



Development of Gas Chromatographic method for Antibacterials

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Abstract

A simple, fast and accurate method has been developed for the determination of Ethanol and Acetone in Clarithromycin granules by Gas Chromatography. The analysis was carried out on Shimadzu GC-2010 Gas Chromatograph. The column used was 30m x 0.32 mm Id fused silica analytical column ZB-624, 1.80 μ m. (6% cyanopropylphenyl 94 % dimethylpolysiloxane as a stationary phase). The detector used was FID detector. The validation of proposed method was also carried out.

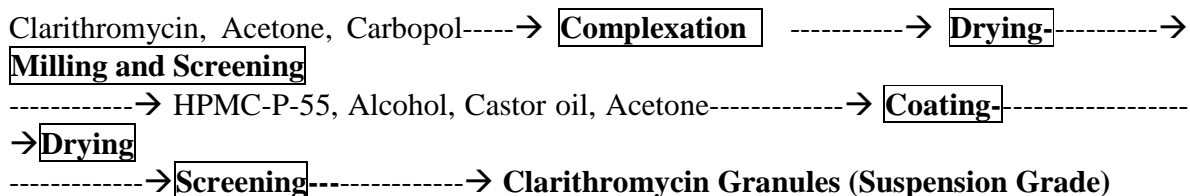
Key words: Gas Chromatography, Clarithromycin granules, Ethanol, Acetone

Manufacturing process

Manufacturing of Chemicals or drugs require use of many solvents. Residual solvents in pharmaceuticals are described as Organic volatile impurities. These solvents are not completely removed by practical manufacturing techniques. Appropriate selection of organic solvent in a synthesis of drugs enhance yield of drugs, or determine characteristic of such critical parameter as crystal form, purity and solubility. It means it plays important role in synthetic process.

Since there are no therapeutic benefits from residual solvents, all solvents should be removed to extent possible to meet product specification, Good manufacturing practices or other quality based requirements. Depending upon toxicity, residual solvents are classified into different classes internationally. Limits for residual solvent are fixed based on TDI (Tolerable daily intake) or PDE (Permitted daily exposure). So it is responsibility of manufacturer to control the solvents before going the formulation for consumption.

Manufacturing Flow for Clarithromycin Granules



In the above Process of manufacturing, Clarithromycin granules are prepared by Clarithromycin by using different operations. Ethanol and Acetone solvents are used in the manufacturing process.

Introduction

In the recent invention granules are provided that may be formulated into a liquid suspension, thereby allowing prompt release of the active agent. The present invention provides granules of a clarithromycin, which include a core comprising clarithromycin and one or more hydrocolloids, a coating over the core, which includes one or more pH dependent polymers. The granules may be used with a suspending medium to form a pharmaceutical composition. The composition provided is sufficiently able to preserve the taste masking effect for a period of at least 14 days after reconstitution.

In yet another general aspect there is provided a method of treating bacterial infections in a mammal in need thereof. The method includes administering a pharmaceutical composition comprising granules, wherein the granules include a core comprising clarithromycin and one or more hydrocolloids, and a coating over the core. The clarithromycin and the one or more hydrocolloids are present in a ratio from about 1:0.2 to about 1:10. The coating comprises one or more pH dependent polymers that release clarithromycin at a pH above 4.5.

Antibacterials should be used only if clinical or laboratory evidence suggests bacterial infection. Use for viral illness or undifferentiated fever is inappropriate, subjects the patient to drug complications without any benefit, and contributes to bacterial resistance. Certain bacterial infections (eg, abscesses, infections with foreign bodies) require surgical intervention and do not respond to antibiotics alone.

Cultures and antibiotic sensitivities are essential for selecting a drug for serious infections. However, treatment often must begin before culture results are available, necessitating selection according to the most likely infecting organisms (empiric antibiotic selection). Whether chosen according to culture results or empirically, drugs used should possess the narrowest spectrum of activity that will control the infection. For empiric treatment of serious infections that may involve any one of several pathogens (eg, fever in a neutropenic patient) or that may be due to multiple pathogens (eg, polymicrobial anaerobic infection), a broad spectrum of activity is desirable. The most likely organisms and the organisms' susceptibility to antibacterial vary according to geography (within cities or even within a hospital) and can change from month to month.

Bactericidal drugs kill bacteria in vitro. Bacteriostatic drugs slow or stop in vitro bacterial growth but depend on body defenses to kill bacteria.

