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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF MARAVIROC IN BULK AND ITS PHARMACEUTICAL FORMULATION

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ABSTRACT

An isocratic reverse phase liquid chromatography (RP-LC) method has been developed and subsequently validated for the determination of Maraviroc in Bulk and its pharmaceutical formulation. Separation was achieved with a Develosil ODS HG-5 (Make: Nomura Chemicals (Japan); 150 mmx4.6 mm I.D; particle size 5 μ m) and Potassium di-hydrogen orthophosphate (pH adjusted to 2.2 with diluted orthophosphoric acid): Methanol (600:400 v/v) as eluent at flow rate 1.2 mL/min and the Column temperature was 40°C. UV detection was performed at 210nm. The method is simple, rapid and selective. The described method of Maraviroc is linear over a range of 14.925 μ g/mL to 89.550 μ g/mL. The method precision for the determination of assay was below 2.0% RSD. The percentage recoveries of active pharmaceutical ingredient (API) from dosage forms ranged from 99.1 to 99.7%. The method is useful in the quality control of Bulk and pharmaceutical formulations.

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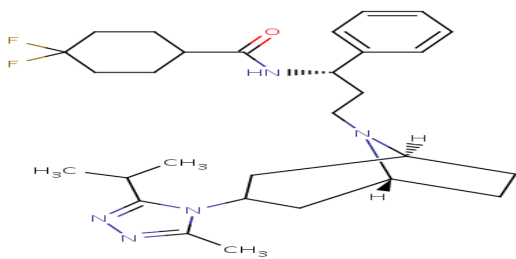
Key Words

LC Determination, Maraviroc

INTRODUCTION

Maraviroc is an entry inhibitor and works by blocking HIV from entering human cells. Specifically maraviroc is a selective, slowly reversible, small molecule antagonist of the interaction between human CCR5 and HIV-1 gp120. Maraviroc selectively binds to the human chemokine receptor CCR5 present on the membrane of CD4 cells (T-cells), preventing the interaction of HIV-1 gp120 and CCR5 necessary for CCR5-tropic HIV-1 to enter cells. Maraviroc has the chemical name 4,4-difluoro-N-[(1S)-3-[(1R,5S)-3-[3-methyl-5-(propan-2-yl)-4H-1,2,4-triazol-4-yl]-8-azabicyclo[3.2.1]octan-8-yl]-1-phenylpropyl]cyclohexane-1-carboxamide. Maraviroc is a white to yellowish or brownish powder with a molecular formula of $C_{29}H_{41}F_2N_5O$ and a molecular weight of 513.67. Maraviroc is practically insoluble in aqueous media, slightly soluble in ethanol, Dimethyl sulfoxide and soluble in PEG 400.

Its Chemical structure is given below¹⁻²



It is not official in any pharmacopoeia, few liquid chromatography procedures have been reported for the determination of Maraviroc³⁻⁴. The author have developed a liquid chromatographic method which would serve as a rapid and reliable method for the determination of Maraviroc in Bulk and pharmaceutical dosage forms.

EXPERIMENTAL⁵⁻¹²

Instrumentation:

The analysis of the drug was carried out on a waters LC system equipped with 2695 pump and 2996 photodiode array detector was used and a Reverse phase HPLC column Develosil ODS HG-5 (Make: Nomura Chemicals (Japan); 150 mmx4.6 mm I.D; particle size 5 μ m) was used. The output of signal was monitored and integrated using waters Empower 2 software.

Chemicals and solvents

HPLC Grade water (Millipore), Methanol (HPLC Grade), Orthophosphoric acid (HPLC Grade) and Potassium di-hydrogen orthophosphate of GR Grade were obtained from E. Merck (India) Ltd., Mumbai.

Buffer preparation:

Accurately weigh and transfer about 1.36 grams of Potassium di-hydrogen orthophosphate in 1000 mL of purified water and mix. Adjust pH to 2.2 (± 0.05) with dilute orthophosphoric acid solution. Filter the solution through 0.45 μ m membrane filter.

Mobile phase preparation:

Prepare a mixture of Buffer and Methanol in the ratio of 600:400 v/v respectively. Filter the solution through 0.45 μ m membrane filter.

Standard preparation:

Accurately weigh and transfer 60.0mg of Maraviroc working standard into a 100 mL volumetric flask, add 60 mL of Methanol and sonicate to dissolve. Cool the solution to room temperature and dilute to volume with Methanol.

