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Determination of mevalonolactone in capsules by capillary gas–liquid chromatography

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Abstract: A wide bore capillary gas chromatographic method was developed to determine mevalonolactone in capsule formulations. The method uses β,β-dimethyl-γ-hydroxymethyl-γ-butyrolactone as an internal standard and has been validated for its accuracy, precision and linearity. The method has been applied for stability testing of the capsule formulation. High-performance liquid chromatographic and gas chromatographic studies demonstrated cyclization of mevalonic acid (open-chain form) to mevalonolactone (cyclic form) under the described gas chromatography conditions. Mass spectrometric analysis indicated that mevalonolactone prepared in water or an organic solvent emerged from the gas chromatographic column as the intact cyclic lactone.

Keywords: *Mevalonic acid; mevalonolactone; capillary GLC; dehydromevalonolactone; cholesterol; mass spectrometry and high-performance liquid chromatography.*

Introduction

Mevalonic acid, which has been detected [1] and identified [2] as a growth factor in certain bacteria, is an important substance in a number of biosynthetic processes. The chemistry of mevalonic acid and its biotransformation to cholesterol in the body has been reviewed [3].

Mevalonic acid exists in two forms as shown in Fig. 1, corresponding to an open-chain form (mevalonic acid, **I**) and a cyclic form (mevalonolactone, **II**) at physiological pH [4] and in aqueous media at pH 2 [5]. Mevalonic acid is prone to cyclization and therefore only its salt form can be isolated.

A variety of analytical techniques have been used to isolate and identify mevalonolactone; namely, anion exchange chromatography [4], thin layer chromatography [6–11], NMR [12] and gas chromatography (GC) [6, 13]. Recently, a capillary column GC method was reported for the simultaneous determination of mevalonolactone and cholesterol in animal feeds [14]. There is no information, however, about any reliable analytical methodology which is accurate, precise and rugged for the determination of mevalonolactone in pharmaceutical formulations and specific against dehydromevalonolactone (**III**, Fig. 1), a potential thermally induced degradation product. This work describes a rapid and accurate determination of mevalonolactone in capsules. The mevalonolactone capsules were used as a control substance in clinical studies of lovastatin [15], a potent inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, an enzyme essential in cholesterol biosynthesis (Fig. 2).

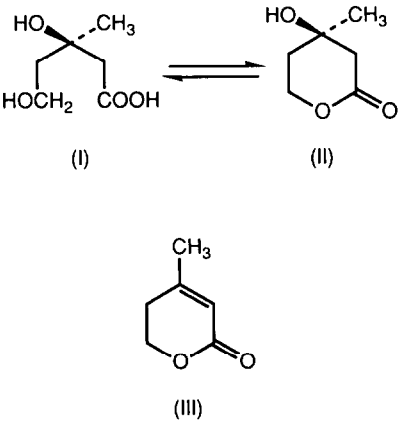

Figure 1
The structures of mevalonic acid (**I**), mevalonolactone (**II**) and dehydromevalonolactone (**III**).

Experimental

Reagents

D,L-Mevalonolactone was purchased from Sigma Co. (St. Louis, Missouri, USA). De-

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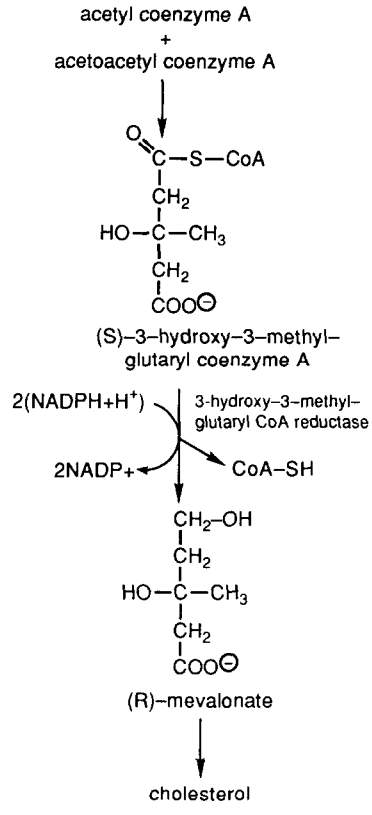

Figure 2
Mevalonate synthesis.

