

to 101.4. The reproducibility of the method was checked by ten replicate determination at 2 µg/ml level of sulpha drug and the relative standard deviation (%) was found to vary between 0.30 to 0.80. The present method has been applied for the analysis of sulpha drugs in pharmaceutical preparations. The results of the analysis of septran, sulphadiazine, albucid and locula compare favourably with those of the official method<sup>3</sup> and the reported methods<sup>8,9</sup>.

The proposed method is found to be simple, rapid and sensitive. Thus the method can be adopted as an alternative to the existing spectrophotometric methods. The recommended procedure is well-suited for the assay and evaluation of drugs in pharmaceutical preparations to assure high standard of quality control.

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## An Improved Gas Chromatographic Assay Method for HMG-CoA Reductase and Mevalonic Acid

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P. BHARATHI DHASAN, R. SUNDARAM, R. SURESH KUMAR, S. K. MITRA AND KALA S. KULKARNI\*  
R&D Center, The Himalaya Drug Company, Makali, Bangalore-562 123.

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**A simple and accurate gas chromatographic method for the *in vitro* estimation of mevalonic acid (as mevalonolactone) was developed. The method showed good sensitivity, accuracy and linearity over a wide concentration range. Detection at lower concentrations without the use of any radioactivity measurements, and more rapid analysis than other existing methods, are the advantages of this modified method.**

Cholesterol is biosynthesised in the body from acetyl-CoA. Though it involves many steps, the most important, rate-limiting step is the formation of mevalonic acid from acetyl-CoA in the presence of enzyme, 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase<sup>1</sup>. Drugs such as lovastatin, simvastatin and guggulipid, which inhibit HMG-CoA

reductase, are used to treat hypercholesterolemia. Several methods have been used to detect and estimate mevalonic acid from biological samples. But these methods include complicated procedures like derivative formation and crystallization to constant specific radioactivity<sup>2</sup>, hydroxamate formation<sup>3</sup> or paper chromatography followed by assay for radioactivity<sup>4</sup>. Since crystallization of derivatives of mevalonic acid to constant specific radioactivity is time consuming, another method is sought to quantify the mevalonic acid

\*For correspondence  
E-mail: dr.kala@himalaya.ac

formed from acetyl-CoA ester. This method is proven to be very satisfactory for the assay of mevalonic acid (as mevalonolactone) from biological systems.

DL-mevalonic acid lactone and acetyl-CoA were purchased from M/s. Sigma Chemicals, St. Louis, MO, USA. Other chemicals were obtained from commercial sources as analytical grade reagents and used without further purification. Micro-9100 Chromatograph of Netel Chromatographs was used for the analysis. Polyethylene glycol (PEG) (10%) packed on diatomite AW 80/100 2 m x 1/8' was used as the column for gas chromatography.

Rat livers were excised and chilled for a few minutes in an ice bath. Each liver was dabbed with filter paper to remove excess water. Then the livers were weighed, chopped into small pieces and homogenized in a Potter-Elvehjem type homogenizer, with ice cold 0.1 M phosphate buffer, pH 7.4, containing nicotinamide 2.4  $\mu\text{mol/ml}$ , ethylenediamine tetra acetate (EDTA) 1.2  $\mu\text{mol/ml}$ , and magnesium chloride 40  $\mu\text{mol/ml}$  to get a 50% w/v suspension<sup>5</sup>. The crude homogenate was centrifuged at 10,000 rpm for 30 min to remove cellular debris. The supernatant was filtered through 0.4  $\mu\text{m}$  syringe filter. The filtrate containing microsomal and soluble enzymes were used as the enzyme source<sup>6</sup>. The protein concentration of this mixture was determined by using the Folin phenol reagent<sup>7</sup>.

The incubation mixture contained acetyl-CoA-300  $\mu\text{mol/ml}$ , as substrate, adenosine triphosphate (ATP)-10  $\mu\text{mol/ml}$ , nicotinamide adenine dinucleotide phosphate (NADP)-2.4  $\mu\text{mol/ml}$ , glucose-1-phosphate (G-1-P)-60  $\mu\text{mol/ml}$ , magnesium chloride, 40  $\mu\text{mol/ml}$ , reduced glutathione (GSH)-50  $\mu\text{mol/ml}$ , phosphate buffer (pH 7.4)-600  $\mu\text{mol/ml}$  and enzyme equivalent to 20 mg of protein of rat liver homogenate. The above mixture was diluted to a volume of 3 ml and incubated for 2 h at 38°.

The Lynen and Grassl method<sup>9</sup> was used to extract mevalonic acid from the incubation mixture with minor modifications. Briefly, the incubation mixture was deproteinized by heat denaturation and the supernatant solution was acidified with 12 M sulphuric acid to pH 1. Mevalonic acid was lactonized by incubation at 38° for 15 min. About 1 g of anhydrous sodium sulphate was added to the aqueous solution. Then the mevalonic acid lactone was extracted with 3x10 ml of chloroform. The combined extracts were evaporated to dryness, reconstituted with 0.1 ml diethyl ether and injected into the gas chromatograph.

