Analysis of an Anti-Obesity OTC Drug alli® (orlistat) using a High Throughput Reversed Phase Column

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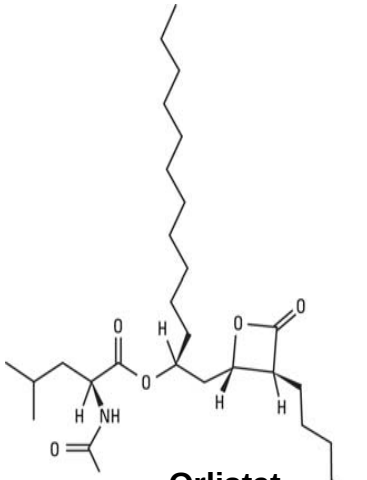
Introduction

Orlistat, is marketed by Roche as a prescription drug under the trade name Xenical® and as an over-the-counter weight loss drug under the trade name alli by GlaxoSmithKline. Orlistat is a lipstatin drug used to treat obesity by preventing the adsorption of dietary fats[1]. Orlistat, a gastrointestinal lipase inhibitor, reduces weight by around 3kg on average and decreases progression to diabetes in high-risk patients[2].



Orlistat

[1]Bodkin J, Humphries E, McLeod M (2003). "The total synthesis of (−)-tetrahydrolipstatin". *Australian Journal of Chemistry* 56 (8): 795–803. doi:10.1071/CH03121

[2]Padwal RS, Majumdar SR. Lancet. 2007 Jan 6;369(9555):71-7.

Scope of generic drugs

- Pharmaceuticals are among the most highly regulated products in the United States.
- After a patent expires many generic manufacturers may produce it as a less expensive product.
- An estimated $64 billion of pharmaceutical products are coming off-patent in the near future[3].
- The retail market for generic pharmaceuticals is also expected to increase, particularly from the competitive pressure of producing quality products at lower cost.

[3]http://www.fda.gov/NewsEvents/ Speeches/ucm051966.htm

Challenge to generic manufacturers

- Xenical has recently come off of patent protection in 2009[4].
- alli is available in the market as the FDA approved non-prescription weight loss aid[5,6].
- The drug is used for prevention of lipid absorption by inhibition of pancreatic lipase[7].
- The challenge for generic makers is to develop validated chromatographic methods for a number of similar drugs coming off patent protection in the near future.

[4]http://drugtopics.modernmedicine.com/drugtopics/Supplements/Drug-patent-expirations-2007 2009/ArticleStandard /Article /detail/414709

[5]Pommier A, Pons M, Kocienski P (1995). "The first total synthesis of (-)-lipstatin". Journal of Organic Chemistry 60 (22): 7334–7339. doi:10.1021/jo00127a045.

[6]Padwal R, Li SK, Lau DC (2004). "Long-term pharmacotherapy for obesity and overweight". Cochrane Database Syst Rev (3): CD004094. doi:10.1002/14651858.CD004094.pub2. PMID 15266516.

[7]Barbier P, Schneider F (1987). "Syntheses of tetrahydrolipstatin and absolute configuration of tetrahydrolipstatin and lipstatin". Helvetica Chimica Acta 70 (1): 196–202. doi:10.1002/hlca.19870700124.

Challenge to generic manufacturers

- Reversed phase liquid chromatography (RPC) is an analytical technique widely used in the R&D and QC departments of drug manufacturers.
- In this era of high throughput analysis, the need to obtain shorter retention times while maintaining or improving resolution from closely eluting impurities is very important for quality control analysis.
- Here we report the separation of Orlistat and the corresponding OTC drug alli using TSK-GEL® ODS-140HTP, 2.3µm, 2.1mm ID x 5cm and 10cm columns.

Objective

To show the usefulness of the 5 and 10cm x 2.1mm ID silica-based TSK-GEL ODS-140HTP (2.3µm) reversed phase columns for high throughput analysis of an Orlistat drug standard and separation of it from a FDA approved OTC drug alli drug standard using a conventional HPLC system.

Material and methods

Analyses were carried out using an Agilent 1200 HPLC system run by Chemstation (ver B.04.02), except in the case of studies shown in figures 9-11 in which an Agilent 1100 HPLC system run by Chemstation (ver B.03.01) was used.