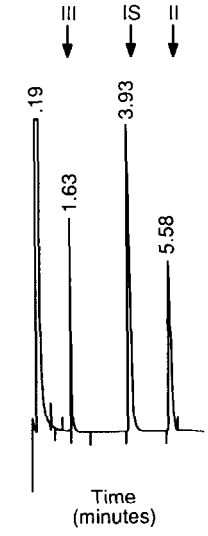

Figure 3
Capillary gas–liquid chromatogram of a standard mixture of mevalonolactone (**II**, 0.25 mg ml^{-1}), internal standard (**IS**, 0.275 mg ml^{-1}) and dehydromevalonolactone (**III**, 0.15 mg ml^{-1}).

hydromevalonolactone was prepared at Merck Sharp and Dohme Research Laboratories (Rahway, NJ, USA). β,β-Dimethyl-γ-hydroxy-methyl-γ-butyrolactone (the internal standard) was purchased from Aldrich Co. (Milwaukee, WI, USA). HPLC grade acetonitrile, potassium phosphate monobasic and phosphoric acid (85%) were purchased from Fisher.

Apparatus

The GC apparatus was a Hewlett–Packard 5890 gas chromatograph (HP Avondale, PA, USA) equipped with a hydrogen flame ionization detector and a model 7673 HP autosampler. A fused silica capillary column (RTX-225, 15 m × 0.53 mm i.d.) with a film thickness of 1.0 μm purchased from Restek Corp. (Bellefonte, PA, USA) was employed. Helium was used as the carrier gas (flow 15.5 ml min^{-1}). The temperature of the oven was 160°C; the injection port was at 190°C (direct injection) and the detector was at 300°C. A typical GC chromatogram is shown in Fig. 3.

Stock standard solution

Individual stock solutions containing mevalonolactone and β,β-dimethyl-γ-hydroxy-methyl-γ-butyrolactone were prepared at concentrations of 1.1 and 0.55 mg ml^{-1} in acetonitrile, respectively.

Working standard solutions

Ten millilitre working standard solutions of the following concentrations were prepared in acetonitrile by suitable dilution of the mevalonolactone stock solution: 0.28, 0.41, 0.55, 0.66 and 0.83 mg ml^{-1}. Calibration standards were prepared by mixing a 2.0-ml aliquot of these mevalonolactone working standard solutions with a 2.0-ml aliquot of the internal standard stock solution.

Sample preparation and assay

For composite assay, the contents from at least five capsules were combined. Duplicate, weighed aliquots were diluted with acetonitrile to a theoretical concentration of 0.5 mg ml^{-1}. A 2.0-ml aliquot of the diluted solution was then mixed with 2.0 ml of the internal standard stock solution for analysis. 1.0-μl aliquots of samples and standards were injected for GC analyses.

Quantification

A peak area ratio (mevalonolactone–internal standard) was calculated for the un-

knowns containing added internal standard. This was compared against a standard calibration curve. By regression analysis, data from typical standard curve demonstrated a regression equation of

$$y = 3.2243x + 0.0032253 \quad (1)$$

and a coefficient of correlation of 0.999.

Results and Discussion

GC assay development

Initially, assay conditions were developed using packed column (3% OV-225) GC to support preclinical samples of mevalonolactone in water. For analysis of feed samples which contain cholesterol and mevalonolactone, a simultaneous assay was developed [14] using a capillary column (DB 210) which yielded better peak shape than the packed column.

In order to support the analysis of mevalonolactone in capsules for clinical studies, the benefits obtained from using a wide-bore capillary column were investigated. A wide-bore capillary column with a film thickness of 1.0 μm provided enhanced column capacity over a narrow-bore capillary column and facilitated the injection of a larger volume of solution for rugged use. Additionally, it allowed direct transfer of packed column method with minor modifications. A fused silica capillary column (RTX 225) with a film thickness of 1.0 μm demonstrated excellent chromatography and separation of all three compounds of interest (**II**, **III** and the **IS**) and was the column of choice.