Transfer 5.0 mL of the above solution into a 50 mL volumetric flask and dilute to volume with Mobile Phase.

Sample preparation: (For Maraviroc Tablets 300mg)

Accurately weigh and transfer the powder equivalent to 300 mg of Maraviroc into a 100 mL volumetric flask add about 60 mL of Methanol, shake for 15 minutes on orbital shaker and sonicate for 20 minutes with occasional shakings. Cool the solution to room temperature and dilute to volume with Methanol and mix. Filter the solution through 0.45 μ m PVDF Filter.

Transfer 2.0 mL of the above solution into a 100 mL volumetric flask and dilute to volume with Mobile Phase.

Chromatographic conditions: A Develosil ODS HG-5 (Make: Nomura Chemicals (Japan); 150 mmx4.6 mm I.D; particle size 5 μ m) Column was used for analysis at column temperature 40°C. The mobile phase was pumped through the column at a flow rate of 1.2 mL/min. The sample injection volume was 10 μ L. The photodiode array detector was set to a wavelength of 210 nm for the detection and Chromatographic runtime was 15 minutes.

RESULTS AND DISCUSSION

Method development⁵⁻¹²:

To develop a suitable and robust LC method for the determination of Maraviroc different mobile phases were employed to achieve the best separation and resolution. The method development was started with Symmetry C18; 250 mmx4.6 mm I.D; particle size 5 μ m with the flow rate of 1.0mL/min. Mobile phase was Buffer (Accurately weigh and transfer about 1.369 grams of Potassium di-hydrogen orthophosphate and 1.729 grams of Di Potassium di-hydrogen orthophosphate in 1000 mL of purified water and mix. Adjust pH to 7.0 (\pm 0.05) with Triethyl amine. Filter the solution through 0.45 μ m membrane filter) and Acetonitrile in the ratio of 400:600 v/v. Column temperature was Ambient and the wavelength was 210nm. The retention time of Maraviroc is 3.7 minutes and the peak shape was broad. For better peak shape the mobile phase pH and

Composition was changed. The Mobile phase composition was Potassium di-hydrogen orthophosphate (pH adjusted to 2.2 with diluted orthophosphoric acid): Methanol (600:400 v/v) as eluent at flow rate 1.2 mL/min and the Column temperature was 40°C. UV detection was performed at 210nm and the HPLC Column was Develosil ODS HG-5 (Make: Nomura Chemicals (Japan); 250 mmx4.6 mm I.D; particle size 5 μ m). The retention time of Maraviroc is 6.956 minutes. The retention time was too long. To reduce the runtime the column changed to Develosil ODS HG-5 (Make: Nomura Chemicals (Japan); 250 mmx4.6 mm I.D; particle size 5 μ m) to Develosil ODS HG-5 ((Make: Nomura Chemicals (Japan); 150 mmx4.6 mm I.D; particle size 5 μ m)). The retention time of Maraviroc is 5.414 minutes (Refer Fig-1.) and the peak shape was good.

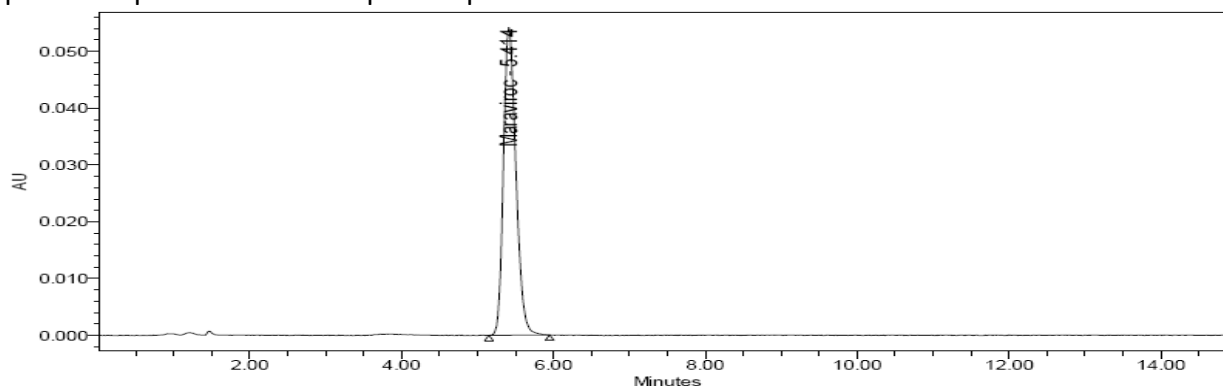


Fig-1. HPLC Chromatogram of Maraviroc Standard.