The present invention relates to taste masked granules comprising poorly soluble, bitter tasting pharmaceutical ingredients, and oral suspension compositions thereof. Also provided are processes for the preparation of taste masked granules and use of the granules to treat a bacterial infection.

Experimental Section

Reagents and chemicals:

Dimethyl sulphoxide HPLC grade	: E Merck India Ltd.
Ethanol HPLC grade	: E Merck India Ltd.
Acetone HPLC grade	: E Merck India Ltd.

Chromatographic Conditions :

Column type	: 30.0m x 0.32 mm ID, 1.8 fused silica capillary column (J & W DB-624 is suitable). (6% Cyanopropyl phenyl, 94% dimethyl polysiloxane)
Carrier gas	: Helium
Flow rate	: 2.50 ml/min.
Detector	: FID
Detection range	: 0
Injector port temperature	: 140°C
Detector temperature	: 250°C
Column oven temperature	: Hold at 40°C for 8 min., then raise to 240°C at a rate of 30°C/min. and hold for 10 min.
Equilibration time	: 1 min.
Split ratio	: 5 : 1

The headspace conditions are as follows :

Cycle	: HS-inj.
Syringe	: 2.5 ml - HS
Sample volume	: 750 µl of head space
Incubation temperature	: 90.0 °C
Incubation time	: 20.0 min.
Agitation speed	: 500 rpm
Agitation on time	: 5 sec.
Agitation off time	: 2 sec.
Syringe temperature	: 100 °C
Fill speed	: 150 µl/s
Pull up delay	: 500 MS.
Inject to	: GC injector
Inject speed	: 200 µl/sec.
Pre inject delay	: 500 ms

Post inject delay	: 500 ms
Syringe flushing	: 00:10:00
GC run time	: 00:34:00

Preparation of Standard solution :

Preparation of standard solution A :

Weigh accurately each about 1.0 g of Ethanol GC standard, 1.0 g of Acetone GC standard into a 100 ml volumetric flask containing about 60 ml of Dimethyl sulphoxide. Mix and dilute to volume with Dimethyl sulphoxide. Further dilute 5 ml of this solution to 100 ml with Dimethyl sulphoxide. (This solution contains 5000 ppm of Ethanol & 5000 ppm of Acetone w.r.t. sample concentration).

Preparation of standard solution B :

Weigh accurately each about **1.0** g of Ethanol GC standard, 1.0 g of Acetone GC standard into a 100 ml volumetric flask containing about 60 ml of Dimethyl sulphoxide. Mix and dilute to volume with Dimethyl sulphoxide. Further dilute 5 ml of this solution to 100 ml with Dimethyl sulphoxide. (This solution contain 5000 ppm of Ethanol & 5000 ppm of Acetone w.r.t. sample concentration).

Test solution

Weigh accurately 0.5 g of the sample and transfer into a vial (vial of 20 ml capacity is suitable). Add 5 ml of Dimethyl sulphoxide and dissolve, equip the vial with a crimp cap and seal.

Procedure

Using a gas tight syringe separately inject equal volumes (about 1 ml) of the gaseous headspace of blank solution, standard A and standard B (six injection), followed by Test solution and record the chromatogram and measure the peak area response. Record the resolution and the peak area response. If blank interference is observed, inject blank solution three times and take mean response. Blank interference should not be more than 15 %. Under the prescribed conditions the residual solvents elutes in the sequence of Ethanol and Acetone. A suitable system is one that yields chromatogram in which all the components in the resolution solution are well resolved. Identify based on the retention time any interfering peak from blank solution corresponding to the listed solvents from the standard and sample solutions and calculate the quantity of the residual solvents detected.

System suitability requirements:

- 1) The similarity factor should be between 0.85 to 1.15.

Area of solvent peak in the chromatogram obtained with standard solution A x Wt. of respective solvent in standard B

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Area of solvent peak in the chromatogram obtained with standard solution B (1st injection) x Wt. of respective solvent in standard A

Similarity factor should be between 0.85 to 1.15. If the similarity factor is found to be within limits continue chromatography with further injections of standard preparation 2. If the similarity factor does not fall within 0.85 to 1.15 prepare fresh standard solution in duplicate, measure the Peak area response and calculate similarity factor again as above.

- 2) The relative standard deviation for peak area responses and retention time of solvents for 6 replicates of the standard B should not be more than 15 % and 2.0 % respectively.
- 3) The resolution between two adjacent peaks in standard solution A should not be less than 2.0 .

