High throughput analysis of a number of common drugs using TSK-GEL ODS-140HTP columns

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Objective:

- To show the usefulness of the silica based TSK-gel ODS-140HTP (2.0mm ID x 5cm, 2.3µm) reversed phase column for high-throughput analysis of common drugs with a wide variety of hydrophobicities which are coming off-patent using a conventional HPLC system.

Scope of generic drugs

- Pharmaceuticals are among the most highly regulated products in the United States.
- Newly developed brand drugs have patent protection until the expiration date.
- After the expiration of the patent protection many generic manufacturers may produce a less expensive product.
- An estimated $64 billion of pharmaceutical products are coming off-patent in the near future.
- The retail market for generic pharmaceuticals is also expected to increase particularly from the competitive pressure of producing quality products at lower cost.

Table 1: Off-patent Drug Schedule (partial list)

	Generic or Chemical name	Brand name	Class	Mechanism of Action	Disease	Degradation Products	Patent expiration
1	Levofloxacin LEV	Levaquine	Ofloxacin, quinolone	Synthetic broad-spectrum antibacterial agent	Bacterial Infection	Decarboxy ofloxacin, 9- piperazino ofloxacin, des-methyl ofloxacin, and ofloxacin-N-oxide	2011
2	Lamotrigine LTG	Lamictal™	Phenyl Triazine	NA	Anti-epileptic	Arene Oxides, N- chloro products by HOCl, N-Oxide	2009
3	Desloratadine DSL	Clarinex™, Claramax, Neo-Clarityn, Aerius™	Tricyclic Antihistamine	Peripheral H1 receptor antagonist	Allergy	NA	2009
4	Lansoprazole LSP	Prevacid™	Omeprazole Substituted benzimidazole	PPI* Gastic acid suppression	Acid related stomach problems	5 metabolites – acid degradation	2009
5	Losartan Potassium LOP	Cozaar™	Angeotensin II receptor (type AT1) antagonist	Blocks the binding of angiotensin II to the receptor (AT1)	hypertension	Imidazole ring breaks down by photo-degradation or by UV	2010
6	Orlistat	Xenical Orlistat™, alli™	lipstatin	Inhibitor of gastric and pancreatic lipases	Obesity	Prevention of lipid absorption by inhibition of pancreatic lipase	2010
7	Ramipril	Altace™	2-aza-bicyclo [3.3.0]-octane-3-carboxylic acid derivative	Inhibit angiotensin converting enzyme (ACE)	Cardio vascular, hypertension	NA	2009

Challenge to generic manufacturers

- The drugs listed in Table 1 cover a wide variety of diseases from simple bacterial infection to serious illnesses like epilepsy, hypertension, allergy etc.
- The structure and function of these drug compounds are diverse. Their chemical and physical properties vary widely.
- These pharmaceutical compounds have very different hydrophobicities.
- The challenge for generic makers is to develop validated chromatographic methods for these drugs (viz. lamotrigine, lansoprazole, levofloxacin, losartan potassium, desloratadine, orlistat).
- The orlistat generic version, alli, is already available over-the-counter.
- Reversed phase liquid chromatography (RPC) is an analytical technique widely used in the R&D and QC departments of drug manufacturers.

Challenge to generic manufacturers

- In this era of high throughput analysis, the need to obtain lower retention times while maintaining or improving resolution from closely eluting impurities is very important for quality control analysis.

- The availability of a column useful to separate a number of drug products having a wide variety of hydrophobicities is useful.

- Here we report the separation of a number of common drugs coming off patent (Table 1) using a TSKgel ODS-140HTP (2.0mm I.D. × 5cm, 2.3µm) column

Material and methods

All analyses were carried out using an HP-1100 HPLC system run by Chemstation (ver B.03.01)

Optimal chromatographic conditions:

Columns:	TSKgel ODS-140HTP, 2.3µm, 2.0mm ID × 5cm
	Thermo Hypersil GOLD, 1.9µm, 2.1mm ID x 5cm
	Phenomenex Luna C18(2)-HST, 2.5µm, 2.0mm ID x 5cm
Mobile Phase:	Gradient – A: water with 0.15% TFA; B: 100% ACN with 0.15% TFA
	Isocratic – Acetonitrile (percentage as mentioned in the respective chromatograms) in water containing 0.15%TFA
Temperature: 40	℃, unless otherwise noted
Injection volume:	10µL
Detection:	wavelength as mentioned in the respective chromatograms
Flow rate:	as mentioned in the respective chromatograms

Material and methods

- High purity Sigma-Aldrich brand drug standards (lamotrigine, lansoprazole, levofloxacin, losartan potassium, desloratadine, orlistat) were used for the preparation of stock standards.

- All the standards and samples were filtered through a 0.45µm membrane before injecting into the column.

- Working standards were prepared by dilution of the stock standard in water or 50% MeOH as necessary and used to generate the calibration curve.

- The over-the counter drug alli (distributed by GlaxoSmithKline Consumer Healthcare, L.P., Moon Twp, PA 15108) 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 water, filtered through a 0.45µm membrane and stored at – 20°C. The working standards were prepared by 1: 10 dilution in 50% MeOH and directly used for the chromatographic analysis.

Material and methods

- The limit of detection (LOD), is a parameter to measure the lowest concentration of an analyte in a sample that can be detected, but not necessarily quantitated under the stated experimental conditions. This 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).

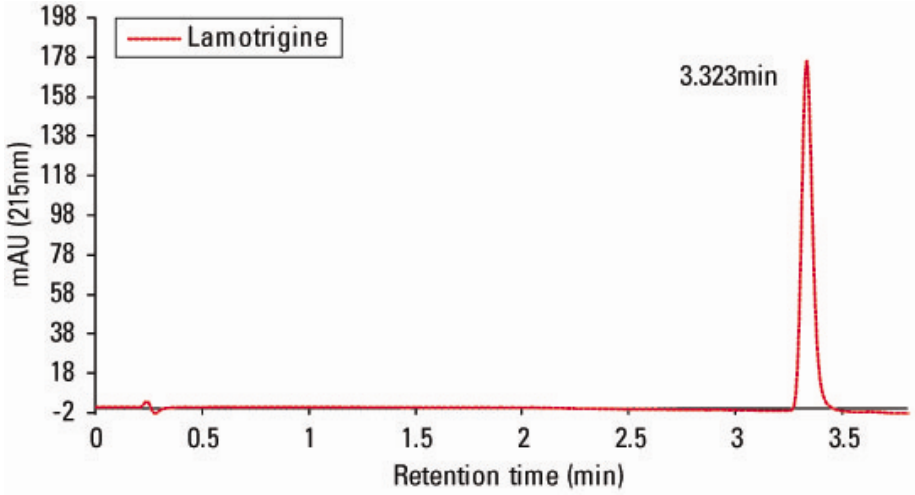
- The LOD is subsequently validated by the analysis of the sample near that limit.

