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Note

Determination of mevinolin and mevinolinic acid in plasma and bile by reversed-phase high-performance liquid chromatography

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Mevinolin (I, Fig. 1) is a fungal metabolite that was isolated from cultures of *Aspergillus terreus*. Its full chemical name is 1′,2′,6′,7′,8a′-hexahydro-3,5-dihydroxy-2′,6′-dimethyl-8′-(2″-methyl-1″-oxobutoxy)-1-naphthalene heptanoic acid 5-lactone. Mevinolin can be converted to its hydroxy acid form and the resultant compound will be referred to as mevinolinic acid (II, Fig. 1).

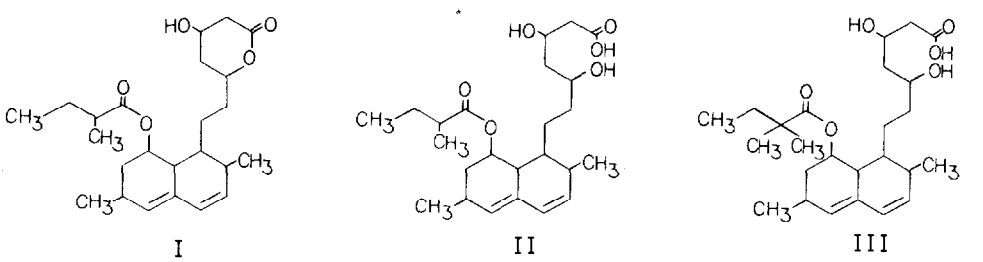

Fig. 1. Chemical structures of mevinolin (I), mevinolinic acid (II) and the internal standard, methyl mevinolinic acid (III).

Mevinolin and mevinolinic acid are potent in vivo inhibitors of hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase which is the enzyme responsible for the conversion of HMG-CoA to mevalonate. This is the major rate-limiting step in the synthesis of cholesterol and both compounds are, therefore, cholesterol synthesis inhibitors. The compounds also produce a significant lowering of serum cholesterol in normal human volunteers; reductions of 23—27% have been reported [1]. Similar results were reported for patients with heterozygous familial hypercholesterolemia [2, 3].

Both of the above compounds are currently undergoing clinical trials in man.

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In addition to this, metabolism studies in rat and dog are in progress. An enzyme inhibition assay capable of measuring HMG-CoA reductase inhibitors has been reported [4] for biological samples. The enzyme inhibition assay only measures hydroxy acids having inhibitory activity. Because the assay measures all of the inhibitors present, it is not specific. Mevinolin is a lactone and not an in vitro inhibitor of HMG-CoA reductase and, therefore, not measured by this assay. Mevinolinic acid is the corresponding hydroxy acid of mevinolin and is an in vitro inhibitor of HMG-CoA reductase. Mevinolin is converted in vivo to mevinolinic acid as well as being present itself. An assay was, therefore, required that was capable of measuring both compounds simultaneously in biological samples.

Preliminary metabolism studies in dog and rat have shown that mevinolin and mevinolinic acid are excreted via the bile into the faeces. An assay capable of measuring these two compounds in bile was, therefore, needed in addition to a plasma assay. No evidence for urinary excretion has so far been found.

A reversed-phase high-performance liquid chromatographic (HPLC) method with ultraviolet detection is described for the determination of mevinolin and mevinolinic acid. Plasma samples are prepared for analysis using a bonded-phase extraction cartridge and isocratic HPLC elution. Bile samples are injected directly and gradient elution HPLC is used. This method has been successfully used to assay plasma and bile samples from rat and dog receiving doses of mevinolin and mevinolinic acid, respectively. It has also been used to measure plasma levels in man following an oral dose of mevinolinic acid.

EXPERIMENTAL

Materials

Acetonitrile, methanol, ammonium phosphate (monobasic), phosphoric acid, potassium phosphate (monobasic) and potassium hydroxide were all HPLC grade or reagent grade and supplied by Fisher Scientific (Pittsburgh, PA, U.S.A.). Milli-Q water was used throughout for the preparation of reagents. Mevinolin and mevinolinic acid were supplied by Merck Sharp and Dohme Research Labs. (Rahway, NJ, U.S.A.). Methyl mevinolinic acid (III, Fig. 1), the internal standard (I.S.), is an analogue of mevinolinic acid and was also supplied by Merck. Human control plasma was supplied by Sera-Tec Biologicals (North Brunswick, NJ, U.S.A.). C_2 Bond-Elut cartridges (100 mg) were supplied by Analytichem International (Harbor City, CA, U.S.A.).