Order of Elution : Ethanol, and Acetone

Calculation

$$\frac{(\text{Peak area response of solvent in test solution} - \text{Peak area response of solvent interference from blank solution corresponding to RT}) \times \text{Weight of standard (g)} \times 5 \times 5 \times 10^6}{(\text{Average peak area response of solvent in standard solution B} - \text{peak area response of solvent interference from blank solution corresponding to RT}) \times 100 \times 100 \times \text{weight of sample (g)}}$$

Results and Discussion

Linearity Experiment

The plot peak area of Ethanol and Acetone standards Vs respective concentration of linearity levels are found linear in the range of 100 ppm to 7500 ppm for both solvents with correlation 0.9996 for Ethanol and 0.9998 for Acetone. Also LOQ Level and Higher level (Linearity 150%) injected in six replicates and determine the relative standard deviation which shows that method is precise for LOQ Level and Higher level (Linearity 150%) To determine the precision of the proposed method, Six samples (as such) were analyzed and determine the %RSD of Ethanol and Acetone content in Clarithromycin granules. The content is well within the limit.

Recovery (Accuracy)

To study the accuracy, reproducibility and the precision of the proposed method, recovery experiment was carried out by adding standard Ethanol and Acetone at four different levels in pre-analyzed sample. The study was carried out with spiking of LOQ level for Ethanol And Acetone. This was determined in triplicate. Recovery observed shown in following table.

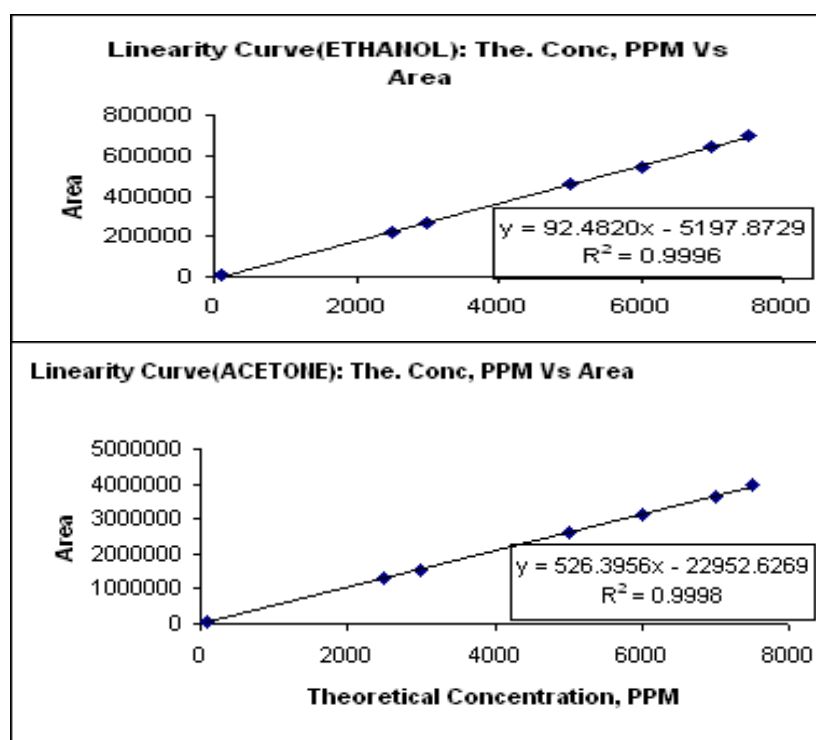
Conclusion

The linearity regression coefficient was found 0.9996 for Ethanol and 0.9998 for Acetone which shows that response is linear from 100 ppm to 7500 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 Clarithromycin granules for monitoring Ethanol and Acetone content.

Linearity

Sample Name	Conc. of Ethanol	Area of Ethanol	Conc. of Acetone	Area of Acetone
Linearity LOQ	100	7458.89	100	45745.67
Linearity 50%	2500	224614.5	2500	1282256.5
Linearity 60%	3000	271263	3000	1551309.5
Linearity 100%	5000	456353	5000	2607763
Linearity 120%	6000	542816.5	6000	3113314
Linearity 140%	7000	639448	7000	3648906
Linearity 150%	7500	697850.5	7500	3960941.8