Validation

The GC method has been validated for its recovery, linearity, precision and limit of quantitation, using the procedures recommended in the USP [16]. A three-point recovery study was performed by analysing samples spiked with known amounts of analyte at 80, 100 and 120% levels of claim. The results shown in Table 1 indicate that an average recovery of 98.4% was obtained. The method was linear over a concentration range of 0.138–0.414 mg ml^{-1} with a coefficient of correlation of 0.999. This range corresponds to approximately 50–150% of the method concentration. Injection precision based on 10 replicate injections of a standard solution (0.28 mg ml^{-1}) was determined to be 0.24% (RSD). The results are summarized in Table 2. Method precision was performed by analysing 10 aliquots from the pooled contents of five capsules (125 mg/cap). Results are shown in Table 3 and demonstrate an RSD of 1.0%. The method can detect as little as 0.0034 mg ml^{-1} with good reproducibility.

Table 1
Recovery data

% Claim	Added (mg)	Found (mg)	% Recovery
80	403.2	397.0	98.5
100	502.5	494.9	98.5
120	605.9	595.1	98.2
		Mean =	98.4%

Table 2
Injection precision

Area ratio of mevalonolactone–internal standard	
	1.910
	1.901
	1.899
	1.897
	1.900
	1.899
	1.904
	1.908
	1.897
	1.899
Mean =	1.901
RSD =	0.24%

Table 3
Method precision

Sample	Assay (mg/cap)
1	128.0
2	127.7
3	127.5
4	129.8
5	127.0
6	126.7
7	129.7
8	129.8
9	127.0
10	130.0
Mean =	128.3
RSD =	1.0%

Solution stability

Mevalonolactone and the internal standard (β,β-dimethyl-γ-hydroxymethyl-γ-butyrolactone) were found to be stable in chloroform and acetonitrile under laboratory conditions for 24 h. The observed total ion chromato-

grams (TIC) (Figs 4A and 4B) from GC–MS analysis of mixtures of these compounds in the respective solvent systems showed two major components eluting at 9.11 and 10.32 min. The corresponding mass spectra (Figs 5A and 5B),