The chromatogram of Maraviroc standard using the proposed method is shown in Fig-1. System suitability results of the method are presented in Table-1.

Table-1(SYSTEM SUITABILITY RESULTS)

Compound	Retention Time * (min.)	Maraviroc area/response*	USP Tailing*	USP Plate count*	%RSD*
Maraviroc	5.399	616014	1.23	5168	0.31

*Number of standard injections analysed are six

Column selection:

Based on the retention and better peak shape of the compound Develosil ODS HG-5 (Make: Nomura Chemicals (Japan); 150 mmx4.6 mm I.D; particle size 5

Maraviroc show significant UV absorbance at Wavelength 210nm. Hence this wavelength has been chosen for detection in analysis of Maraviroc in Maraviroc tablets.

μ m) Column was selected as a suitable column for analysis of Maraviroc.

Method validation¹²⁻¹⁵ :

The developed LC method extensively validated for assay of Maraviroc using the following parameters.

Specificity

Blank and Placebo interference: A study to establish the interference of placebo was conducted. Assay was performed on placebo in triplicate equivalent to about the weight of placebo in portion of test preparation as per test method. Chromatograms of Blank and Placebo solutions showed no peaks at the retention time of Maraviroc peak. This indicates that the excipients used in the formulation do not interfere in estimation of Maraviroc in Maraviroc tablets.

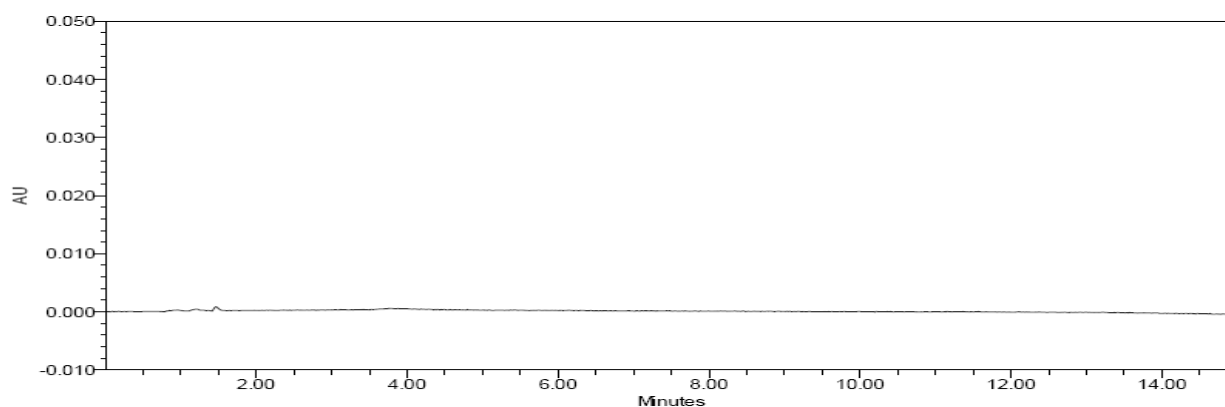
The chromatogram of Maraviroc Blank and Placebo using the proposed method is shown in Fig- 2 & Fig-3.

Table-2 (LINEARITY TABLE REPORT)

S.No.	Concentration (mcg/mL)	Area	y-Best fit	(Difference) ²	Correlation Coefficient (R)=	1.0000
25%	14.925	151555	151362	37212	Regression Coefficient (R ²)=	1.0000
50%	29.850	304125	304563	191602	y-Intercept=	-1838.5
75%	44.775	458888	457763	1264832	Slope of Regression line=	10265
100%	59.700	611125	610964	25927	Residual Sum of squares=	13477672
125%	74.625	761255	764165	8465828	Minimum Con in mcg/mL =	14.9250
150%	89.550	919234	917365	3492271	Maximum Con in mcg/mL =	89.5500
					y-Intercept at 100 % level =	-0.301

Linearity of detector response: Linearity of detector response was established by plotting a graph to concentration *versus* area and determining the correlation coefficient.

