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RESEARCH ARTICLE

GC-HS Method Development for the Estimation of Benzene Content in Lovastatin

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ABSTRACT

A simple, fast and accurate method has been developed for the estimation of benzene content in Lovastatin by Gas Chromatography. The analysis was carried out on Perkin Elmer Clarus 600 GC-HS Chromatograph. The column used was DB-624 30m X 0.32 mm X 1.8 µm fused silica analytical column (6% cyanopropylphenyl 94 % dimethylpolysiloxane as a stationary phase).The detector used was FID detector.

KEYWORDS

Gas Chromatography, Lovastatin, Benzene.

INTRODUCTION

Lovastatin is a member of the drug class of statins, used in combination with diet, weight-loss, and exercise for lowering cholesterol (hypolipidemic agent) in those with hypercholesterolemia to reduce risk of cardiovascular disease. Lovastatin is a naturally occurring drug found in food such as oyster mushrooms[1] and red yeast rice[2]. Lovastatin is also naturally produced by certain higher fungi, such as *Pleurotus ostreatus* (oyster mushroom) and closely related *Pleurotus* spp[3]. A major bulk of work in the synthesis of lovastatin was done by M. Hirama in the 1980s[4,5] Hirama synthesized compactin and used one of the intermediates to follow a different path to get to lovastatin.

The primary use of lovastatin is for the treatment of dyslipidemia and the prevention of cardiovascular disease and is recommended to be used only after other measures, such as diet, exercise, and weight reduction, have not improved cholesterol levels[6] Compactin and lovastatin, natural products with a powerful inhibitory effect on HMG-CoA reductase, were discovered in the 1970s, and taken into clinical development as potential drugs for lowering LDL cholesterol[7,8]. Lovastatin and other statins have recently been studied for their chemo preventive and chemotherapeutic effects in certain cancers. However, based on clinical evidence, such effects could not be demonstrated[9]. In principle, independent of their hydroxymethyl glutaryl (HMG)-CoA reductase inhibition, lovastatin and other statins reduce proteasome activity, leading to an accumulation of cyclin-dependent kinase inhibitors p21 and p27, and G1 phase arrest in breast cancer cell lines. For that purpose, lovastatin is also used experimentally[10].

Analytical techniques for benzene have advanced significantly in recent years, and development continues. Modern technologies include gas chromatography (GC), mass spectrometry (MS), infrared (IR), ultraviolet (UV), and fluorescence spectroscopy, and combined techniques, such as GC–MS and GC–

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Fourier transform infrared (FTIR), etc. [11–20]. Among these techniques capillary GC with flame ionization detection (FID) is most important and most widely used for analysis of benzene content[4–7, 10, 11].

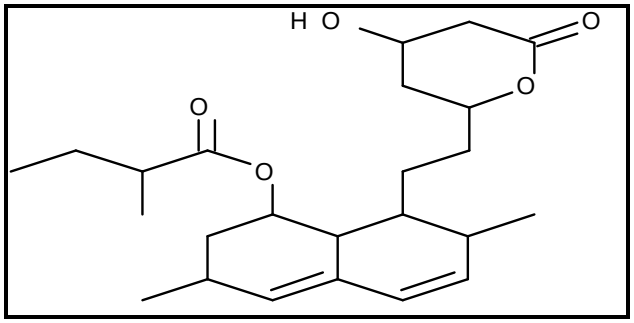

Figure 1: Lovastatin Structure

MATERIALS AND METHODS

Table 1: Reagents and chemicals

Sr. No	Reagents and Chemicals	B. No./ Manufacturer	Purity
01	Lovastatin	PG/LOV/57/60 In house	99.39
02.	Benzene	605A-0203-1504-31/SDFINECHEM	99.70
03	Dimethyl Sulphoxide	K42708900 217 Merck	99.80

Table: 2 Chromatographic conditions

Instrument	:	PE, Clarus 600 Gas chromatography with Headspace injector.
Column	:	DB-624 30m X 0.32mm X 1.8 µm
Carrier gas	:	Nitrogen
Gas flow/Pressure	:	1.0 ml/min
Injector temperature	:	200°C
Detector temperature	:	250° C (hold for 10 min)
Oven program		Ramp 10°C/min upto 220°C (hold for 10.0 min)
Sensitivity	:	10^{-6}
Range	:	1
Total run time	:	38
Split ratio	:	5:1
Head Space Parameter		
Oven temperature	:	85°C
Needle temperature	:	95°C
Transfer line temperature	:	110°C
GC Cycle time	:	40.0 min
Thermostatic time	:	30.0 min
Injection time	:	0.2 min
Withdrawal time	:	0.2 min
Vial venting	:	ON
Column pressure	:	18 PSI
Pressurizing time	:	3.0 min

Preparation of Solutions

Blank Preparation

Transfer carefully 2 ml of Dimethyl Sulphoxide (DMSO) into the headspace vial and crimp the vial with aluminum cap and PTEF septa.