- For determination of limit of quantitation (LOQ), the LOD sample concentration is multiplied by a factor of 10.

Table 2: Comparative chart of the properties of the columns used in this study

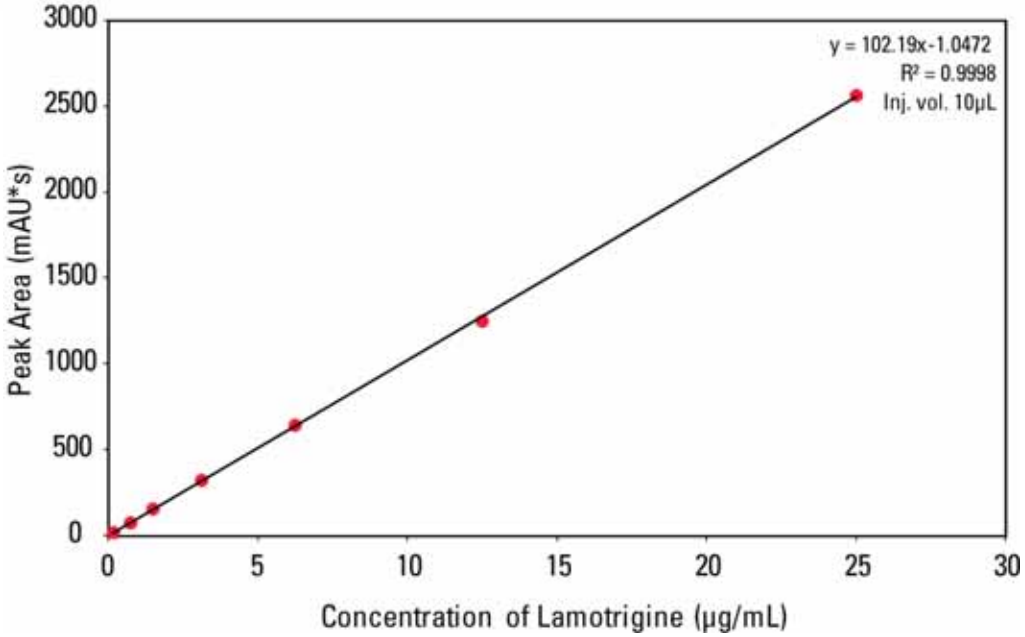
	TSKgel ODS-140HTP	Hypersil GOLD	Luna
Carbon Content	8%	10	17.5
Endcapped	Yes[2]	Yes	-
Particle Size (µm)	2.3	1.9	2.5
Pore Size (Å)	140	175	100
Preferred Sample Type	Hydrophobic	Medium strength	-
Bonded Phase Structure	Polymeric	-	-
Specific Surface Area (m^2/g)	-	220	400
*Asymmetry Factor	0.90 - 1.3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

Figure 1: Separation of lamotrigine using a TSKgel ODS-140HTP column



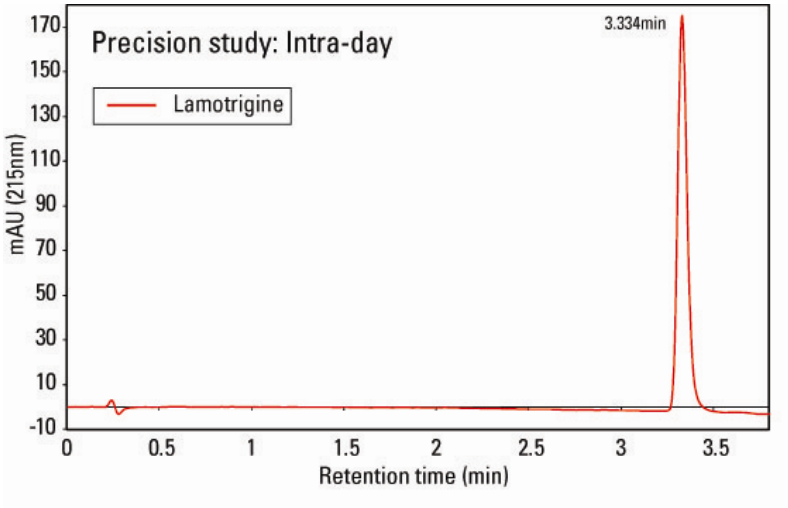
Equipment:	HP1100 series HPLC
Column:	TSKgel ODS-140HTP 5cm
Mobile phase:	A Water + 0.15% TFA B 100% Acetonitrile with 0.15% TFA, 15 minute linear gradient from 4%A – 100%B
Flow rate:	0.8mL/min
Detection:	UV@ 215nm
Temperature:	40°C
Injection vol.:	10µL
Sample:	lamotrigine 12.5µg/mL

Figure 2: Lamotrigine calibration curve

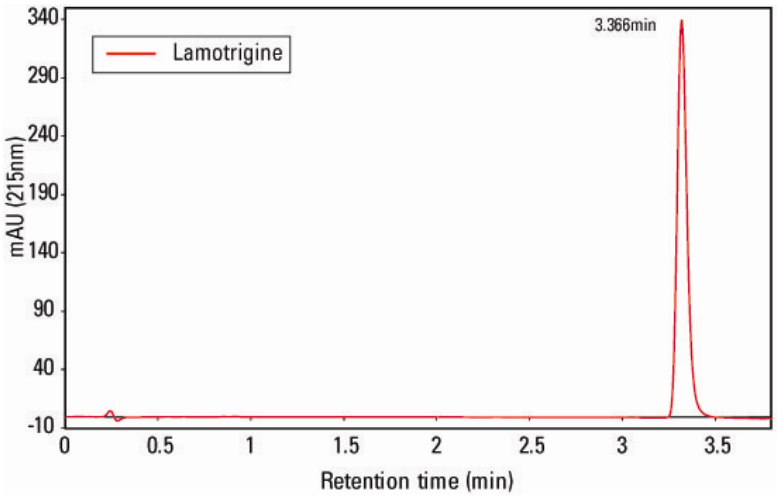


Calibration was linear in the concentration range of 2 – 20µg/mL

Figure 3: Precision study: Intra-day and inter-day variation in retention time of lamotrigine