A series of solutions of Maraviroc standard were prepared in the concentration range of about 14.925 µg/mL to 89.550 µg/mL. A graph was plotted to concentration in µg/mL on X-axis *versus* response/Area on Y-axis. The detector response was found to be linear with a correlation coefficient of 1.0000. Linearity graph is shown in Fig-4. Linearity results of the method are presented in Table-2.

**Fig-2.** HPLC Chromatogram of Maraviroc Blank.

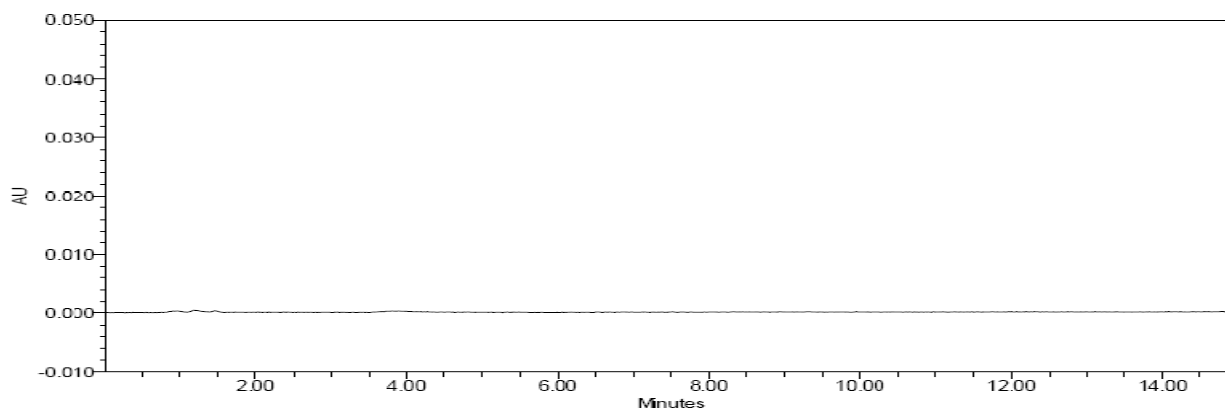


Fig-3. HPLC Chromatogram of Maraviroc placebo.

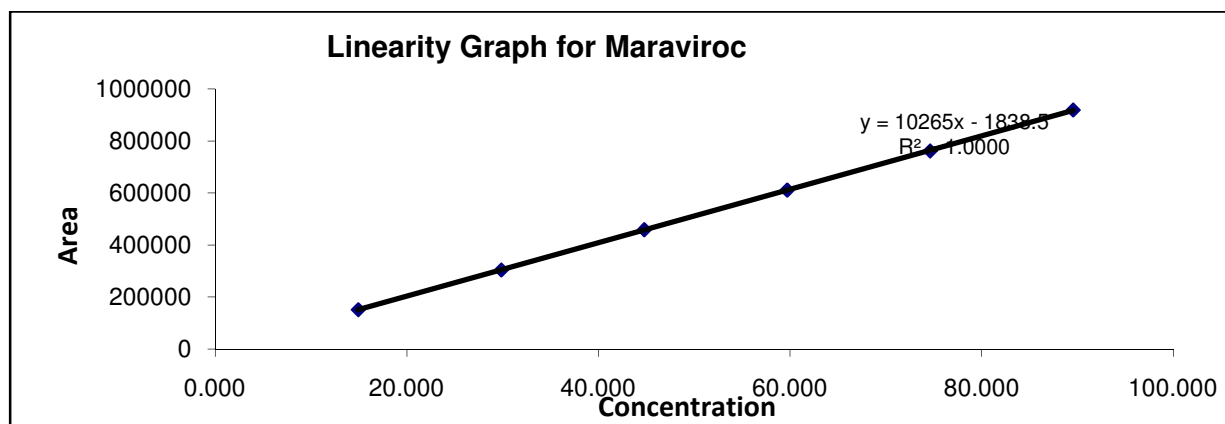


Fig-4. Linearity of detector response graph.

Precision of test Method: The precision of test method was conducted by assay in six samples of Maraviroc Tablets. The average % assay of Maraviroc in Maraviroc

Tablets was found to be 99.7 for 300mg tablets and the %RSD is 0.5. The results were given in Table-3. A typical LC Chromatogram is shown in Fig-5.

