internal standard to yield concentrations of 5.0 and 50 ng/ml for MK-462 and sumatriptan or 10 ng/ml for their respective internal standards (L-743,214 and L-737,404). After solid-phase extraction 10 ng of L-743,214 or L-737,404 were added to extracts containing MK-462 or sumatriptan, respectively, and 5.0 and 50 ng of MK-462 or sumatriptan to extracts containing 10 ng/ml of L-743,214 or L-737,404, respectively. Samples were assayed by LC–MS–MS and the peak area ratios compared with those obtained from unextracted standards. The mean absolute recoveries were 92.2% for MK-462, 86.1% for sumatriptan, 108% for L-743,214 and 101% for L-737,404.

2.4. LC–MS–MS

LC–MS–MS was performed on a Sciex (Thornhill, Ont., Canada) Model API III triple quadrupole mass spectrometer, equipped with an upgraded collision cell and interfaced via a Sciex heated nebulizer probe to a liquid chromatograph consisting of a Hewlett Packard 1050 quaternary pump and autoinjector. MK-462 was chromatographed on a Spherisorb CN (25 cm×4.6 mm I.D., 5 μm) from Thomson Liquid Chromatography (Springfield, VA, USA). The mobile phase was acetonitrile–methanol–water (54:4:42, v/v) containing 0.1% TFA at a flow-rate of 1 ml/min. The chromatographic column for the determination of sumatriptan was a Beckman CN (25 cm×4.6 mm I.D., 5 μm) from Beckman Instruments (San Ramon, CA, USA) and was used with a mobile phase consisting of acetonitrile–methanol–water (36:6:58, v/v) containing 0.1% TFA at a flow-rate of 1.2 ml/min. The nebulizer probe temperature setting was 500°C. The nebulizing gas pressure and auxiliary flow were set at 80 p.s.i. (ca. 552 kPa) and 2.0 l/min, respectively. Chemical ionization was effected by a corona discharge needle set at +3 μA and positive ions were sampled into the quadrupole mass analyzer via a 0.0045 in. (0.1143 mm) orifice.

The product ion mass spectra of MK-462, sumatriptan and their respective internal standards are shown in Fig. 2 and Fig. 3. Quantitation was conducted using multiple reaction monitoring (MRM). The mass spectrometer was programmed to transmit the protonated molecules $[M+H]^+$ through the first quadrupole (Q1) at m/z 270 for MK-462, m/z 298 for the internal standard L-743,214, m/z 296 for sumatriptan and m/z 324 for the internal standard L-737,404. Following collision induced fragmentation in Q2 (collision gas argon 120×10^{13} atoms cm^2) product ions were selected by Q3 at m/z values of 201, 229, 157 and 251 for MK-462, L-743,214, sumatriptan and L-737,404, respectively. The orifice potential settings for the assays of MK-462 and sumatriptan were 45 V and 40 V, respectively. The dwell time was 400 ms. Peak-area ratios of the analyte to internal standard were computed using MacQuan version 1.3 software from Sciex. The calibration curves were constructed using a weighed $(1/x)$ linear regression of plasma concentrations and measured area ratios. Plasma concentrations of MK-462 or sumatriptan in unknown samples were determined by interpolation from the appropriate standard curve.

3. Results and discussion

Our objectives were to develop and validate convenient (and preferably automated) assays which