Day	Retention Time (min)
1	3.33
2	3.29
3	3.32
4	3.31
5	3.28
Average	3.31
Std. Dev.	0.02
%RSD	0.62

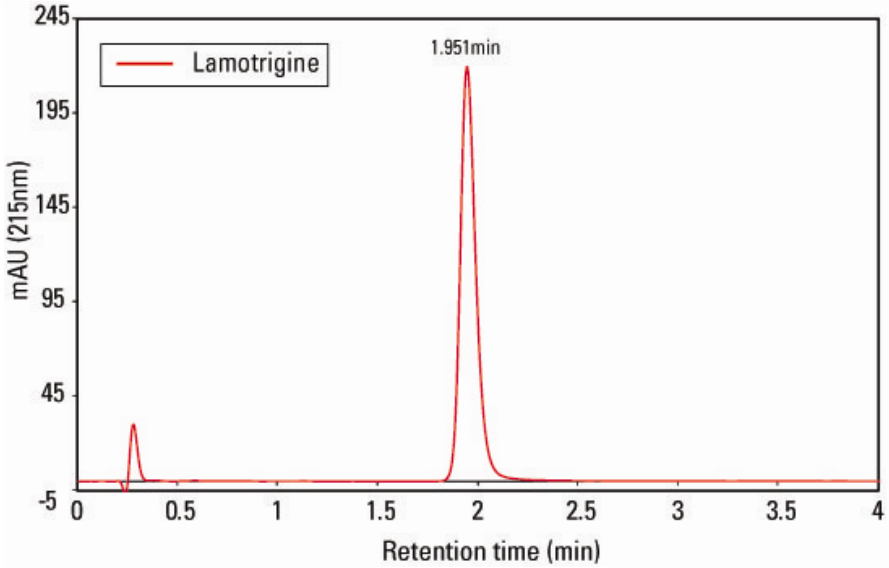


Equipment: HP1100 series HPLC
Column: TSKgel ODS-140HTP, 5 cm
Mobile phase: A Water + 0.15% TFA
B 100% Acetonitrile with 0.15% TFA,
15 minute linear gradient from
4%A – 100%B
Flow rate: 0.8mL/min
Detection: UV@ 215nm
Temperature: 40°C
Injection vol.: 10µL
Sample: lamotrigine 12.5µg/mL

Conditions apply for both chromatograms

Lamotrigine was separated with low retention time.

Figure 4: Isocratic elution of lamotrigine using a TSKgel ODS-140HTP column



Equipment: HP1100 series HPLC
Column: TSKgel ODS-140HTP, 5cm
Mobile phase: 10% Acetonitrile with 0.15% TFA
Flow rate: 0.8mL/min
Detection: UV@ 215nm
Temperature: 40°C
Injection vol.: 10µL
Sample: lamotrigine 12.5µg/mL

Consecutive Injections	Retention Time (min)
1	1.95
2	1.96
3	2.02
Average	1.98
Std. Dev.	0.04
%RSD	1.87

Lamotrigine was separated with low retention time (< 2 minutes).

Figure 5: Lamotrigine limit of detection (LOD) and limit of quantitation (LOQ) using a TSKgel ODS-140HTP column

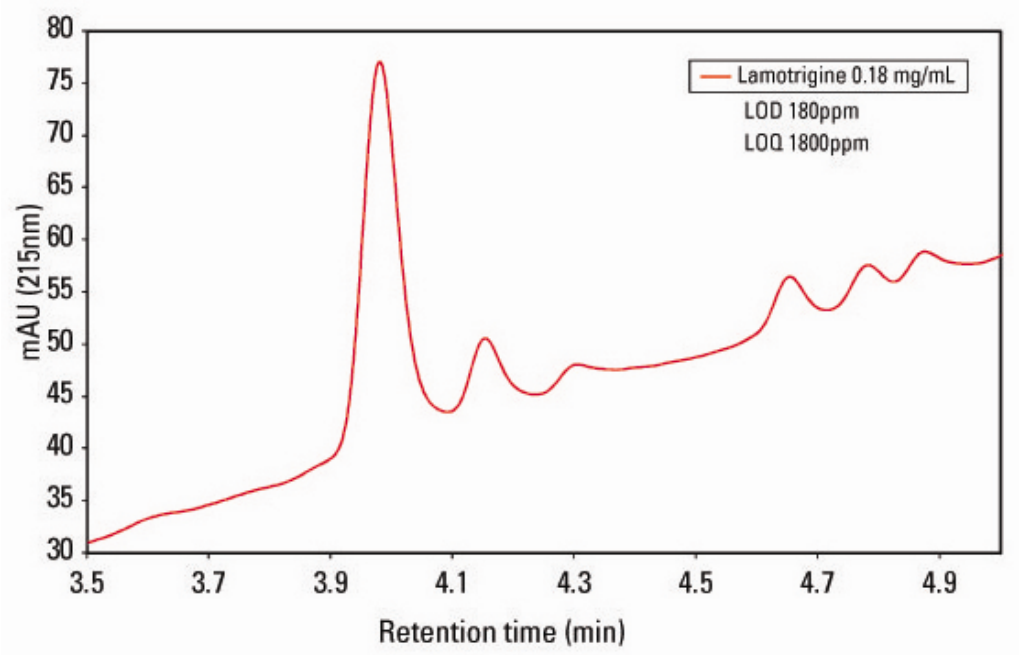
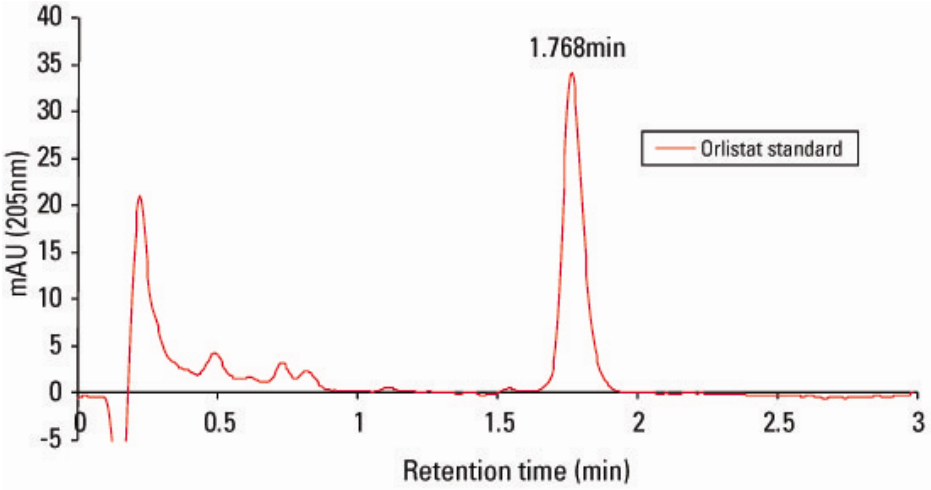