Optimal chromatographic conditions:

- Columns:
  - TSKgel ODS-140HTP, 2.3µm, 2.1mm ID x 5cm
  - TSKgel ODS-140HTP, 2.3µm, 2.1mm ID x 10cm
  - Hypersil GOLD® C18, 1.9µm, 2.1mm ID x 5cm
  - Luna® C18(2)-HST, 2.5µm, 2.0mm ID x 5cm
- Detection: 205nm
- Column temp: 50˚C unless mentioned otherwise
- Flow rate: as mentioned in respective figures
- Injection volume: as mentioned in respective figures
- Mobile phase (Isocratic): ACN in H_20 as mentioned in respective figures

Material and methods

- High purity Sigma-Aldrich brand orlistat was used for the preparation of stock standards using methanol as a diluent.
- Working standards were prepared by dilution of the stock standard in 50% MeOH.
- The over-the-counter drug alli was purchased from a local pharmacy.
- A total of 0.1145g of the white, cube shaped drug material was weighed out of a single 60mg capsule, dissolved in 50% MeOH in H_2O, filtered through a 0.45µm membrane and stored at -20°C.
- The standards and samples were filtered through a 0.45µm membrane before injection into the column.

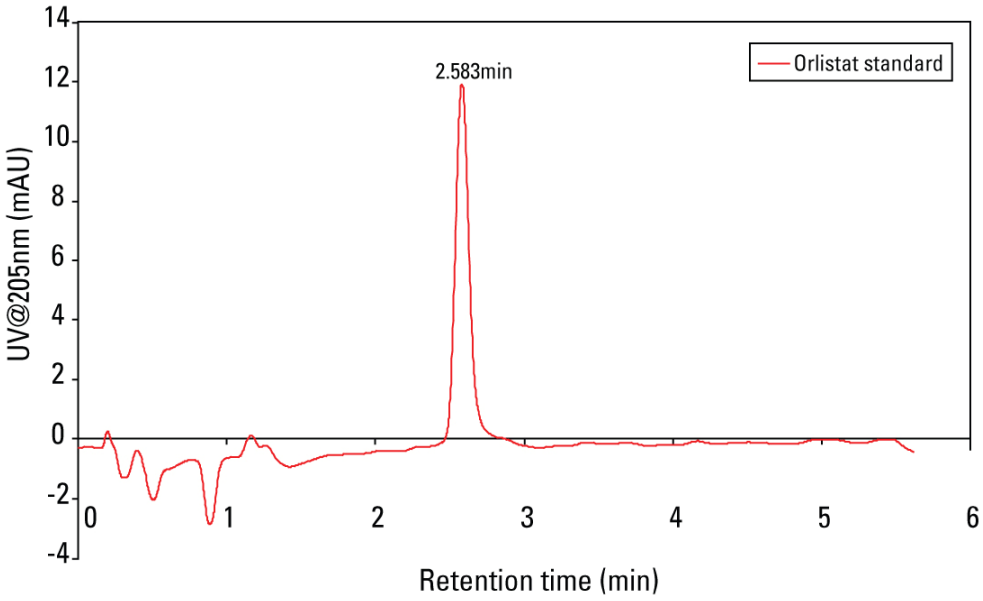
Material and methods

- The limit of detection (LOD), is the lowest concentration of an analyte in a sample that can be detected, but not necessarily quantitated under the stated experimental conditions.
- LOD is measured by a procedure for the validation of compendial methods as mentioned in USP under section 1225.
- The standard deviation of the base line response (mAU at the wavelength selected for detection) using a blank sample is calculated.
- The standard deviation in mAU is multiplied by a factor of 2 to provide an estimate of the limit of detection (LOD).
- The LOD is subsequently validated by the analysis of the sample near that limit.
- For determination of limit of quantitation (LOQ), the LOD sample concentration is multiplied by a factor of 10.

Table 1: Comparative chart of the properties of the columns used in this study

	TSKgel ODS-140HTP	Hypersil GOLD	Luna
Carbon Content	8%	10%	17.5%
Endcapped	Yes[1]	Yes	-
Particle Size (µm)	2.3	1.9	2.5
Pore Size (Å)	140	175	100
Bonded Phase Structure	Polymeric	Monomeric	Monomeric
Specific Surface Area (m^2/g)	-	220	400
*Asymmetry Factor	0.90 - 1.3	-	1.12
*Theoretical Plates	280,000 (plates/meter)	-	160,227 (plates/meter)

Figure 1: Analysis of orlistat standard using a TSKgel ODS-140HTP, 2.3µm, 2.1mm ID X 10cm column



Column:	TSKgel ODS-140HTP, 2.1mm ID x 10cm
Mobile phase:	86% ACN:14% H_2O
Flow rate:	1.0mL/min
Detection:	UV@205nm
Temperature:	50°C
Injection vol.:	2µL
Sample:	orlistat standard (100µg/mL)