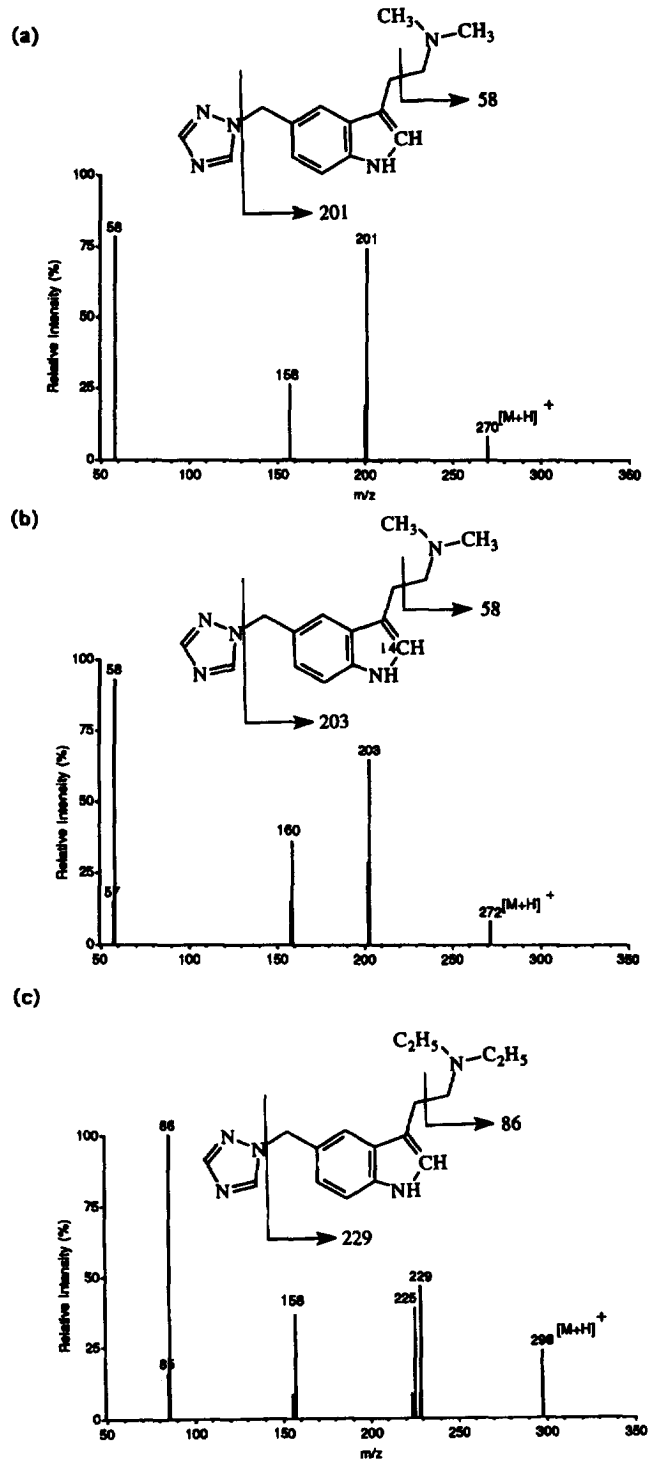


Fig. 2. Positive product ion mass spectra of the protonated molecular ions of (a) unlabeled MK-462 (m/z 270), (b) ^{14}C-labeled MK-462 (m/z 272) and (c) L-743,214 (m/z 298).