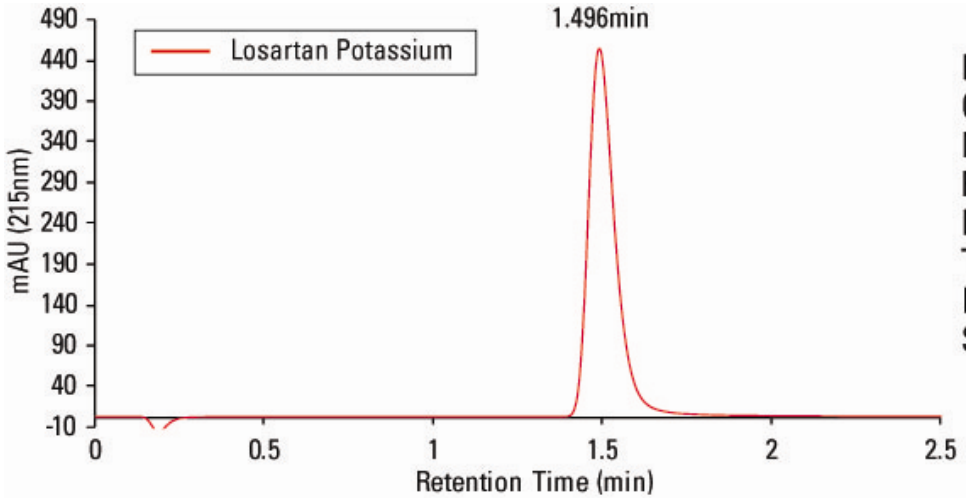
Figure 6: Isocratic elution of an orlistat standard using a TSKgel ODS-140HTP column



Equipment: HP1100 series HPLC
Column: TSKgel ODS-140HTP, 5cm
Mobile phase: 70% Acetonitrile with 0.15% TFA
Flow rate: 1.5mL/min
Detection: UV@ 205nm
Temperature: 40°C
Injection vol.: 10µL
Sample: orlistat 100µg/mL

Orlistat was separated with low retention time (< 2 minutes).

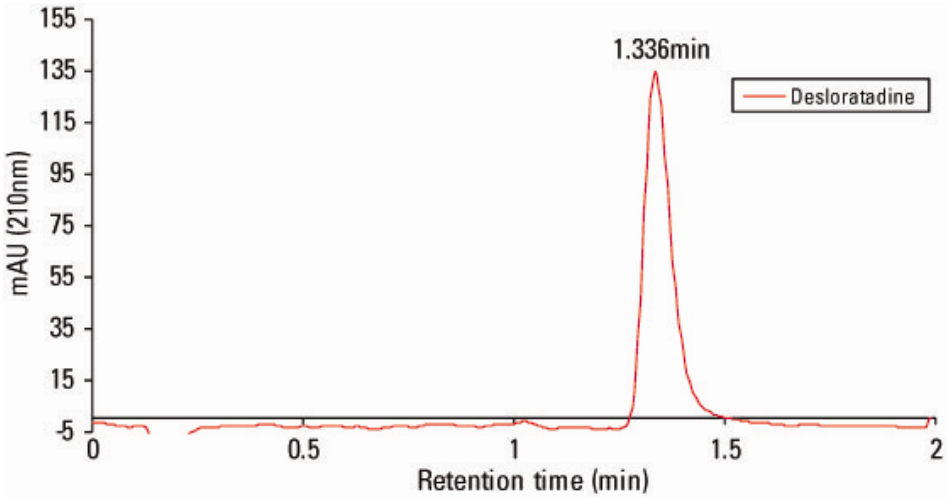
Figure 7: Isocratic elution of losartan potassium standard using a TSKgel ODS-140HTP column



Equipment: HP1100 series HPLC
Column: TSKgel ODS-140HTP, 5cm
Mobile phase: 20% acetonitrile with 0.15% TFA
Flow rate: 1.2mL/min
Detection: UV@ 215nm
Temperature: 40°C
Injection vol.:10µL
Sample: losartan potassium 14µg/mL

Losartan Potassium was separated with low retention time (< 2 minutes).

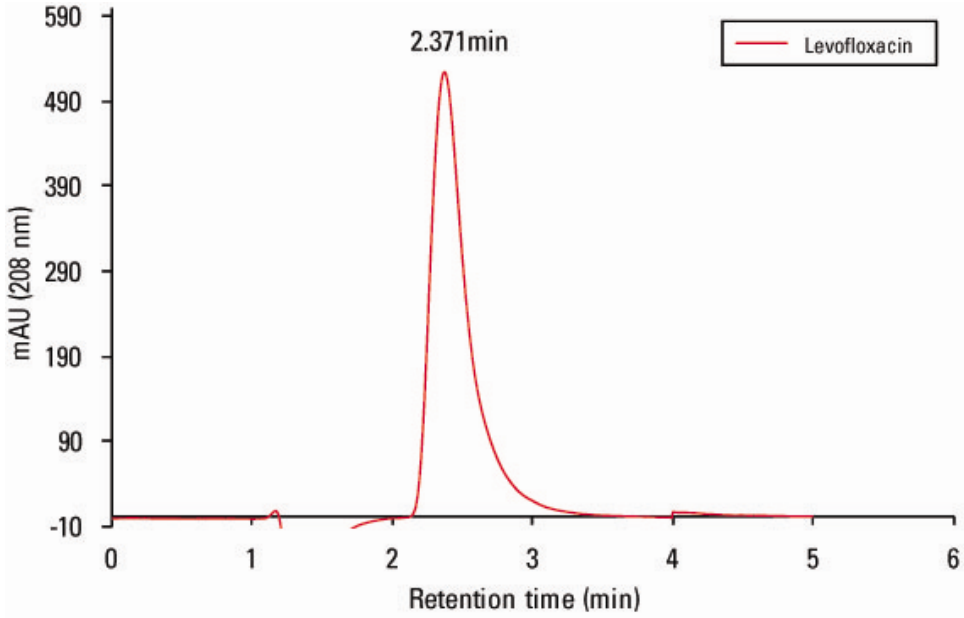
Figure 8: Isocratic elution of desloratadine standard using a TSKgel ODS-140HTP column



Equipment: HP1100 series HPLC
Column: TSKgel ODS-140HTP, 5cm
Mobile phase: 15% acetonitrile with 0.15% TFA
Flow rate: 1.5mL/min
Detection: UV@ 210nm
Temperature: 50°C
Injection vol.: 10µL
Sample: desloratadine 10µg/mL

Desloratadine was separated with low retention time (< 2 minutes).