Instrumentation

A Hewlett-Packard 1090 liquid chromatograph with a variable-volume injector, autosampler and column heater was used for this analysis. A Kratos Model 783 variable-wavelength ultraviolet detector was used to monitor the mobile phase. A Spectra-Physics SP4270 computing integrator was used to measure peak areas.

Standard solution

A stock solution of mevinolin was prepared in acetonitrile (1 mg/ml). Mevinolinic acid and methyl mevinolinic acid, the internal standard, were

supplied as ammonium salts and stock solutions were prepared in acetonitrile–water (60:40, v/v) at equivalent free acid concentration (1 mg/ml). Working standards for mevinolin and mevinolinic acid were prepared in the same solvent as the stock solution at concentrations of 10, 5, 2.5, 1, 0.5 and 0.25 μg/ml. This produced equivalent plasma concentrations of 1000, 500, 250, 100, 50 and 25 ng/ml for each compound. The stock solution of internal standard was diluted to 5 μg/ml in the same solvent as the stock standard solution to give a working internal standard solution.

HPLC conditions

The HPLC column used for this analysis was a C_{18} Sepralyte column (5 cm × 4.6 mm I.D., 3-μm packing) supplied by Analytichem International. The mobile phase consisted of an aqueous buffer and an organic modifier. The aqueous buffer was ammonium phosphate (0.05 *M*) and phosphoric acid (0.01 *M*) and the organic modifier was acetonitrile. For plasma analysis, an isocratic mixture (50%, v/v) of these two solvents was used. For bile analysis a linear gradient from 20 to 75% acetonitrile in 10 min was used. The column was maintained at 50°C and a flow-rate of 1.5 ml/min was used. The ultraviolet detector was set to monitor 238 nm at a sensitivity of 0.01 a.u.f.s. for plasma and 0.1 a.u.f.s. for bile with an 0.2-s time constant. The integrator was run at a chart-speed of 0.5 cm/min for plasma and 1 cm/min for bile at an attenuation of 8 mV f.s.d.

Analysis of plasma

Plasma standards were prepared by taking blank human plasma (500 μl) and potassium phosphate (500 μl, 0.1 *M*, pH 7.2) and keeping this mixture in an ice–water mixture. The range of mevinolin working standard solutions (50 μl of each, respectively) and mevinolinic acid working standard solutions (50 μl of each, respectively) were added to the buffered plasma. Working internal standard solution (50 μl) was added to each tube and the contents were mixed.

A C_2 Bond-Elut extraction column (1 ml) was rinsed with methanol (1 ml) and potassium phosphate buffer (1 ml, 0.1 *M*, pH 7.2). The buffered plasma sample was loaded onto the column and a vacuum applied. The column was further washed with phosphate buffer (2 × 1 ml) and acetonitrile–phosphate buffer (20:80, v/v; 2 × 1 ml) and all washings were discarded. Each column was then eluted with acetonitrile–water (75:25, v/v; 400 μl) and the eluates were collected. An aliquot (30 μl) of this solution was injected for HPLC analysis.

Patient plasma samples were prepared for analysis in the same way as plasma standards substituting patients' plasma (500 μl) for blank plasma and acetonitrile–water (60:40, v/v; 100 μl) for the two working standard solutions.

Analysis of bile

Blank rat bile was spiked with mevinolin and mevinolinic acid to give 10 μg/ml of each. An aliquot (10 μl) of this solution was injected directly for HPLC analysis. Rat bile (10 μl) from dosed rats was injected directly for HPLC analysis.

RESULTS AND DISCUSSION

Plasma

The method described has been successfully used to quantify mevinolin and mevinolinic acid in human, rat and dog plasma. The plasma clean-up involved passing buffered plasma through a C_2 extraction cartridge. Under these conditions, the mevinolin and mevinolinic acid were retained. The cartridge was then washed with 20% acetonitrile—phosphate buffer. This removed many of the polar species that might otherwise interfere with the determination of the compounds of interest. The 20% acetonitrile in the wash solvent was the highest percentage of acetonitrile that could be used. Any increase gave partial elution of the mevinolinic acid. The 75% acetonitrile used to elute the compounds of interest gave a high recovery of mevinolin and mevinolinic acid with minimum elution of interfering species.