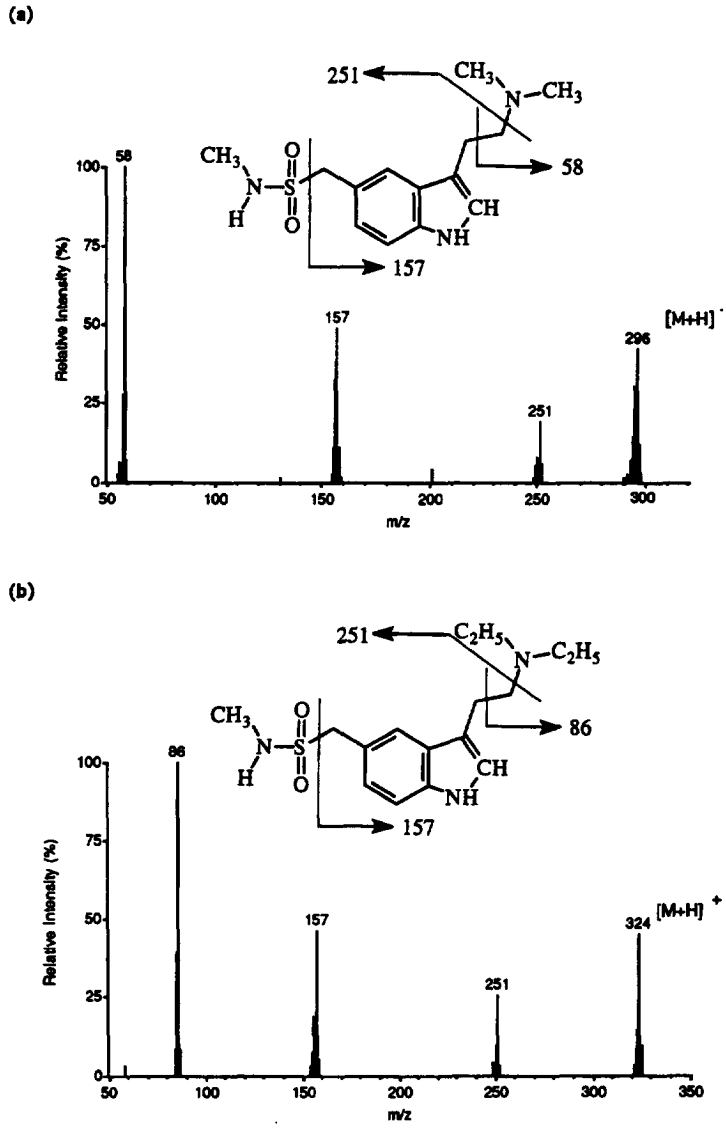


Fig. 3. Positive product ion mass spectra of the protonated molecular ions of (a) sumatriptan (*m/z* 296) and (b) L-737,404 (*m/z* 324).

were capable of measuring MK-462 and sumatriptan at the range of concentrations needed for the analysis of clinical plasma samples. The use of LC–MS–MS for sumatriptan improved the lower limit of quantification when compared to previously published methods using HPLC with electrochemical [4] or thermospray mass spectrometry [5] detection. For most clinical trials a lower limit of quantification of 0.5 ng/ml was acceptable. Representative chromatograms of plasma extracts from volunteers receiving MK-462 and sumatriptan are shown in Fig. 4 and Fig. 5.

3.1. Calibration

The validation data for MK-462 and sumatriptan showed acceptable precision, linearity and reproducibility over the concentration ranges studied (Table 1 and Table 2). There were no interferences