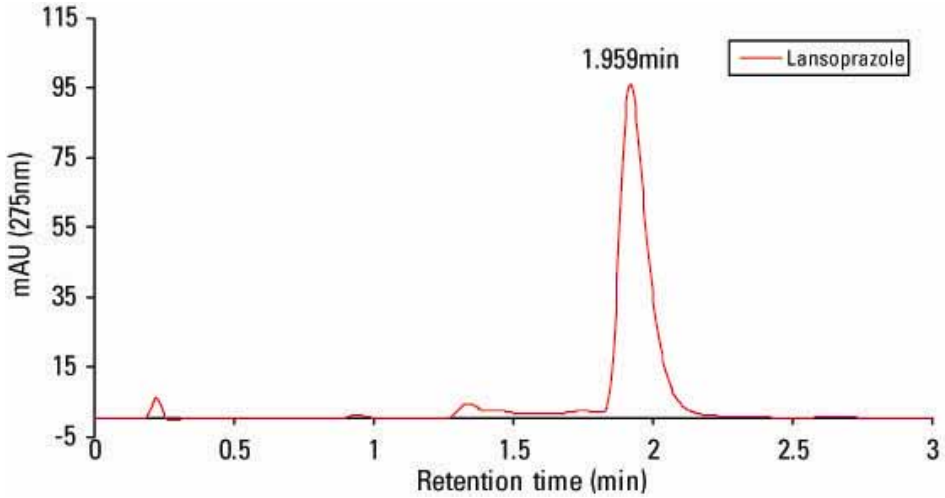
Figure 9: Isocratic elution of levofloxacin standard using a TSKgel ODS-140HTP column



Equipment: HP1100 series HPLC
Column: TSKgel ODS-140HTP, 5cm
Mobile phase: 5% acetonitrile with 0.15% TFA
Flow rate: 0.2mL/min
Detection: UV@ 208nm
Temperature: 40°C
Injection vol.:10µL
Sample: levofloxacin 100µg/mL

Levofloxacin was separated with low retention time.

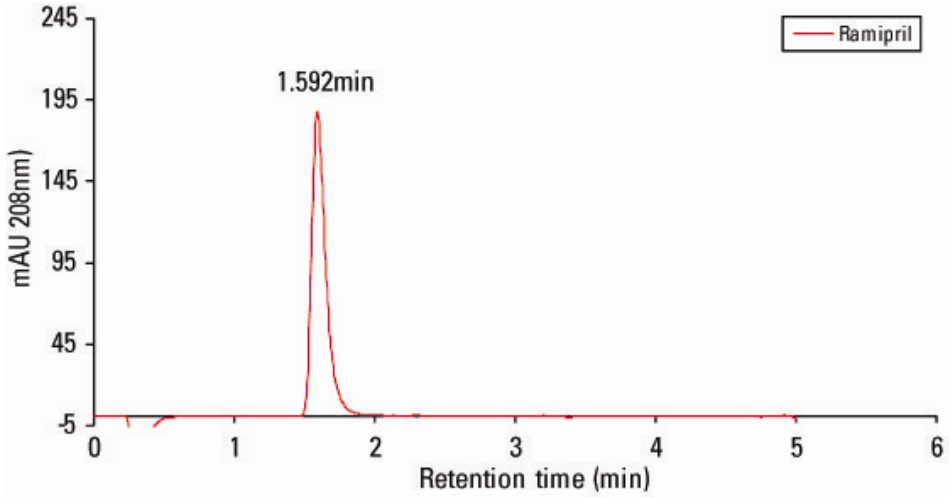
Figure 10: Isocratic elution of lansoprazole standard using a TSKgel ODS-140HTP column



Equipment: HP1100 series HPLC
Column: TSKgel ODS-140HTP, 5cm
Mobile phase: 15% acetonitrile with 0.15% TFA
Flow rate: 1.0µL/min
Detection: UV@ 275nm
Temperature: 50°C
Injection vol.:10µL
Sample: lansoprazole 11µg/mL

Lansoprazole was separated with low retention time (< 2 minutes).

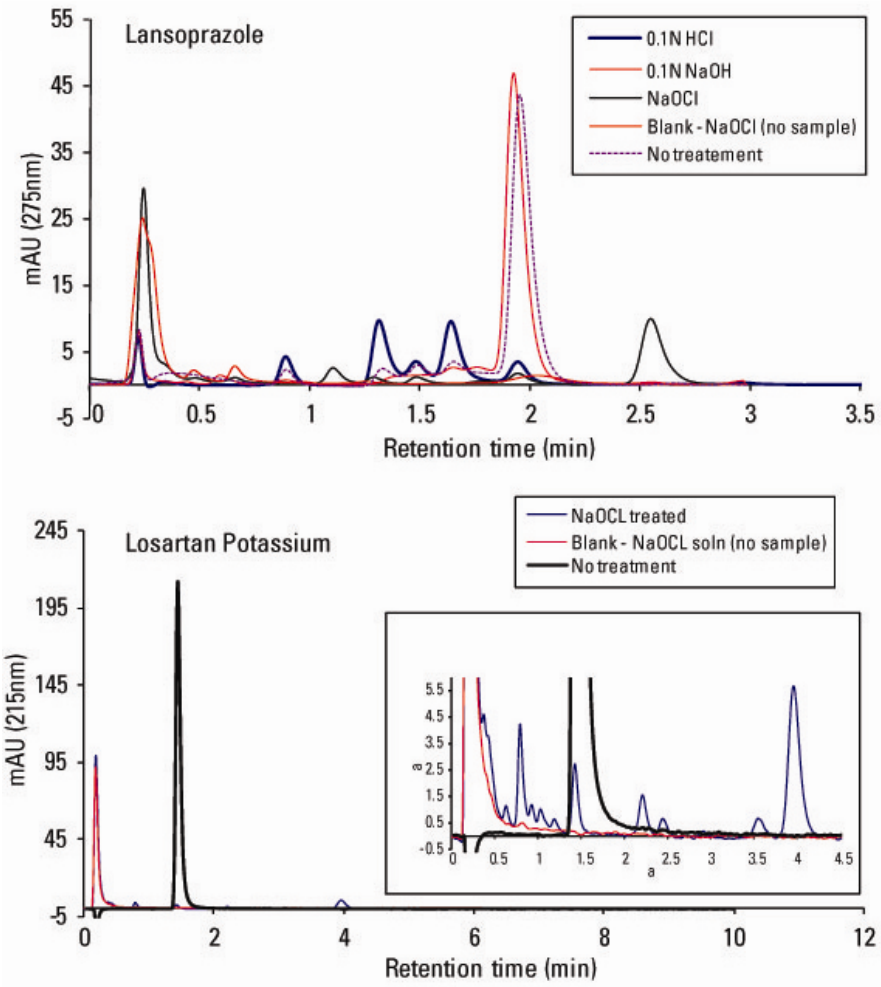
Figure 11: Isocratic elution of ramipril standard using a TSKgel ODS-140HTP column