Standard Stock Solution Preparation

Weigh accurately about 10 mg of Benzene into 100 ml volumetric flask containing about 20 ml of diluent, Mix and dilute to volume with diluent. Take 10 ml of this solution into 100 ml volumetric flask containing about 20 ml of diluent, Mix and dilute to volume with diluent.

Standard Solution Preparation

Pipette out 5 ml of Stock solution into another 100 ml volumetric flask containing about 20 ml of diluent, Mix and dilute to volume with diluent. Transfer 2 ml each standard preparation into the six head space vials and crimp each vial with aluminum cap and PTFE septa.

Test Solution Preparation

Weigh and transfer carefully about 500 mg of test sample in the head space vial, add 2 ml of diluent and mix well to dissolve. Crimp the vial with aluminum cap and PTFE septa.

Procedure

1. Inject blank preparation in to the chromatograph.
2. Inject six standard preparation in to the chromatograph, integrate peak corresponding to the standard component and record the chromatogram.
3. Inject two test preparation in the chromatograph and integrate peak corresponding to the retention time of benzene that obtained with standard preparation.

System Suitability

The test is valid only if in standard preparation solution, % RSD of peak area for Benzene is not more than 15.0%.

Calculation

$$\text{Residual content (ppm)} = \frac{At \times Ws \times Dt \times 1000000}{As \times Ds \times Wt}$$

Where,

At - Average peak area of Benzene in the test preparation.

As - Average peak area of Benzene in the standard preparation.

Ws - Weight of Benzene in the standard preparation.

Wt - Weight of test sample.

Ds - Dilution of Standard.

Dt – Dilution of Test.

RESULTS AND DISCUSSION

Construction of Linearity

The plot peak area of Benzene Vs respective concentration of linearity levels are found linear in the range of 0.10 ppm to 3.03 ppm for Benzene with correlation 0.999.Also LOQ Level and Higher level injected in three replicates and determine the relative standard deviation which shows that method is precise for LOQ Level and Higher level to determine the precision of the proposed method, three samples (as such) were analyzed and determine the % RSD benzene content in Lovastatin. The content is well within the limit. Linearity curves are shown in figure: 1 and the results are shown in table no: 3 and 4.

Recovery (Accuracy)

To study the accuracy, reproducibility and the precision of the proposed method, recovery experiment was carried out by adding standard Benzene at three different levels in pre-analyzed sample. The study was carried out with spiking of LOQ level solution for benzene. This was determined in triplicate. Recovery observed shown in table No 5.

Table 3: Linearity of Benzene

Sr.No	Level	Area	Average	Std. Dev	% RSD	Concentration(ppm)
1	LOQ	641	659	29.7377	4.5125	0.10
	LOQ	693				
	LOQ	642				
2	50	5535	5494	56.3590	1.03	1.01
	50	5430				
	50	5518				
3	75	8966	8776	230.9091	2.63	1.62
	75	8519				
	75	8843				
4	100	11298	11342	129.7228	1.14	2.02
	100	11488				
	100	11240				
5	120	13279	13336	165.4459	1.24	2.42
	120	13522				
	120	13206				
6	150	16336	16893	535.0069	3.17	3.03
	150	17403				
	150	16939				

Table 4: Slope, Intercept and Coefficient correlation

Slope	5549
Y-Intercept	-17.55
Coefficient correlation (R^2)	0.999

Table 5: Recovery (Accuracy) for Benzene

% Level of Recovery	Area of Benzene	Content (ppm)	% Recovery
80 %	8000	2	97 %
100 %	9801	2	95%
120 %	11731	2	94%