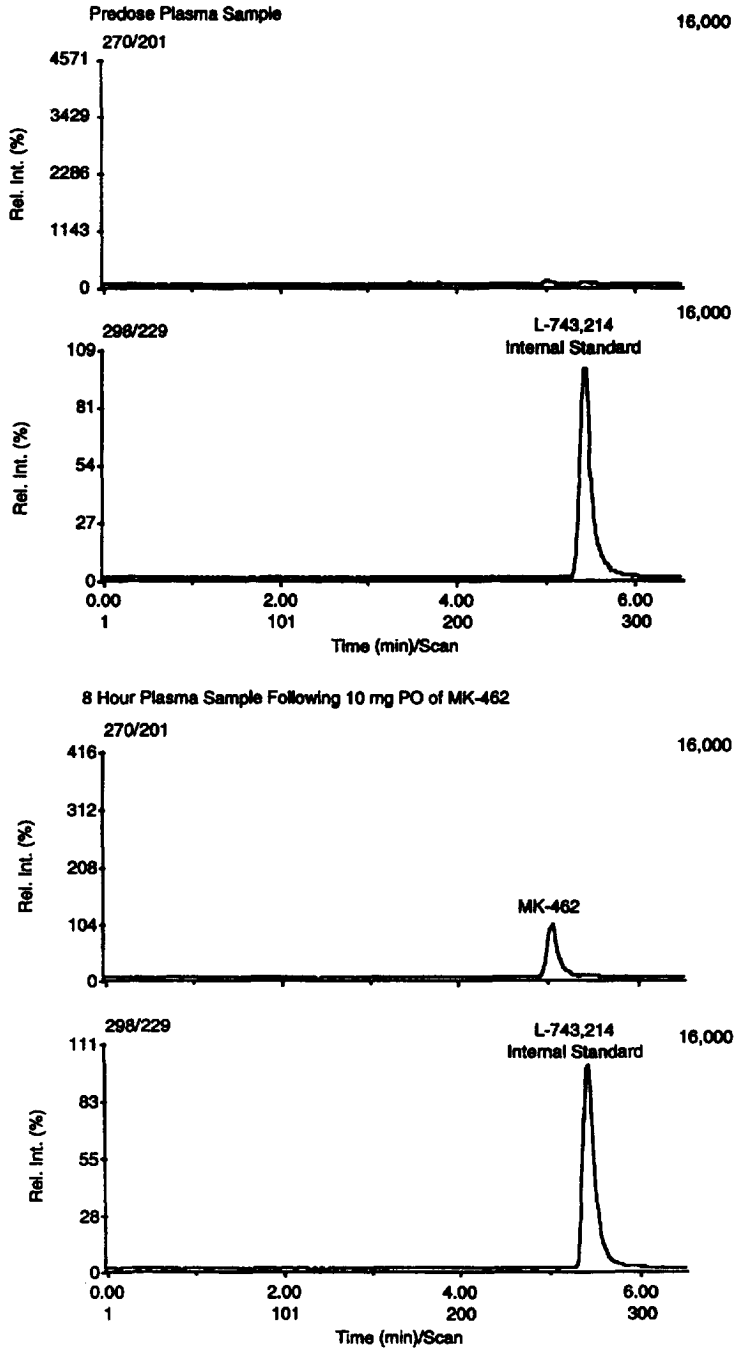


Fig. 4. Chromatograms obtained by multiple reaction monitoring of plasma extracts from a volunteer receiving MK-462 (10 mg p.o.). Top: predose plasma extract. Bottom: extract of plasma collected 8 h post administration. (Concentration=ca. 1 ng/ml).

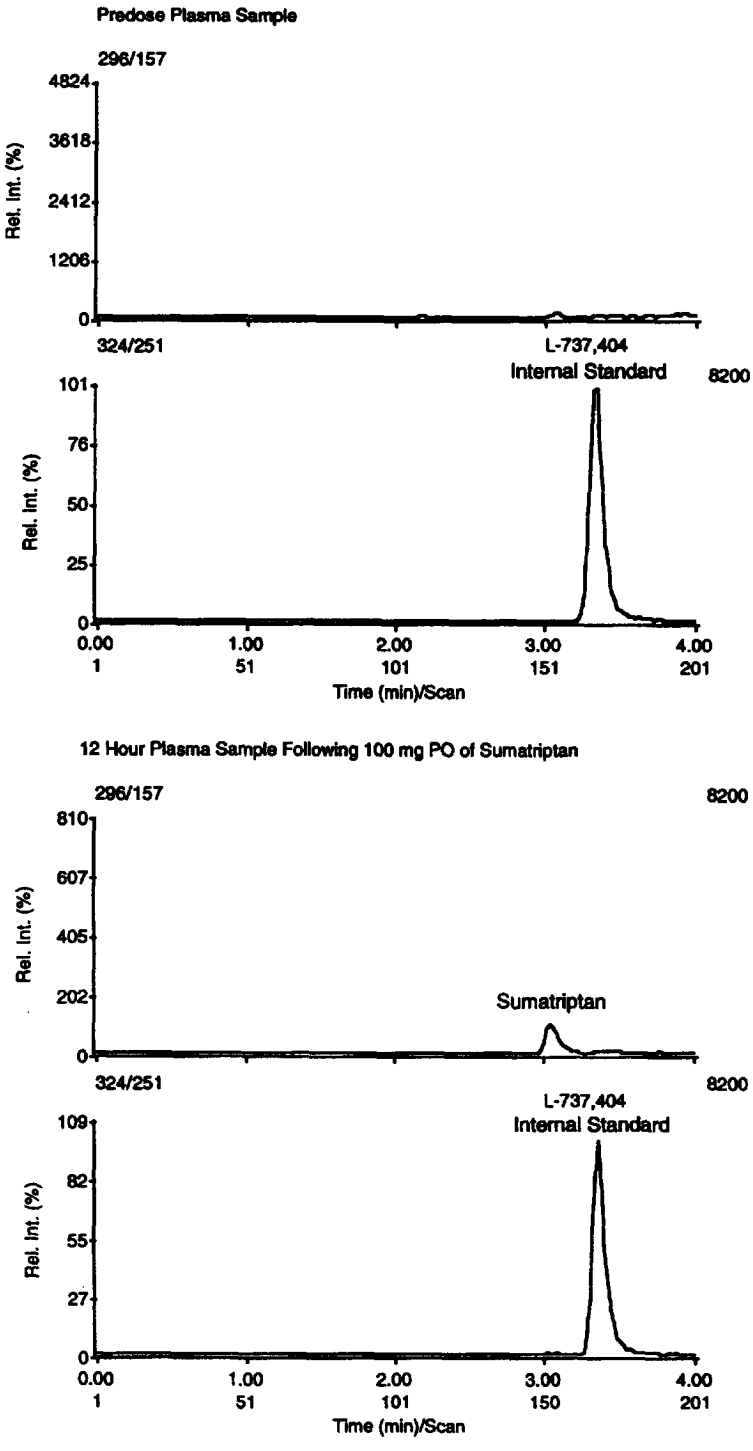


Fig. 5. Chromatograms obtained by multiple reaction monitoring of plasma extracts from volunteer receiving 100 mg orally of sumatriptan. Top: predose plasma extract. Bottom: extract of plasma collected 12 h post administration of 100 mg (p.o.) of sumatriptan. (Concentration= ca. 0.8 ng/ml).