Equipment: HP1100 series HPLC
Column: TSKgel ODS-140HTP, 5cm
Mobile phase: 25% acetonitrile with 0.15% TFA
Flow rate: 0.8mL/min
Detection: UV@ 208nm
Temperature: 50°C
Injection vol.:10µL
Sample: ramipril 11.8µg/mL

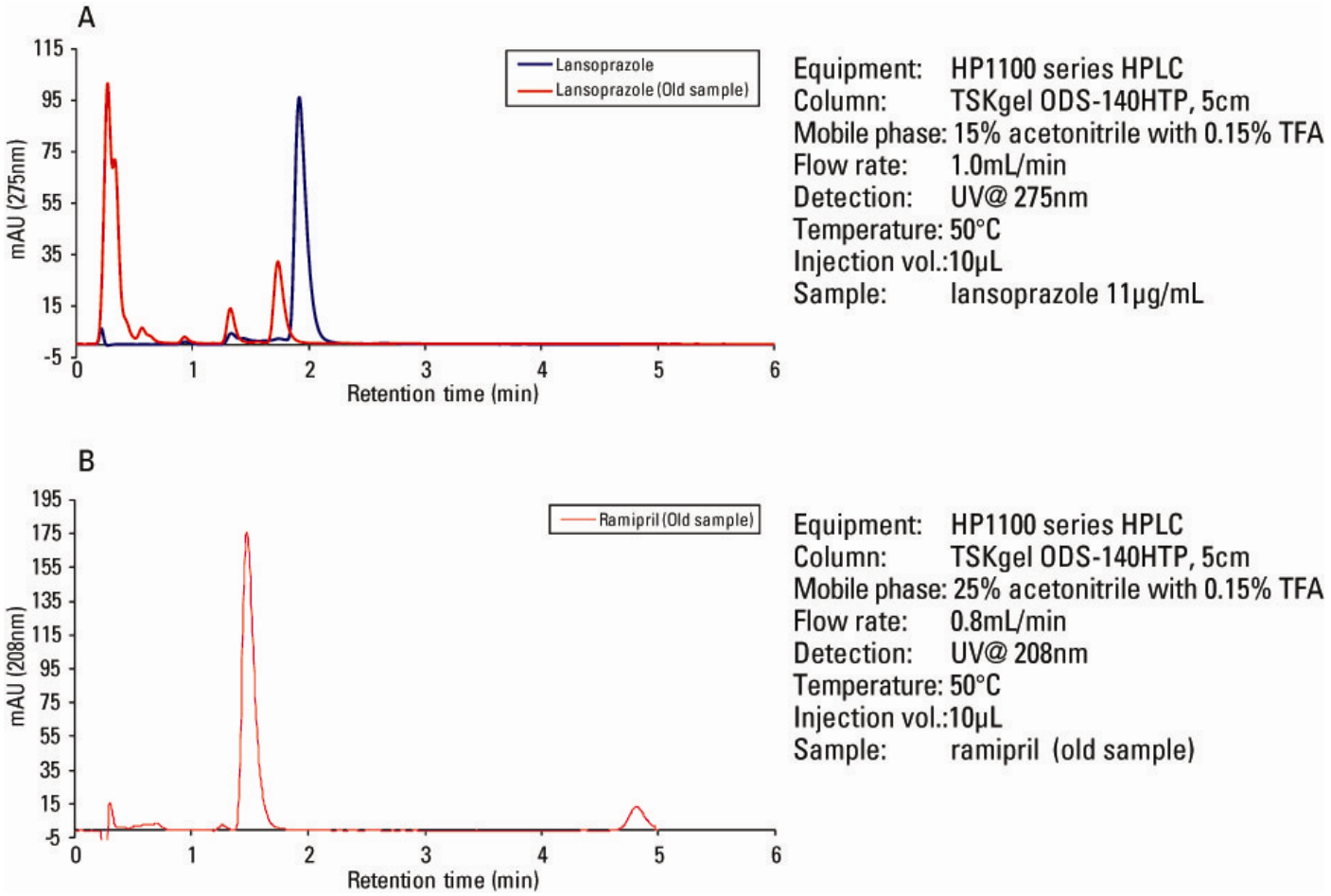
Ramipril was separated with low retention time (< 2 minutes).

Figure 12: Separation of the forced degradation products of (a) lansoprazole (b) losartan potassium using a TSKgel ODS-140HTP column



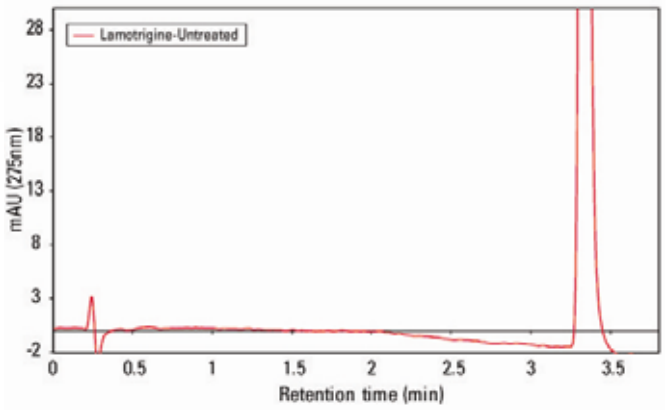
- In forced degradation (also known as a stress test) the drug compounds are subjected to extreme chemical and environmental conditions to produce and then identify breakdown products.
- The ICH guidelines indicate that forced degradation is designed to help determine the intrinsic stability of the molecule. The purpose is to establish probable degradation products and to validate the stability-indicating power of the analytical method used.
- Selectivity is a measure of relative retention of two sample components. The selectivity of the TSKgel ODS-140HTP column was always >1.0 for the new peaks obtained from forced degradation.