Recovery (Accuracy)**Ethanol**

Sample Name	Sample Area of ethanol	% Recovery	Std Dev	Average	%RSD	Amount Observed	Actual amount Recovered	Actual amount Added
LOQ Accuracy	10827	88.50	2.989	9.53	3.33	111.39	84.08	95
LOQ Accuracy	11233	92.90				115.57	88.26	95
LOQ Accuracy	10706	87.19				110.14	82.83	95
50% Accuracy	225009	96.32	0.772	96.87	0.797	2314.9	2287.59	2375
50% Accuracy	227531	97.41				2340.8	2313.53	2375
100% Accuracy	468350	100.86	1.243	99.45	1.250	4818.3	4791.08	4750
100% Accuracy	457537	98.52				4707.1	4679.84	4750
100% Accuracy	459615	98.97				4728.5	4701.22	4750
150% Accuracy	689959	98.00	0.945	97.34	0.971	7098.3	7071.00	7215
150% Accuracy	680582	96.67				7001.8	6974.53	7215
Mean		98.11						
Std		1.71						
RSD		1.74						

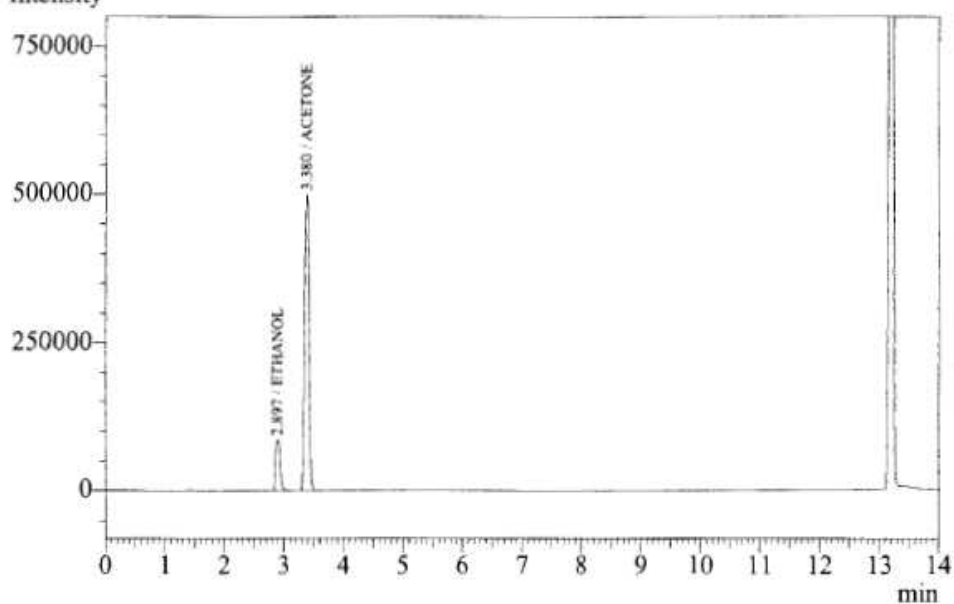
Acetone

Sample Name	Sample Area Of acetone	% Recovery	Std Dev	Average	%RSD	Amount Observed	Actual amount Recovered	Actual amount Added
LOQ Accuracy	52344	94.12	1.587	93.54	1.69	95.25	90.35	96.0
LOQ Accuracy	52678	94.75				95.86	90.96	96.0
LOQ Accuracy	51090	91.74				92.97	88.07	96.0

50% Accuracy	1250379	94.60	1.716	95.82	1.79	2275.3	2270.48	2400
50% Accuracy	1282387	97.03				2333.6	2328.73	2400
100% Accuracy	2623886	99.37	1.107	98.12	1.12	4774.8	4769.93	4800
100% Accuracy	2568570	97.28				4674.1	4669.27	4800
100% Accuracy	2580070	97.71				4695.1	4690.20	4800
150% Accuracy	3891135	98.28	2.075	96.81	2.14	7080.9	7076.01	7200
150% Accuracy	3775028	95.34				6869.6	6864.73	7200
Mean		97.09						
Std		1.71						
RSD		1.77						