Table 1
Intra- and inter-day accuracy and precision for the determination of MK-462 and ^{14}C-labeled MK-462 in human plasma by LC–MS–MS

	Nominal conc. (ng/ml)	MK-462 accuracy (% found/actual)	MK-462 precision (R.S.D., %)	Nominal conc. (ng/ml)	^{14}C-MK-462 accuracy (% found/actual)	^{14}C-MK-462 precision (R.S.D., %)
Intra-day	0.5	96.1	4.3	0.1	112.9	7.7
(n=5)	1	105.1	5.1	0.2	104.1	1.0
	2	108.5	2.0	0.4	98.0	4.7
	5	98.0	2.8	1	96.3	2.8
	10	102.0	3.1	2	91.4	4.5
	20	104.1	2.1	4	92.1	3.5
	50	106.5	2.3	10	105.1	1.9
Inter-day	2	90.0	2.6	0.44	95.6	3.0
(n=10)	10	102.8	2.3	4.44	91.0	5.2
	50	92.9	4.5			

from endogenous plasma components with the measurement of MK-462, sumatriptan or their respective internal standards.

3.2. Precision and accuracy

Intra-assay precision was determined by analysis of replicates (n=5) of control plasma containing known concentrations of MK-462 and sumatriptan over the concentration ranges of 0.5 to 50 ng/ml. The percent coefficients of variation at the lower quantifiable limit (0.5 ng/ml) were 4.3 and 5.6% for MK-462 and sumatriptan, respectively. Inter-assay accuracy and precision were calculated from replicate quality control data. The results are shown in Table 1 and Table 2 for MK-462 and sumatriptan, respectively.

The stability of MK-462 was determined in human plasma at −20°C and during consecutive freeze/thaw cycles. Quality control samples were prepared at concentrations of 1.03 and 10.3 ng/ml in human plasma and stored at −20°C. The stability samples were analyzed on Day 1 and subsequently at 10 and 71 weeks following preparation (Table 3). MK-462 showed satisfactory stability when stored at −20°C for a period of up to 71 weeks. The stability of MK-462 was also determined in human plasma following freeze/thaw cycles. The samples were

Table 2
Intra- and inter-day accuracy and precision for the determination of sumatriptan in human plasma by LC–MS–MS

	Nominal conc. (ng/ml)	Sumatriptan accuracy (% found/actual)	Sumatriptan precision (R.S.D., %)
Intra-day	0.5	112.9	5.6
(n=5)	1	95.7	12.5
	2	99.8	6.9
	5	95.7	8.8
	10	93.2	7.0
	20	94.2	6.2
	50	98.2	6.9
	100	98.2	4.3
Inter-day	1	102.6	14.6
(n=10)	10	87.3	6.4
	50	95.5	8.9

Table 3
Stability of MK-462 in human plasma at −20°C and during two freeze/thaw cycles

Actual conc. (ng/ml)	Measured concentration (ng/ml)		
	Day 1	Week 10	Week 71
1.03	1.05	1.02	1.13
10.3	10.0	9.70	11.5
	Freeze/thaw t_0	Freeze/thaw t_1	Freeze/thaw t_2
5.00	5.08	4.94	4.81
50.0	51.3	62.4	55.8

analyzed immediately following sample preparation (t_0) and following two freeze/thaw cycles (t_1 and t_2). The samples were frozen in a dry ice acetone bath and allowed to thaw at room temperature. MK-462 showed satisfactory stability (Table 3).

3.3. Measurement of ^{14}C-labeled MK-462

Following intravenous administration of ^{14}C-labeled MK-462 to volunteers there was a need, because of the high specific activity of the dosing solution, to measure both the unlabeled (^{12}C-) and the ^{14}C-labeled drug in plasma and urine. The assay was accordingly modified to simultaneously monitor the labeled and unlabeled species by including the parent/product ion combination 272→203. The product ion mass spectrum (10% valley for both mass filters) of ^{14}C-labeled MK-462 is included in Fig. 2.

The assay was satisfactory over the concentration range 0.1 to 10 ng/ml, showing good linearity and acceptable inter and intra-assay precision (Table 1). The accuracy of the assay for ^{14}C-labeled MK-462 was determined at 0.4 and 4 ng/ml. The percent recoveries (and C.V.s) were 95.6% (3.0) and 91.0% (5.2), respectively (Table 1).