Typical chromatograms from the determinatin of mevinolin and mevinolinic acid in human plasma are shown in Fig. 2. Mevinolinic acid eluted at 2.58 min, the internal standard at 3.50 min and mevinolin at 4.95 min. As can be seen from the blank plasma sample, there is minimal endogenous interference. The chromatogram showing both compounds is from a 2-h human plasma sample following a 100-mg oral dose of mevinolinic acid. The limits of detection for these two compounds was 25 ng/ml (based on a signal-to-noise ratio of 5:1).

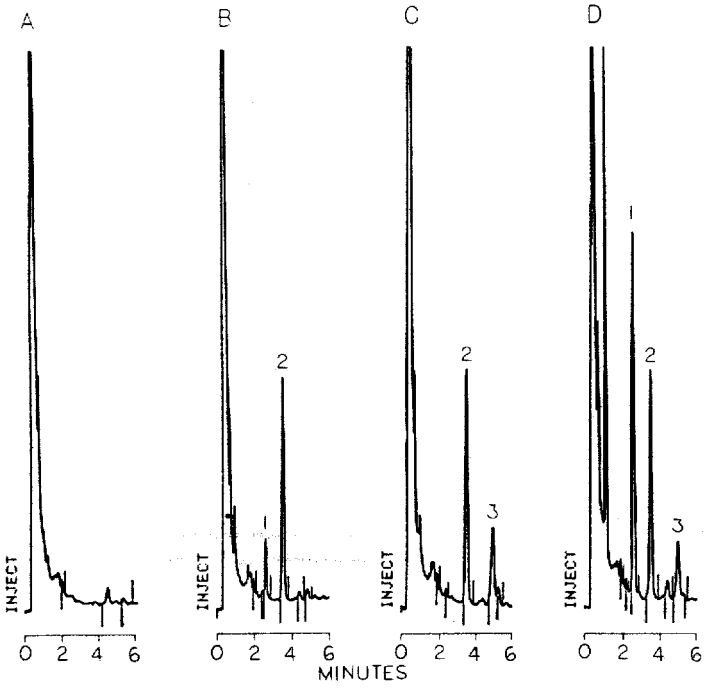

Fig. 2. Typical chromatograms for mevinolin and mevinolinic acid in human plasma. (A) Blank human plasma; (B) blank human plasma containing 100 ng/ml mevinolinic acid (1) and 500 ng/ml internal standard (2); (C) blank human plasma containing 500 ng/ml internal standard (2) and 250 ng/ml mevinolin (3); (D) patient plasma containing 543 ng/ml mevinolinic acid (1), 500 ng/ml internal standard (2) and 162 ng/ml mevinolin (3).

TABLE I

INTER-DAY VARIATION FOR THE ASSAY OF MEVINOLINIC ACID AND MEVINOLIN IN PLASMA

Concentration (ng/ml)	Mevinolinic acid			Mevinolin		
	Mean drug/I.S. ratio (n = 5)	S.D.	R.S.D. (%)	Mean drug/I.S. ratio (n = 5)	S.D.	R.S.D. (%)
25	0.0494	0.0028	5.7	0.0585	0.0046	7.8
50	0.0899	0.0045	5.0	0.1241	0.0062	5.0
100	0.1911	0.0121	6.3	0.2008	0.0116	5.8
250	0.4857	0.0278	5.6	0.4028	0.0119	3.0
500	0.9917	0.0558	5.6	0.8639	0.0273	3.2
1000	2.0278	0.0951	4.7	1.6091	0.0644	4.0

The standard lines used in this analysis covered the range 25–1000 ng/ml for mevinolin and mevinolinic acid. The lines were linear throughout this range and plots of drug concentration against the ratio of peak areas drug/internal standard confirmed this. The reproducibility of this assay was checked by calculating the inter-day variation of each point on the two standard lines over a five-day period. The data are summarized in Table I. All points had relative standard deviation of 7.8% or less. The accuracy of the method was confirmed