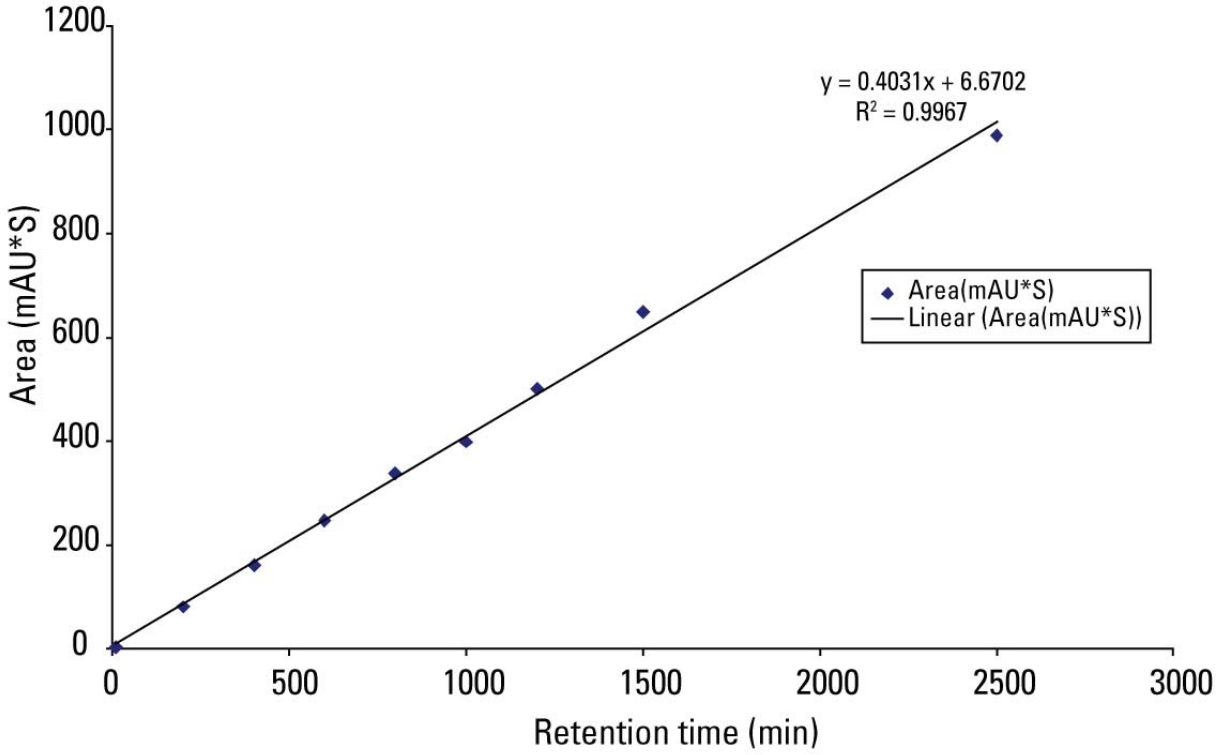
Orlistat eluted with high symmetry and short retention time.

Table 2: System suitability using TSKgel ODS-140HTP, 2.3µm, 2.1mm ID X 10cm column

System Suitability					
Serial number	RT(min)	k	Area (mAU*S)	AF	Plate(N)
1	2.583	9.36	78.694	1.2	3695
2	2.582	9.36	79.044	1.2	3793
3	2.584	9.37	80.59	1.2	3699
4	2.586	9.37	82.902	1.2	3705
5	2.591	9.39	83.955	1.2	3775
Average	2.5852	9.37	81.037	0.826	3733.4
Stdev	0.0035	0.012	2.3267	0.016	46.763
%RSD	**0.1378**	**0.130**	**2.8711**	**2.025**	**1.252**

Low percentage relative standard deviation in the peak parameters show that this column can be used for the separation of orlistat with precision and consistency.

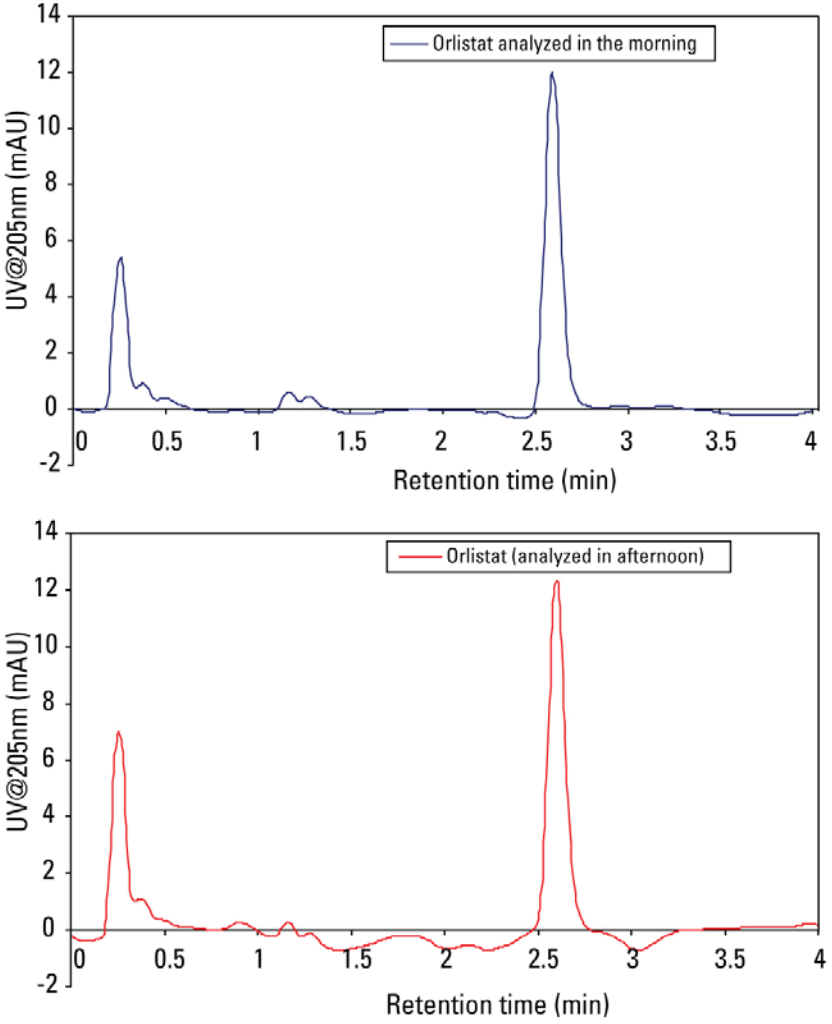
Figure 2: Linearity test using TSKgel ODS-140HTP, 2.3µm, 2.1mm ID X 10cm column