Chromatogram for standard

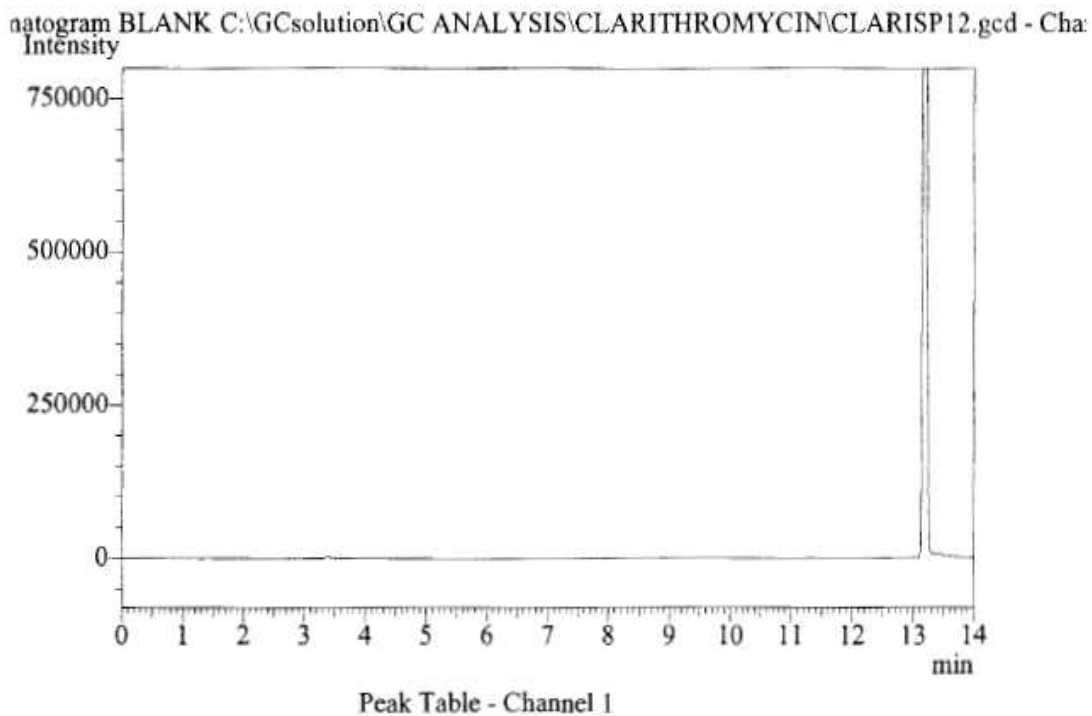
Chromatogram STD C:\GCsolution\GC ANALYSIS\CLARITHROMYCIN\CLARISP05.gcd - Channel 1



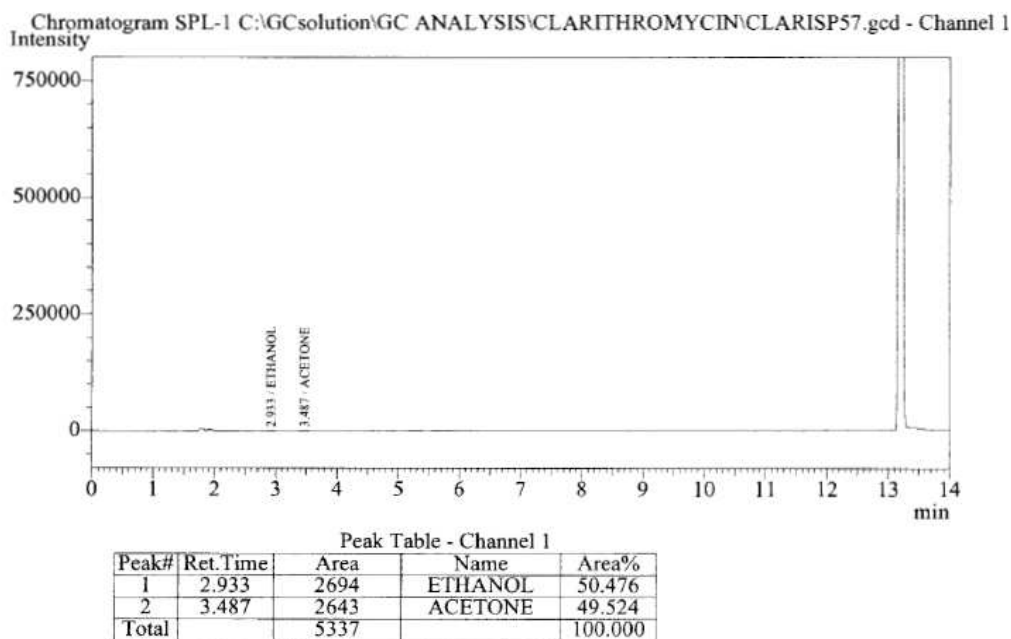
Peak Table - Channel 1

Peak#	Ret. Time	Area	Name	Area%	Theoretical Plates	Tailing Factor	Resolution
1	2.897	465254	ETHANOL	14.926	5938.391	1.179	0.000
2	3.380	2651740	ACETONE	85.074	8447.239	1.133	3.254
Total		3116994		100.000			

Chromatogram for blank



Chromatogram for sample



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