Table-3(RESULTS FOR PRECISION OF TEST METHOD)

Sample No.	% of Maraviroc in Maraviroc Tablets (300mg)
01	99.5
02	99.8
03	100.2
04	100.3
05	99.1
06	99.3
Average	99.7
SD	0.4858
% RSD	0.5

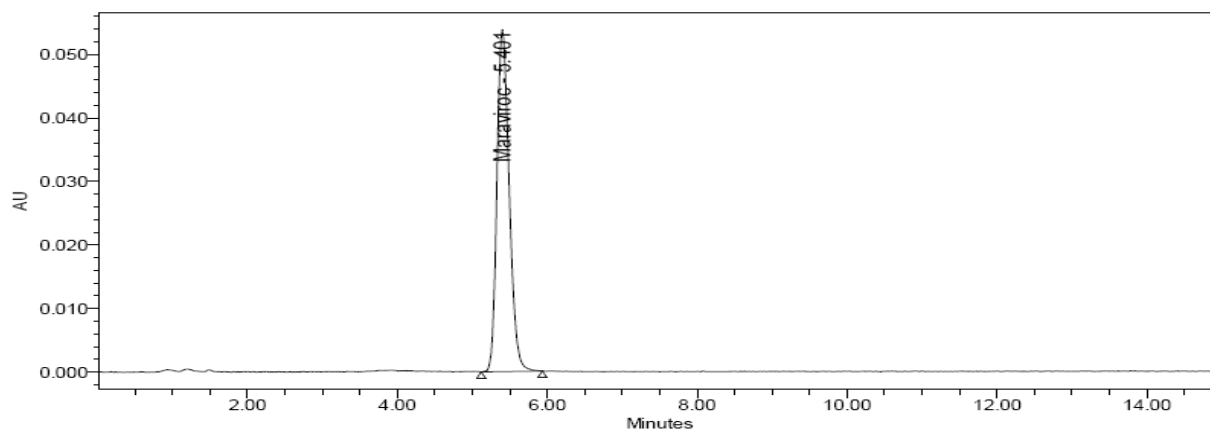


Fig-5. Typical LC Chromatogram of Formulated Maraviroc 300mg Tablets.

Accuracy: A Study of recovery of Maraviroc from spiked placebo was conducted at six different spike levels i.e.25, 50, 75, 100,125 and 150%. Samples were prepared by mixing placebo with Maraviroc raw material equivalent to about the target initial

concentration of Maraviroc. Sample solutions were prepared in triplicate for each spike level and assayed as per proposed method. The % recovery was given in Table-4. The mean recoveries of Maraviroc from spiked were found to be in the range of 99.1 to 99.7%.

Table-4(ACCURACY IN THE ASSAY DETERMINATION OF MARAVIROC)

Sample No.	Spike level	'mcg/mL' added	'mcg/mL' found (recovered)	% of Recovery	Mean % recovery
1.	25%	14.8255	14.7799	99.69	99.70
2.	25%	14.8255	14.7526	99.51	
3.	25%	14.7658	14.7492	99.89	
4.	50%	29.8699	29.5707	99.00	99.40
5.	50%	29.7505	29.4860	99.11	
6.	50%	29.5913	29.5791	99.96	
7.	75%	44.8347	44.4954	99.24	99.3
8.	75%	44.8944	44.5478	99.23	
9.	75%	44.7949	44.4954	99.33	
10.	100%	59.7199	59.1415	99.03	99.5
11.	100%	59.6005	59.2650	99.44	
12.	100%	59.4015	59.3656	99.94	
13.	125%	74.6449	73.9018	99.00	99.1
14.	125%	74.6648	74.1631	99.33	
15.	125%	74.6847	73.8591	98.89	
16.	150%	89.5699	89.0203	99.39	99.3
17.	150%	89.5898	88.8461	99.17	
18.	150%	89.6097	88.9773	99.29	

Ruggedness: A study to establish the stability of Maraviroc in standard and test solutions were conducted on bench top and refrigerator at Initial, 1 day

and 2 day. The assay of Maraviroc in standard and test solutions were estimated against freshly prepared standard each time. The difference in% assay of

standard and test solutions from initial to 1 day and 2 days was calculated and given in Table-5 & Table-6. From the above study, it was established that the

Standard and sample preparations are stable for a period of 48 hours at room temperature ($25^{\circ}\text{C}\pm 2^{\circ}\text{C}$) and at refrigerator condition ($2^{\circ}\text{C}-8^{\circ}\text{C}$).