Amount (ng)	RT (min)	k
10	2.625	9.53
200	2.585	9.37
400	2.581	9.36
600	2.573	9.32
800	2.564	9.29
1000	2.562	9.28
1200	2.564	9.28
1500	2.561	9.27
2500	2.551	9.23
Average	2.574	9.326
Stdev	0.0218	0.088
%RSD	**0.849**	**0.949**

Peak area for orlistat was linear in the range of 0.01 – 2.50µg. relative standard deviations for retention time and capacity factor were below 1% RSD in this range.

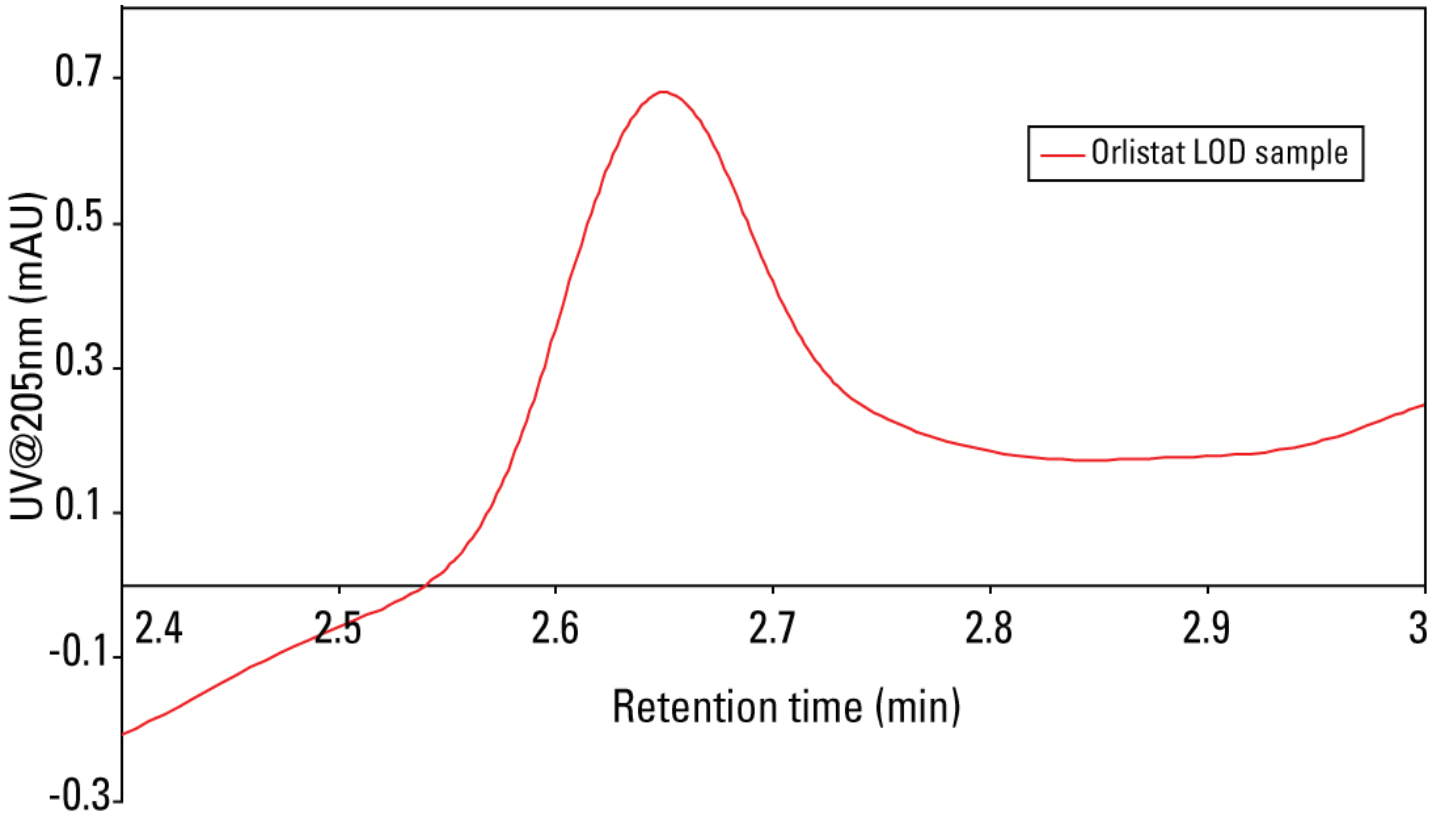
Figure 3: Precision study using TSKgel ODS-140HTP 2.3µm, 2.1mm ID X 10cm column