The cross reactivity of the unlabeled drug on the parent/product channel used to measure the ^{14}C-labeled drug was determined by analysis of unlabeled MK-462 while monitoring both the m/z 270→201 and 272→203 channels. The cross reactivity was approximately 1%; a concentration of 10 ng/ml of unlabeled drug produced an apparent concentration of ca. 0.1 ng/ml of the ^{14}C-labeled species. After analysis of clinical specimens total MK-462 concentrations were reported as the sum of their ^{12}C- and ^{14}C-labeled entities.

3.4. Automation of sample extraction

Due to the high specificity and reliability of the tandem MS–MS detection system, sample throughput is increased dramatically when compared with most conventional HPLC methodologies. The rate-determining step of the overall assay then becomes sample preparation. Accordingly we developed procedures for the automation of sample extraction.

Prior to purchasing the Gilson Aspec XL, all plasma extraction for MK-462 and sumatriptan assays were performed manually. Transfer of these method to the Gilson Aspec XL required minimal modifications to the manual procedures. The Aspec handles all aspects of the extraction from cartridge activation to sample elution, but sample pipetting, evaporation and reconstitution were still performed manually. Although sample preparation time on the Aspec (12 min) is nearly double the analytical run time (6.5 min) the robot is capable of running 24 h per day, virtually unattended, and allows the analyst to process the data generated for previous assays.

3.5. Automation of data processing

DMLIMS+ (Drug Metabolism Laboratory Information Management System Plus; PennComp, Wayne, PA, USA) is an information management system which can be interfaced with Sciex's data system. DMLIMS+ allows the user to pre-establish

a database for a complete clinical study prior to commencement of sample analysis. The database summarizes the clinical study protocol in terms of the dose groups, the number of subjects per group and the allocation number of the individual patients. As analytical results are obtained, they are automatically entered into the database. The user can, at any time, generate summaries of data including plasma concentration time-profiles, tables, pharmacokinetic parameters and assay performance reports. Comparisons between dose groups or sexes can be easily obtained. All the data which are required for reporting can be generated with a few key strokes and readily interfaced with a variety of word processing systems. Additionally DMLIMS+ greatly facilitates setting up Sciex's RAD (routine acquisition and display) software. Implementation of these automated methods for the assays MK-462 and sumatriptan has enabled an increase in our monthly sample through-put by a factor of 2.

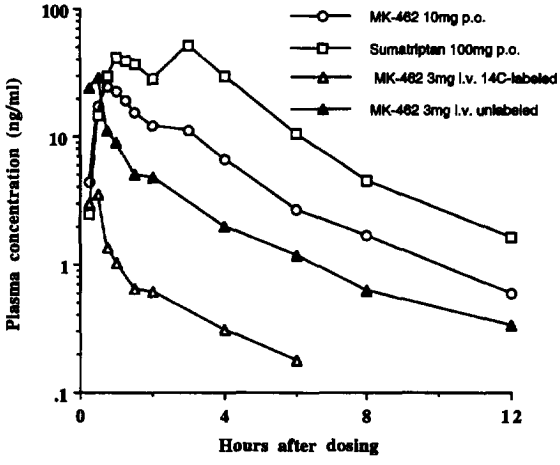


Fig. 6. Plasma concentration-time profiles following oral (p.o.) administration of MK-462 and sumatriptan to healthy male volunteers and (i.v.) intravenous administration of 3 mg of ^{14}C-labeled MK-462. The unlabeled and ^{14}C-labeled species of MK-462 were separately determined.

4. Conclusions

Sensitive, accurate and precise assays based on LC–MS–MS have been developed for the determination of sumatriptan and MK-462 in human plasma with lower quantifiable limits of 0.5 ng/ml. The use of automated sample extraction and a LIMS system greatly increased the assay's efficiency. Plasma concentration–time profiles following single oral administration of MK-462 (10 mg) and sumatriptan (100 mg) to volunteers are shown in Fig. 6.

The assay for MK-462 was modified to include the simultaneous determination of the ^{14}C-labeled species. Plasma concentration–time curves following intravenous administration of ^{14}C-labeled MK-462 (3 mg total free base) to a volunteer are included in Fig. 6.

Acknowledgments

The authors thank Dr. V. Matassa for providing the internal standards (L-743,214 and L-737,404) and Maureen Hetzel for assistance with preparing the manuscript.

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