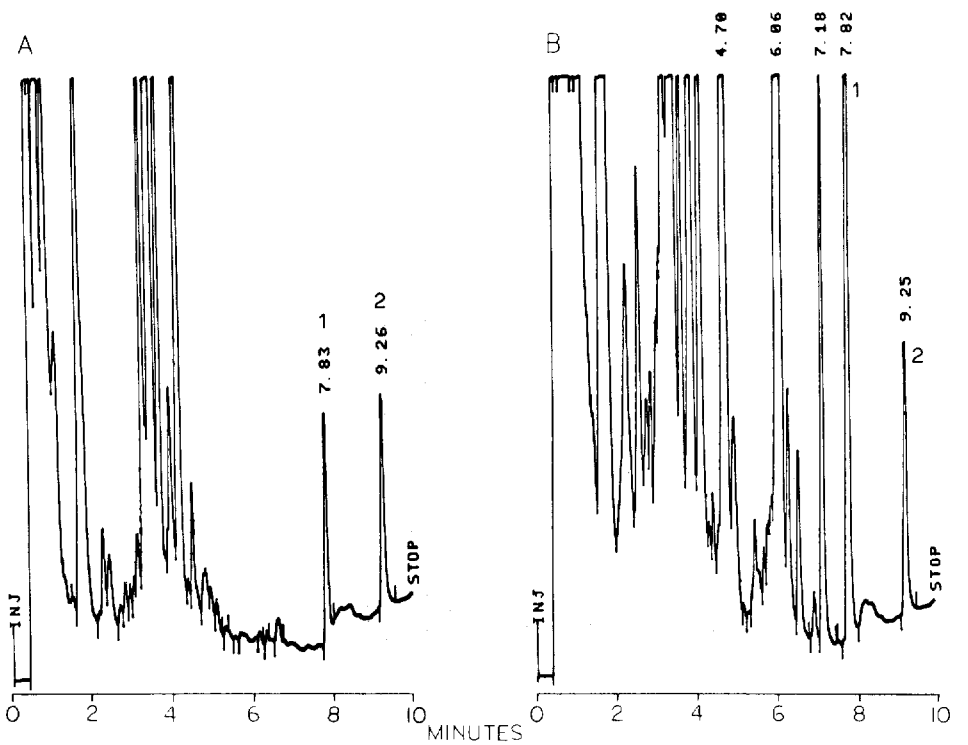

Fig. 3. Typical chromatograms for mevinolin and mevinolinic acid in rat bile. (A) Blank rat bile spiked with 10 μg/ml mevinolinic acid (1) and 10 μg/ml mevinolin (2); (B) rat bile 1 h post-dose (10 mg/kg mevinolinic acid) showing 12.7 μg/ml mevinolinic acid (1) and 47.5 μg/ml mevinolin (2).

by running quality-control samples at low and high values on the standard lines. Correct assays were obtained for these samples.

Recoveries were checked by spiking the two drugs and the internal standard into the appropriate volume of 75% acetonitrile used to elute the cartridges. The absolute recoveries of mevinolinic acid and the internal standard were around 95%. The recovery of mevinolin was around 85%.

Bile

The concentration of mevinolin and mevinolinic acid in bile samples was found to be much higher than in plasma. A major reason for examining bile samples was to look for drug metabolites. For that reason, it was desirable to avoid sample manipulation prior to injection that might lead to loss of possible metabolites. Small volumes (10 μl) of bile samples were, therefore, injected directly for HPLC analysis. Because no sample clean-up was attempted gradient elution HPLC was necessary to give adequate chromatographic resolution of the compounds of interest. No adverse affects on HPLC column performance have been detected using this approach.

This method has been successfully used to develop profiles for dog and rat bile following doses of mevinolin and mevinolinic acid. It has been used as the basis for an HPLC fractionation procedure to develop drug and metabolite profiles. Typical chromatograms of rat bile are shown in Fig. 3. This is from a 1-h post 10 mg/kg intravenous does of mevinolinic acid. Mevinolinic acid eluted at 7.83 min and mevinolin at 9.25 min. The peaks at 4.70, 6.06 and 7.18 min are probably drug-related and further work is in progress to identify these compounds.

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