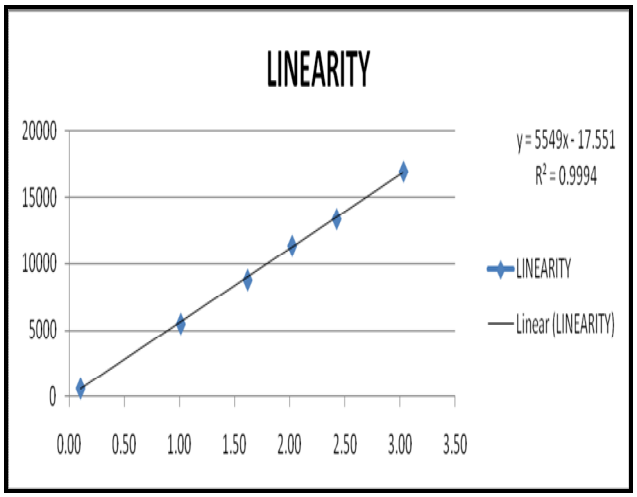

Figure 2: Linearity of Benzene

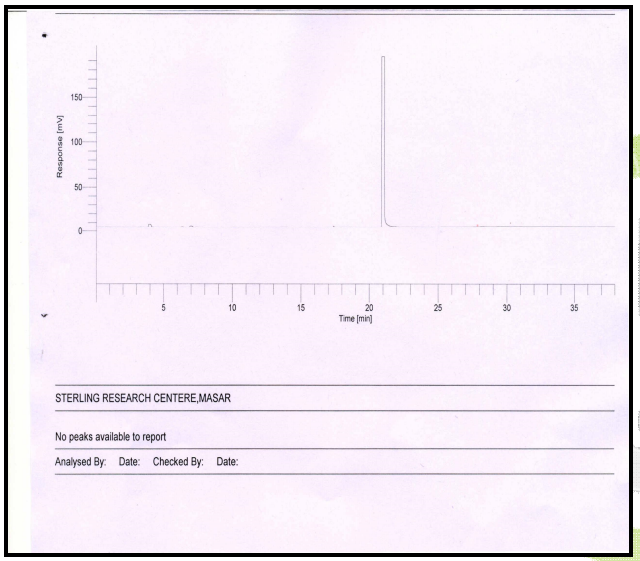

Figure 3: Chromatogram of Blank

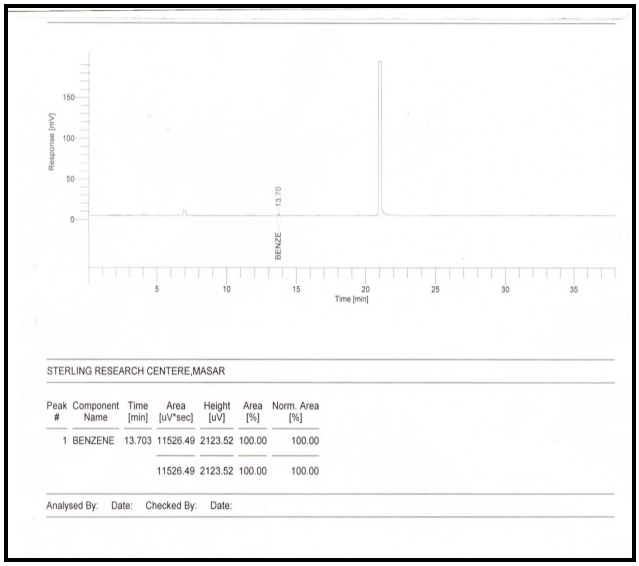

Figure 4: Chromatogram of Standard

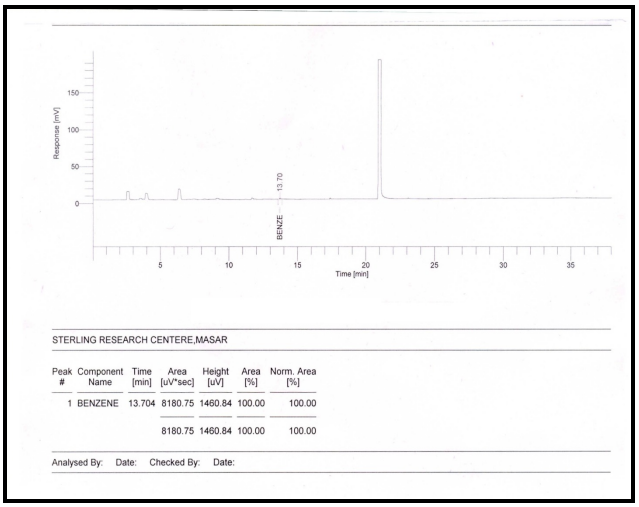

Figure 5: Chromatogram of Sample

CONCLUSION

The linearity regression coefficient was found 0.999 for Benzene content which shows that response is linear from 0.10 ppm to 3.03 ppm. High percentage of recovery shows that the method is free from interference of other raw material. The recovery value proves that method is accurate and reproducible. The proposed method is simple, fast, accurate and precise. Thus proposed method can be used for routine quality control analysis of Lovastatin for monitoring Methanol and Toluene content.

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