The lactonized mevalonic acid was chromatographed

using a previously conditioned column of 10% polyethylene glycol on diatomite AW 80/100 2 m x 1/8' at 200°. The nitrogen gas flow rate was 30 ml/min. The injector temperature and flame ionisation detector (FID) temperature were kept at 210° and 220°, respectively. Standard mevalonic acid solutions were prepared at different concentrations in diethyl ether and analyzed for the linearity. Known concentrations of the standard mevalonic acid were spiked into the biological samples and the compound was extracted and analyzed to study the recovery of extraction. The mevalonic acid formed in the above mentioned incubation mixture was analyzed in a similar way and evaluated for reproducibility.

The new method of analysis of mevalonic acid detected lowest level of 1  $\mu\text{g}$  and the standard curve was linear between 1  $\mu\text{g}$  to 125  $\mu\text{g}$  level (fig. 1). The concentration of mevalonic acid formed in the *in vitro* experiments, ranged from 25 to 50  $\mu\text{g}$ . The modified method of extraction of mevalanolactone from the incubation mixture resulted in a better recovery (75.9  $\pm$  3.38 %) than the previous method

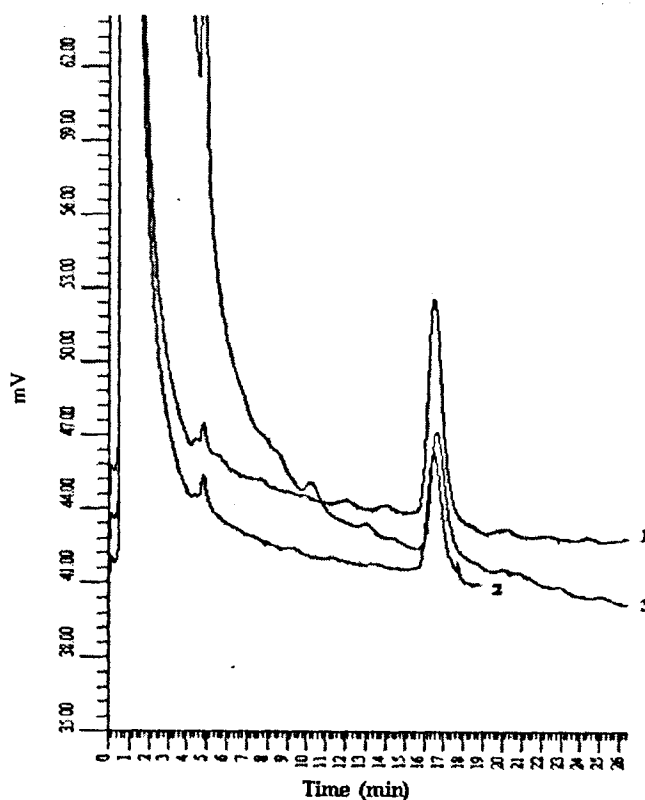


Fig. 1: Analysis of mevalonic acid in biological systems. Standard mevalonic acid (1), mevalonic acid analysis in the spiked biological sample (2), estimation of mevalonic acid in unknown sample (3).

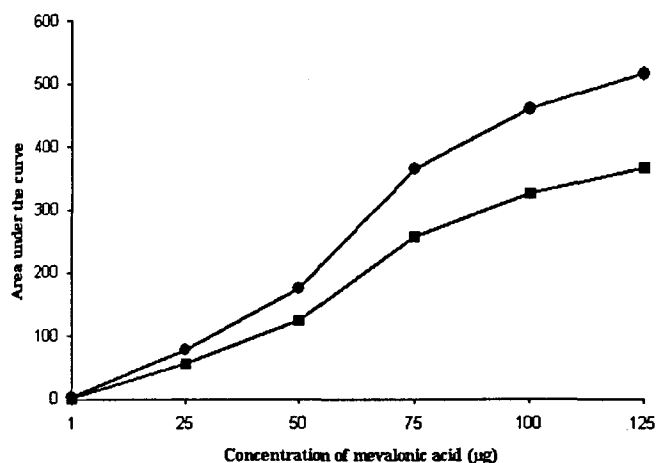


Fig. 2: Standard curve of mevalonic acid.

Mean area under the curve for standard mevalonic acid solution (●), mean area under the curve for mevalonic acid analysed from the spiked samples (■).

(64.5%)<sup>10</sup> (Table 1). Rapid analysis of mevalonic acid is possible due to its short retention time of 16 min, whereas, in the previous methods it was about 35-40 min. No interference in the analysis of mevalonic acid from other biological constituents (fig. 1) occurred using this method.

The procedure is very simple, reproducible and suitable for screening of a large number of samples. The higher sensitivity and detection at lower concentrations of the compound by this method is comparable with existing methods, which involve complex procedures or radioactivity measurements. The proposed method of analysis of mevalonic acid can be applied to quantify mevalonic acid from various biological samples. It would be useful for screening of drugs, which inhibit HMG-CoA reductase, which are used in the management of hypercholesterolemia.

TABLE 1: RECOVERY OF MEVALONIC ACID BY EXTRACTION FROM THE INCUBATION MIXTURE

Sample No.	Labelled amount (µg)	Amount found* (µg)	% Recovery*
1	10	6.73 ± 0.42	67.30 ± 4.2
2	50	49.0 ± 2.4	98.00 ± 4.8
3	100	70.1 ± 5.1	70.10 ± 5.1
4	250	213.0 ± 7.6	85.20 ± 3.04
5	500	331.2 ± 8.3	66.24 ± 1.66
6	1000	690.8 ± 14.8	69.08 ± 1.48

\* Mean and standard deviation of 6 determinations.

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