	RT (min)	k	Area (mAU*S)	Symmetry	Plate (N)
Average from system suitability study	2.5852	9.37	81.03747	0.826	3733.4
	2.593	9.4	78.344	0.85	3726
	2.598	9.42	80.5183	0.86	3741
Average	2.592	9.397	79.967	0.845	3733.467
Stdev	0.006	0.025	1.429	0.017	7.500
%RSD	**0.249**	**0.268**	**1.787**	**2.067**	**0.201**

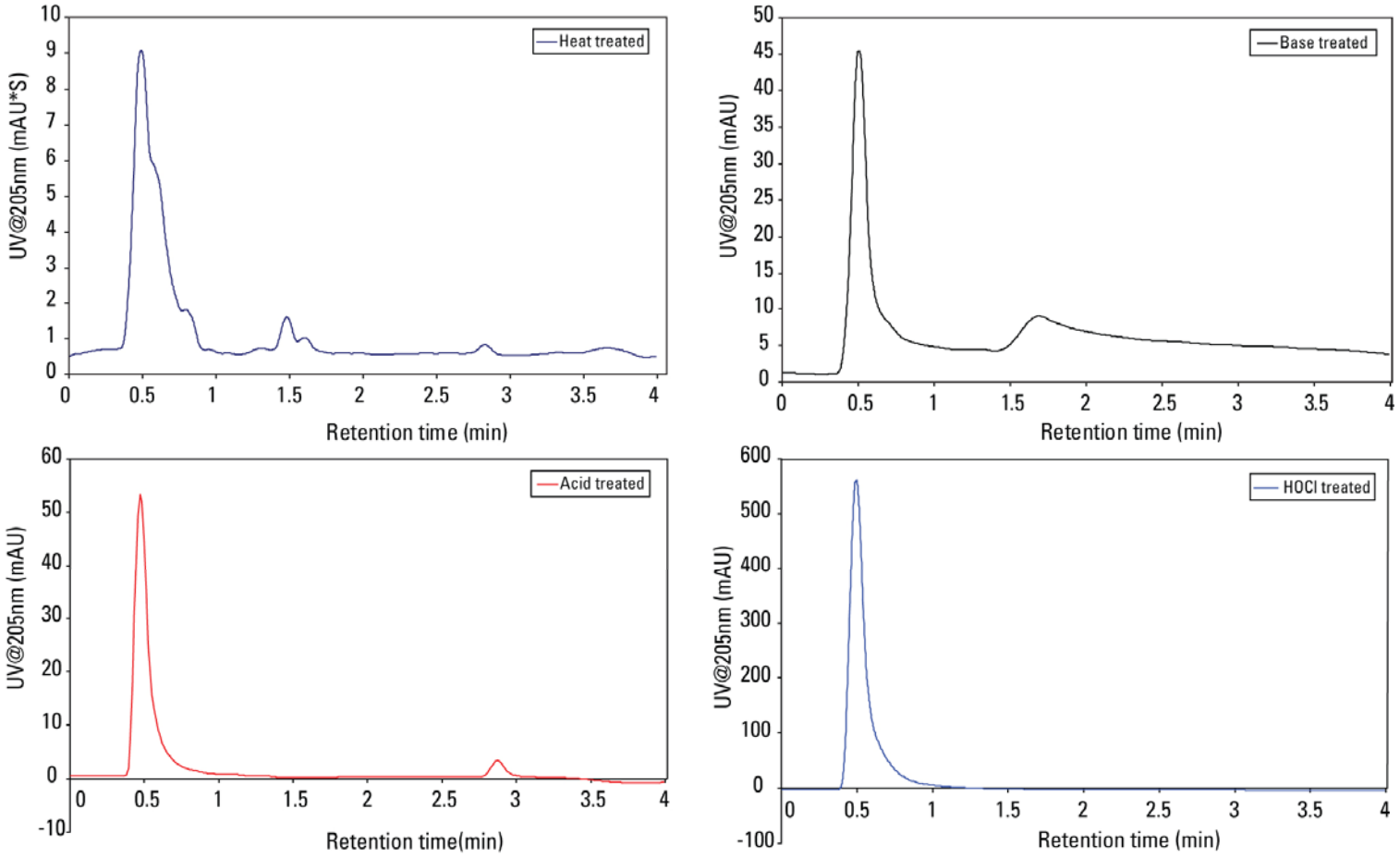
The table shows the high precision in the values of the peak parameters obtained at two times of the day with the average values obtained from the system suitability study (table 2).