TABLE- 5 Bench top Stability of Maraviroc Test preparation and Standard Preparation:

Time	% Assay of Standard preparation	Difference	% Assay of test preparation		Difference	
			Test-1	Test-2	Test-1	Test-2
Initial	99.5 [®]	NA*	99.9	100.1	NA*	NA*
After 24 hours	99.2	0.3	99.5	99.6	0.4	0.5
After 48 hours	98.7	0.8	99.0	99.2	0.9	0.9

NA*----Not Applicable[®]-----Potency of Maraviroc on as is basis.

TABLE- 6 Refrigerator Stability of Maraviroc Test preparation and Standard Preparation:

Time	% Assay of Standard preparation	Difference	% Assay of test preparation		Difference	
			Test-1	Test-2	Test-1	Test-2
Initial	99.5 [®]	NA*	99.9	100.1	NA*	NA*
After 24 hours	99.3	0.2	99.6	99.7	0.3	0.4
After 48 hours	98.8	0.7	99.1	99.4	0.8	0.7

NA*----Not Applicable

[®]-----Potency of Maraviroc on as is basis.

Robustness: A study to establish the effect of variation in mobile phase composition, flow rate, temperature and pH of Buffer in mobile phase was conducted. Standard and test solutions prepared as per proposed method were injected into HPLC system. The system suitability parameters and % assay were evaluated. From the above study the proposed method was found to be robust.

CONCLUSION

The proposed HPLC method is rapid, sensitive, precise and accurate for the determination of Maraviroc and can be reliably adopted for routine quality control analysis of Maraviroc in Bulk and its pharmaceutical formulations.

REFERENCES

1. <http://garcinia-cambogia.101herbs.com/>
2. <http://www.motherherbs.com/garcinia-cambogia.html>
3. Publications & Information Directorate, Council of Scientific & Industrial Research (1986). G. cambogia Desr. The Useful Plants of India. (New Delhi: Publications & Information Directorate, 1986) 229.
4. <http://www.ayurvedicure.com/garcinia-cambogia.htm>
5. <http://www.ncbi.nlm.nih.gov/pubmed/9820262>
6. Dr.KM Nadkarni, The Indian Materia Medica, Vol.I, pg 566
7. Prof P.V Sharma, Dravya Guna Vigyana, Vol II, pg 337
8. http://ijitce.co.uk/download/IJITCE_Mar.pdf
9. http://www.streetdirectory.com/travel_guide/47311/lose_weight/garcinia_cambogia__a_natural_weight_loss_supplement_ingredient.html
10. <http://jama.ama-assn.org/content/280/18/1596.full>
11. <http://ukpmc.ac.uk/abstract/MED/9820262/reload=0;jsessionid=DAE25B774B485FAA7C6504DFDF92E4>

12. <http://forum.lef.org/default.aspx?f=41&m=15160>
13. <http://www.wisegeek.com/what-is-garcinia-cambogia.htm>
14. <http://www.garciniacambogia.net/Garcinia-specification/garcinia-specs.pdf>
15. http://www.indo-world.com/garcinia_cambogia/garcinia_cambogia_extract.htm
16. R. Revathi, R. Ravi, V.S. Saravanan, T. Ethiraj, T. Sudhamani;" International Journal of Pharma Research and Development – Online www.ijprd.com; Publication Ref No.: IJPRD/2010/PUB/ARTI/VOV-2/ISSUE-7/SEP/015
17. "Early Communication about an Ongoing Safety Review of Meridia (sibutramine hydrochloride)".
18. <http://en.wikipedia.org/wiki/Sibutramine>
19. <http://en.wikipedia.org/wiki/Orlistat>
20. <http://archinte.ama-assn.org/cgi/content/extract/171/7/703>
21. "phentermine". The American Society of Health-System Pharmacists. Retrieved 3 April 2011.
22. Nelson DL, Gehlert DR (February 2006). "Central nervous system biogenic amine targets for control of appetite and energy expenditure". *Endocrine* 29 (1): 49–60. doi:10.1385/ENDO:29:1:149.PMID 16622292
23. <http://en.wikipedia.org/wiki/Phentermine>
24. <http://www.drugs.com/npp/guggul.html>
25. <http://www.livestrong.com/article/404681-guggul-and-obesity/>
26. <http://www.livestrong.com/article/125567-side-effects-cissus-quadrangularis/>