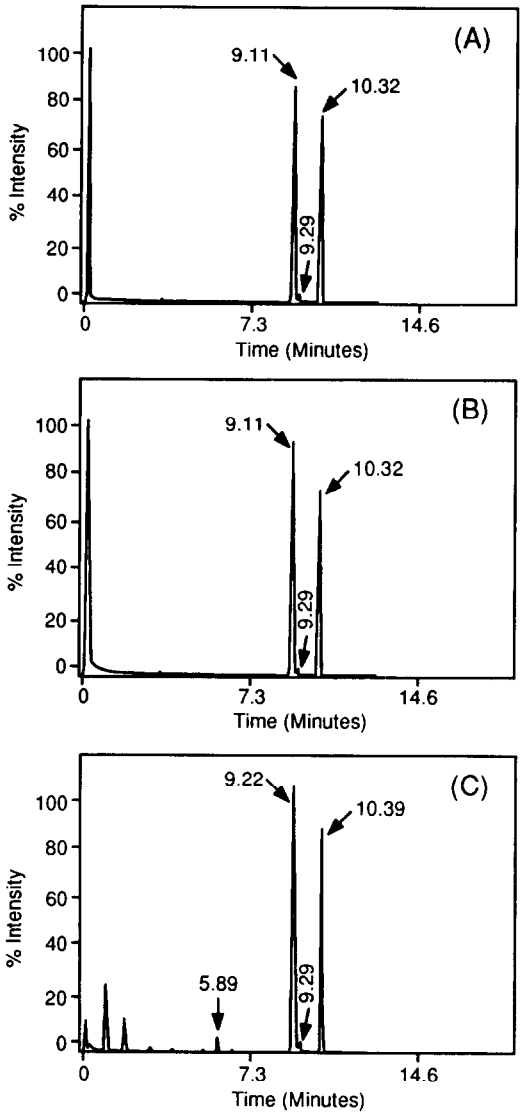

Figure 4
Total ion chromatograms of mevalonolactone and internal standard in (A) chloroform, (B) acetonitrile and (C) water. GC–MS analyses were performed on the VGMM7035 mass spectrometer, interfaced with the HP 5710A gas chromatograph. For these analyses, a 15 m DB 225 fused silica capillary column was heated from 100°C (isothermal for 2 min) to 220°C at 80°C min^{-1}. Injector temperature was kept at 250°C. A 0.1-μl injection (splitless) of 0.1 mg ml^{-1} solution was made for the analyses. Mass spectrometric conditions were set at a source temperature of 200°C, an interface temperature of 220°C and an ionization energy of 60 V. Data acquisition was done via the on-line VGDS2000 series data system. The instrument was calibrated from mass 20–300 and data were acquired at a scan rate of 1 s per decade of mass for all samples analysed.

with molecular ions at *m/z* 144 and 130, respectively, were consistent for the internal standard and mevalonolactone. A minor component with a retention time of 5.89 min (Fig. 4C) was observed in aqueous media. The mass spectrum of this component (Fig. 5C) shows a molecular ion of *m/z* 112 and base peak at *m/z* 82. This, in addition to other mass peaks in the spectrum, was consistent for the dehydromevalonolactone. The internal standard also exhibited a minor component at 9.29 min (Figs 4A, 4B and 4C). This component was found to be present in all solutions containing the internal standard. The mass spectrum of this component exhibited mass peaks at *m/z* 113, 85 and 56, which are characteristic of an isomer of the butyrolactone (the internal standard).

Method specificity

Under the prescribed assay conditions, mevalonolactone is separated from dehydromevalonolactone and the internal standard. The capsule formulations (125 and 500 mg potency) which were assayed by the capillary GC method contain only mevalonolactone and a small amount of water (3%) (water uptake determined from equilibrium study). Based on validation data obtained (see validation section), the assay procedure was specific for mevalonolactone in capsules. Corn oil, which was used to fill the capsules for use as placebo, did not interfere with the assay.

Application

The GC assay method was applied to stability analyses of capsule formulations stored at 30°C for 10 weeks. The results, which are tabulated in Table 4, indicate mevalonolactone in the formulation is chemically unchanged during storage. The 5°C samples were analysed as reference for comparison.

Cyclization study

It has been previously implied that mevalonic acid spontaneously cyclizes at room temperature [4, 8]. It has also been reported [6] that mevalonic acid isolated as the end product in the assay of hydroxymethylglutaryl-CoA reductase activity cyclizes in the presence of 3 M HCl. Quantitation was by thin layer separation and radioactive measurements. The same authors also reported that the use of 9 M HCl leads to the formation of Δ^3,4-methyl-γ-valerolactone (dehydromevalonolactone) and qualitatively demonstrated its formation by