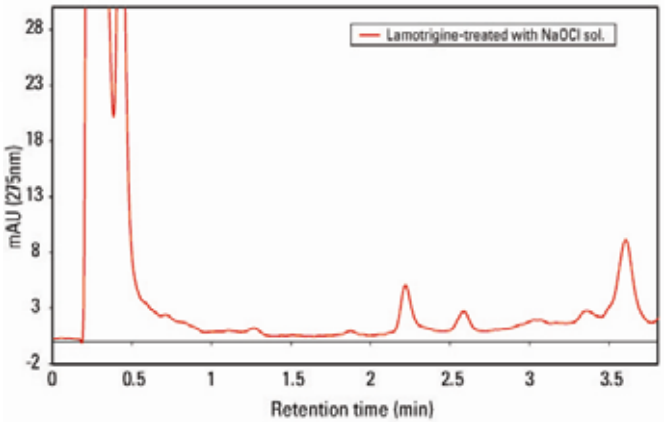
Figure 13: Separation of the degradation products of (a) lansoprazole (b) ramipril upon storage using a TSKgel ODS-140HTP column

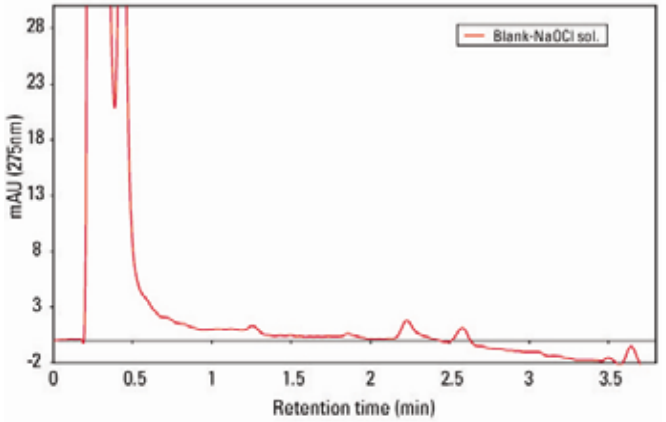


Ramipril degradation product separated as new peak at around 4.9 min. which was not observed in a fresh sample (Figure 11). (The difference in retention times was due to differences in flow rate at which the analysis was carried out)

Figure 14: Separation of the forced degradation products of lamotrigine using a TSKgel ODS-140HTP column







- In this study lamotrigine (25µg/mL; 750µL in mobile phase) was treated with 750µL of 6% NaOCl solution for 1 min – the final concentration of lamotrigine was 12.5µg/mL.

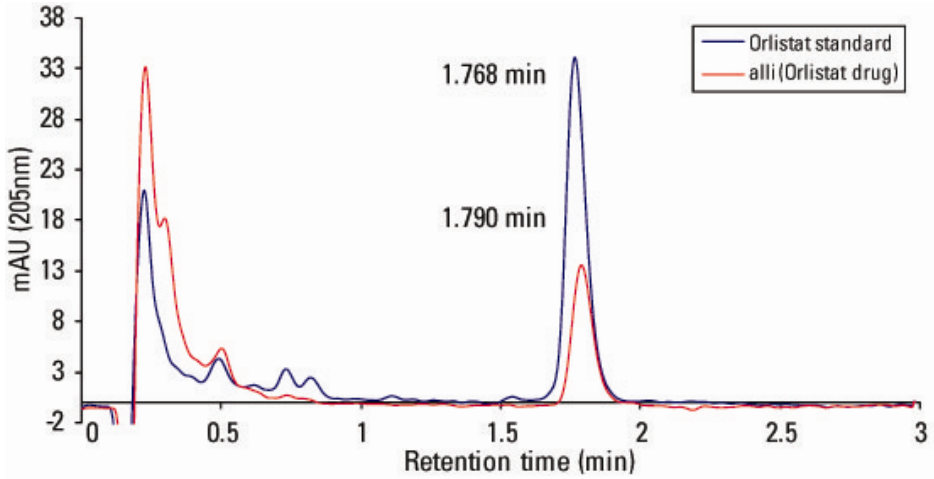
- The chromatogram shows the disappearence of the lamotrigine peak upon treatment with NaOCl (middle panel).

- Lamotrigine is known to form two different *N*-chloro products by NaOCl solution (6%) which have been reportedly identified by X-ray crystallography[1]

- The lower most panel in this figure is the blank NaOCl solution shown as a reference.

[1] DMD 35:1050-1056, 2007

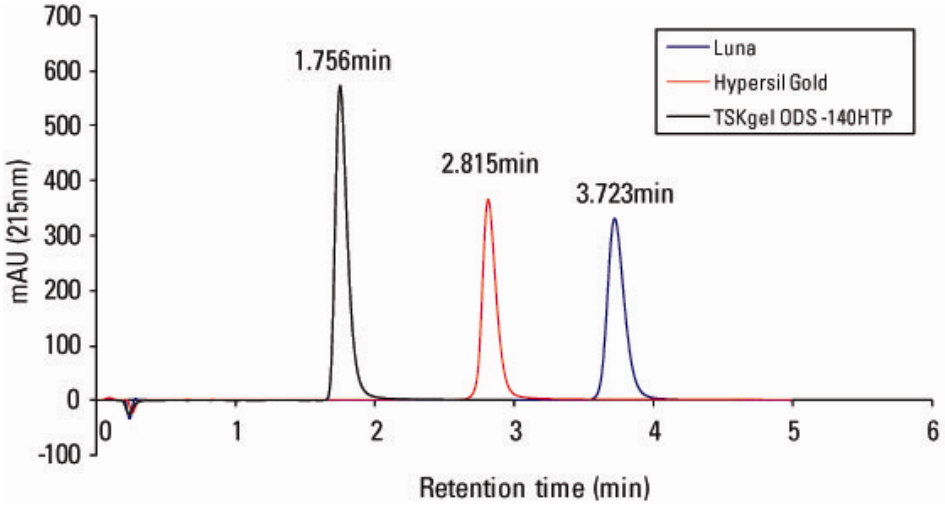
Figure 15: Separation of orlistat from the over-the-counter drug alli using a TSKgel ODS-140HTP column



Equipment: HP1100 series HPLC
Column: TSKgel ODS-140HTP, 5cm
Mobile phase: 70% Acetonitrile with 0.15% TFA
Flow rate: 1.5mL/min
Detection: UV@ 205nm
Temperature: 40°C
Injection vol.:10µL
Sample: orlistat 100µg/mL

The standard orlistat peak eluted at 1.768 minute while the orlistat sample from alli eluted at 1.790 min. This study shows that the column can be used for method development of these generic drugs.