Figure 4: Limit of Detection (LOD) and Limit of Quantitation (LOQ) of orlistat



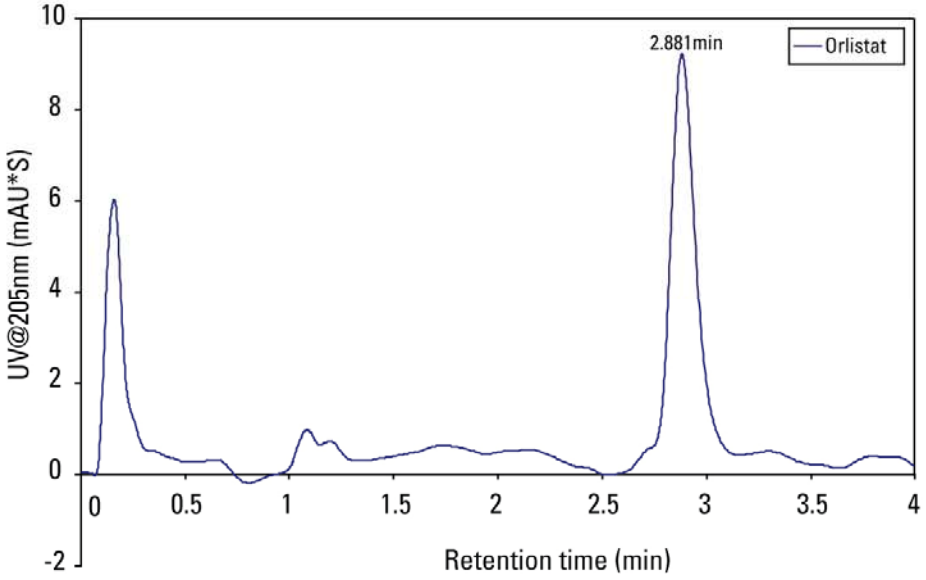
LOD = 5ng
LOQ = 50ng

Figure 5: Degradation study using TSKgel ODS-140HTP, 2.3µm, 2.1mm ID X 10cm column



The orlistat peak disappeared upon forced degradation. This study shows that this column can be used for the forced degradation study of orlistat for quality control purposes.

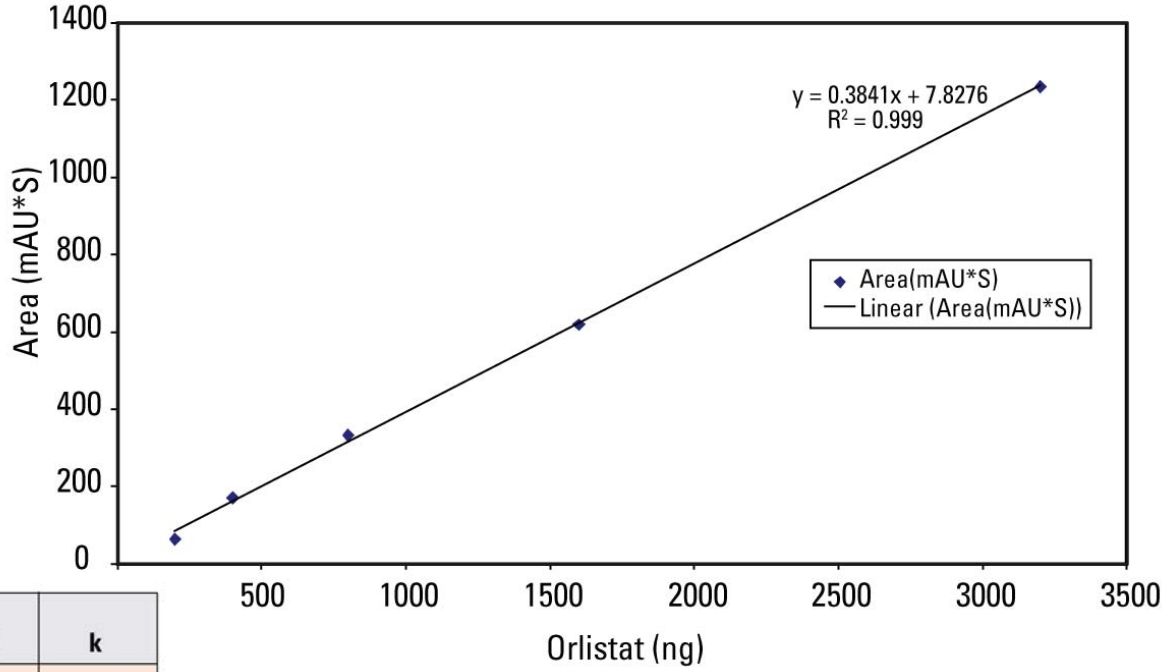
Figure 6: Analysis of Orlistat standard using TSKgel ODS-140HTP, 2.3um, 2.1mm ID X 5cm column