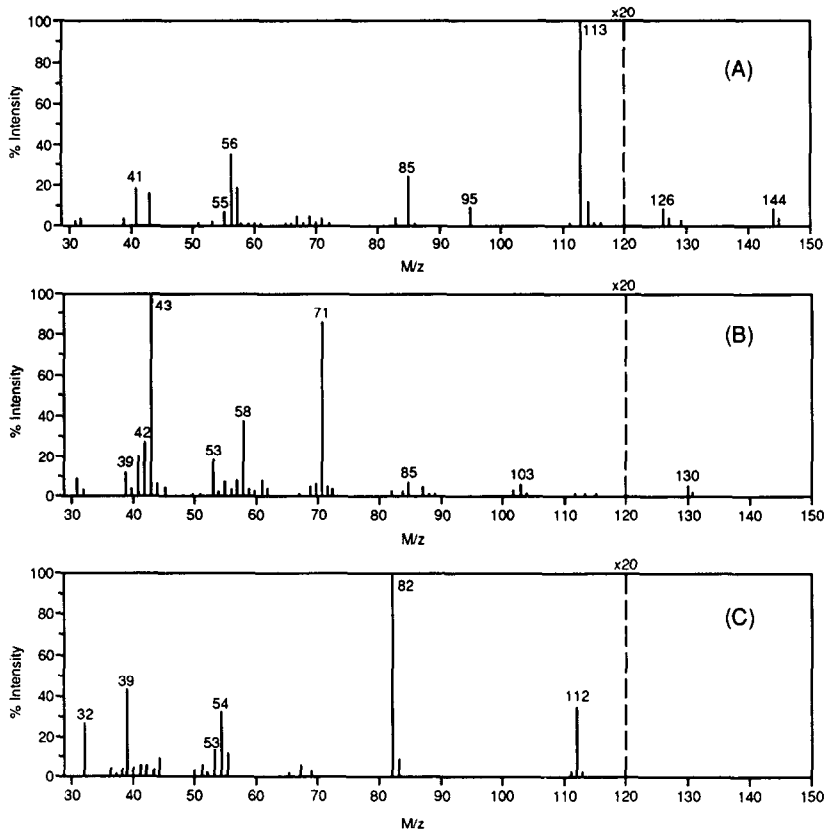

Figure 5
Mass spectra of (A) dehydromevalonolactone, (B) internal standard and (C) mevalonolactone derived from total ion chromatograms (Fig. 4).

Table 4
Assay of mevalonolactone capsules stored for 10 weeks

Capsule formulation	Label (mg)	Storage temperature (°C)		Assay (%)*
A	125	30		97.7
				97.5
				98.5
				98.4
			Mean =	98.0
		5		99.0
				98.7
			Mean =	98.8
B	500	30		98.4
				99.4
				99.0
				98.9
			Mean =	98.9
		5		99.7
				98.9
			Mean =	99.3

*Corrected for K.F.

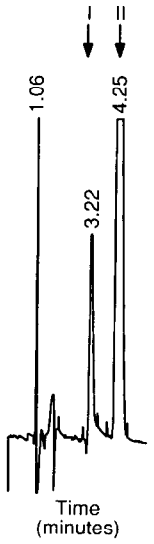

Figure 6
HPLC chromatogram of mevalonic acid (I) and mevalonolactone (II). The HPLC instrument used was a HP 1090LUSI (HP Avondale, PA, USA) with a Spectroflow 783 variable wavelength detector (Applied Biosystems, Foster City, CA, USA) and a HP 3390A integrator. The column used was a Hypersil ODS (5 μm, 100 × 4.6 mm i.d.) obtained from Hewlett-Packard. The mobile phase consisted of 0.05 M potassium phosphate monobasic, adjusted to pH 2.5 with phosphoric acid, and acetonitrile (99:1), premixed and degassed with helium. The injection volume was 50 μl, the oven temperature was ambient, and the detection was by UV at 210 nm. The detector range was set at 0.005 AUFS.

GC. Further evidence is provided here that mevalonic acid also cyclizes to mevalonolactone under the GC conditions.

A 1-mg ml^{-1} aqueous solution of mevalonolactone standard which was stored at room temperature for 72 h was diluted to 0.5 mg ml^{-1} with water and assayed by HPLC. A chromatogram illustrating the separation of mevalonic acid and mevalonolactone by HPLC is shown in Fig. 6. The HPLC assay of the solution showed the presence of about 7% (by area) of mevalonic acid. After an additional 24 h, this diluted solution yielded about 8% mevalonic acid. The same solution (after addition of the internal standard) was injected into the gas chromatograph. A recovery of 98.9% mevalonolactone was obtained. Immediate HPLC analysis of a freshly prepared sample (1 and 0.5 mg ml^{-1} mevalonolactone in water) detected about 0.5% of the mevalonic acid. The above results offer convincing evidence for the cyclization of the open-chain form to the cyclic lactone in the GC injection port.

Conclusions

The GC analytical method described has been shown to be linear, accurate, precise and specific for mevalonolactone. The method has been used to measure mevalonolactone in capsule formulations. Mevalonic acid has been demonstrated to cyclize to mevalonolactone under the prescribed GC conditions.

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