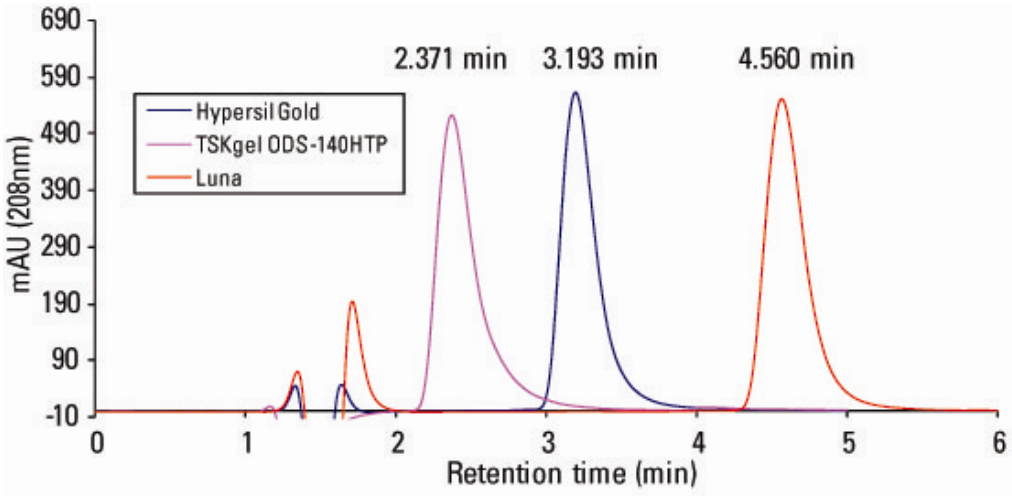
Figure 16: Elution profile of the off-patent drug losartan potassium using a TSKgel ODS-140HTP, Hypersil GOLD and Luna column



Equipment: HP1100 series HPLC
Column: TSKgel ODS-140HTP, 5cm
Mobile phase: 20% acetonitrile with 0.15% TFA
Flow rate: 1.0mL/min
Detection: UV@ 215nm
Temperature: 40°C
Injection vol.: 10µL
Sample: losartan potassium 14µg/mL

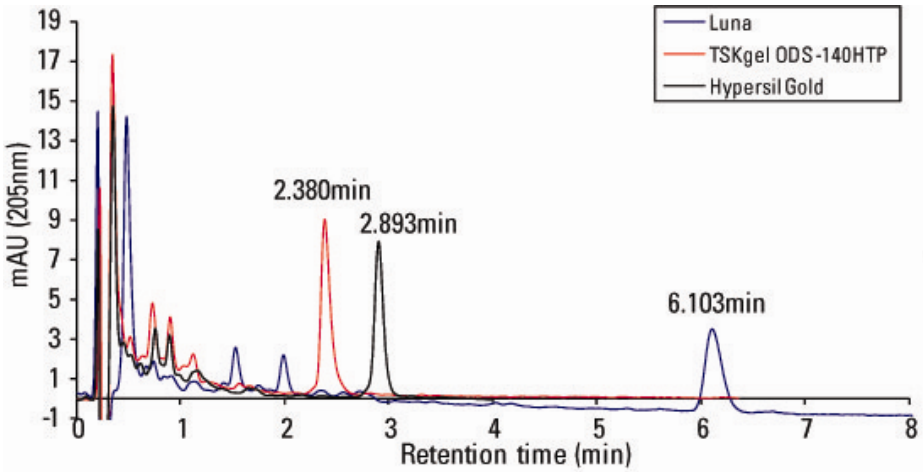
A TSKgel ODS-140HTP column yielded the shortest retention times in comparison to the other two competitive columns tested under identical chromatographic conditions.

Figure 17: Elution profile of the off-patent drug levofloxacin using a TSKgel ODS-140HTP, Hypersil GOLD and Luna column



Equipment: HP1100 series HPLC
Column: TSKgel ODS-140HTP, 5cm
Mobile phase: 5% acetonitrile with 0.15% TFA
Flow rate: 0.2mL/min
Detection: UV@ 208nm
Temperature: 40°C
Injection vol.:10µL
Sample: levofloxacin 100µg/mL

Figure 18: Comparative study of the elution profile of the off-patent drug orlistat



Equipment: HP1100 series HPLC
Column: TSKgel ODS-140HTP, 5cm
Mobile phase: 70% Acetonitrile with 0.15% TFA
Flow rate: 1.2mL/min
Detection: UV@ 205nm
Temperature: 40°C
Injection vol.: 10µL
Sample: orlistat 100µg/mL

Irrespective of the chemical structures and hydrophobicities of the drugs studied here in figures 15,16 and 17, the TSKgel ODS-140HTP column yielded the shortest retention times in comparison to the other two competitive columns tested under identical chromatographic conditions.

Conclusion

- The TSKgel ODS-140HTP, 5cm column was used for the analysis of a number of common drugs with a wide range of hydrophobicities.

- Generic manufacturers can use this column for the separation of the drugs
    - 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

- Shorter run times also have an added benefit in that they reduce the amount of organic waste.

Trademarks

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- Aerius is a registered trademark of Schering-Plough HealthCare Products, Inc.
- Cozaar is a registered trademark of E. I. du Pont de Nemours and Company
- Prevacid is a registered trademark of Takeda Pharmaceuticals
- Xenical Orlistat is a registered trademark of Hoffmann-La Roche Inc.
- Altace is a registered trademark of King Pharmaceuticals Research and Development, Inc.