Column:	TSKgel ODS-140HTP, 2.1mm ID x 5cm
Mobile phase:	70% ACN, 30% H_2O
Flow rate:	1.0mL/min
Detection:	UV@ 205nm
Temperature:	40°C
Injection vol.:	2µL
Sample:	orlistat, 100µg/mL

	System Suitability	
Serial number	**RT (min)**	**k**
1	2.881	26.72
2	2.881	26.73
3	2.881	26.73
4	2.882	26.74
5	2.882	26.73
Average	2.881	26.730
Stdev	0.001	0.007
%RSD	**0.019**	**0.026**

Figure 7: Linearity test using TSKgel ODS-140HTP, 2.3um, 2.1mm ID X 5cm column

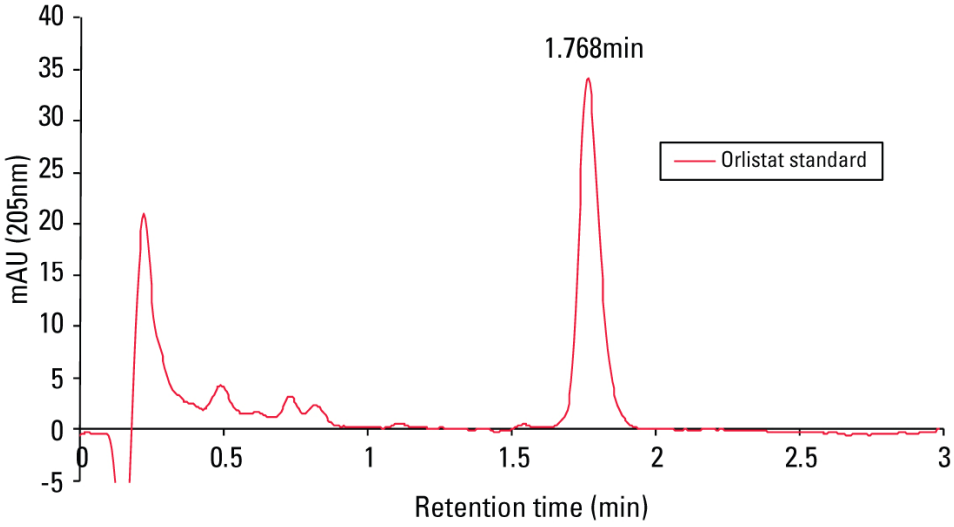


Amount (ng)	RT (min)	k
200	2.882	26.73
400	2.863	26.55
800	2.829	26.23
Average	2.858	26.503
Stdev	0.027	0.253
%RSD	**0.940**	**0.956**

Amount (ng)	RT (min)	k
200	2.882	26.73
400	2.863	26.55
800	2.829	26.23
1600	2.784	25.79
3200	2.715	25.13
Average	2.8146	26.086
Stdev	0.067	0.643
%RSD	**2.380**	**2.464**

Orlistat analysis was linear in the range of 0.2 – 0.8µg with a low %RSD value (<1). Though the coefficient of linear regression (R^2) was 0.999 over a broader range of 0.2 – 3.2µg, %RSD value of retention time and capacity factor increased to 2.4 and 2.5 respectively.

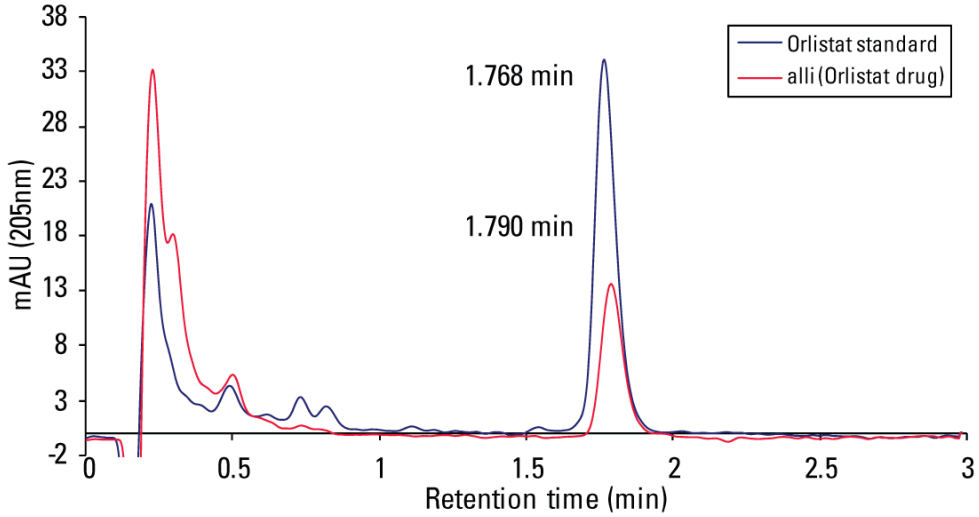
Figure 8: Isocratic elution of an orlistat standard using a TSKgel ODS-140HTP, 2.3um, 2.1mm ID X 5cm column



Column:	TSKgel ODS-140HTP, 2.1mm ID x 5cm
Mobile phase:	70% ACN, 30% H_2O with 0.15% TFA
Flow rate:	1.5mL/min
Detection:	UV@205nm
Temperature:	40°C
Injection vol.:	10µL
Sample:	orlistat, 100µg/mL

Orlistat could be eluted with low retention time (< 2 minutes) using these chromatographic conditions.

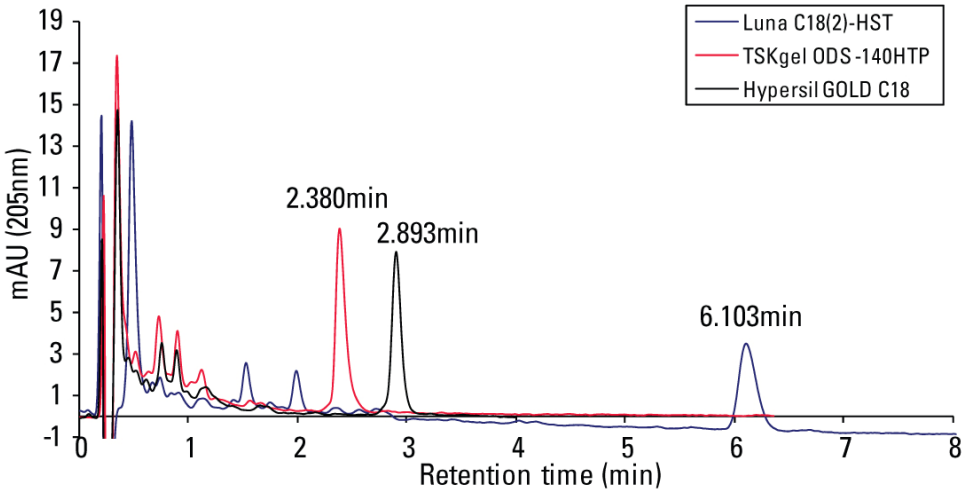
Figure 9: Analysis of orlistat from alli using a TSKgel ODS-140HTP column



Column:	TSKgel ODS-140HTP, 2.1mm ID x 5cm
Mobile phase:	70% ACN with 0.15% TFA
Flow rate:	1.5mL/min
Detection:	UV@205nm
Temperature:	40°C
Injection vol.:	10µL
Samples:	orlistat standard and alli

- **The orlistat standard peak eluted at 1.768 minute, while the orlistat sample from alli eluted at 1.790 minute.**
- **No interference was noticed from the excipients of alli.**
- **This study shows that the column can be used for the method development of these generic drugs.**

Figure 10: Comparative study of the elution profile of the off-patent drug Orlistat



Columns:	TSKgel ODS-140HTP, 2.1mm ID x 5cm Luna C18(2)-HST, 2.0mm ID x 5cm Hypersil GOLD C18, 2.1mm ID x 5cm
Mobile phase:	70% ACN with 0.15% TFA
Flow rate:	1.2mL/min
Detection:	UV@205nm
Temperature:	40°C
Injection vol.:	10µL
Sample:	orlistat, 100µg/mL

The TSKgel ODS-140HTP column yielded the shortest retention times in comparison to the other two competitive columns tested under identical chromatographic conditions.

Conclusions

- TSK-GEL ODS-140HTP columns were successfully used for the analysis of two common hypertension drugs with low retention time.
- This data also shows that generic manufacturers can use this column for the analysis of Orlistat
  - For quality control purposes pertaining to
    - Detection of the sample at low concentrations
    - For the monitoring of the stability of the drug substance
    - For forced degradation studies without any interference from the excipients or the reagents
  - For the separation of active pharmaceutical ingredient (API) from the product
- TSK-GEL ODS-140HTP columns yielded the shortest retention times in comparison to two competitive columns tested under identical chromatographic conditions.
- HPLC systems were not optimized to reduce extra-column band broadening.