Analysis of a Number of Common Drugs using TSK-GEL ODS-140HTP, 2.3µm High Throughput Reversed Phase Columns

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Introduction: Scope of generic drugs

- Pharmaceuticals are among the most highly regulated products in the United States.
- After the expiration of patent protection many competitive manufacturers may produce a less expensive generic product.
- An estimated $64 billion of pharmaceutical products have either recently come off-patent or will come off-patent in the near future.

Objective:

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

Objective

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

Generic or Chemical name	Brand name	Class	Mechanism of Action	Disease	Degradation Products	Patent expiration
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
Lamotrigine LTG	Lamictal™	phenyl triazine	NA	anti-epileptic	arene oxides, N- chloro products by HOCl, N-Oxide	2009
Desloratadine DSL	Clarinex™, Claramax Neo-Clarityn, Aerius™	tricyclic antihistamine	peripheral H1 receptor antagonist	allergy	NA	2009
Lansoprazole LSP	Prevacid™	omeprazole substituted benzimidazole	PPI* gastic acid suppression	acid related stomach problems	5 metabolites – acid degradation	2009
Losartan Potassium LOP	Cozaar™	angeotensin II receptor (type AT_1) antagonist	blocks the binding of angiotensin II to the receptor (AT_1)	hypertension	imidazole ring breaks down by photo-degradation or by UV	2010
Orlistat	Xenical™, alli™ (OTC)	lipstatin	inhibitor of gastric and pancreatic lipases	obesity	prevention of lipid absorption by inhibition of pancreatic lipase	2010
Ramipril	Altace™	2-aza-bicyclo[3.3.0]-octane-3-carboxylic acid derivative	inhibit angiotensin-converting enzyme (ACE)	hypertension	NA	2009

This table shows a partial list of drugs selected for this study which cover:

a. a wide variety of diseases

b. diverse structure and function

c. various chemical and physical properties

d. a diversity of hydrophobicities.

Material and Methods

Instrument: HP-1100 HPLC system run by Chemstation (ver B.03.01)

Optimal chromatographic conditions:

- **Column:** TSKgel ODS-140HTP, 2.3µm, 2.0mm ID x 5cm
- **Detection:** UV (see respective chromatograms)
- **Column temp:** 40˚C unless mentioned otherwise
- **Flow rate:** see the respective chromatograms
- **Injection volume:** 10µL
- **Mobile phases:**
    - Isocratic condition: Acetonitrile in water containing 0.15%TFA
- The limit of detection (LOD) is measured by USP method (Ref: <1225> Validation of Compendial Methods; USP23)
- Limit of quantitation (LOQ) is obtained by multiplying the LOD value by 10.

Material and Methods

Table 2: Properties of the TSKgel ODS-140HTP, 2.3µm, 2.0mm ID x 5cm reversed phase column used in this study

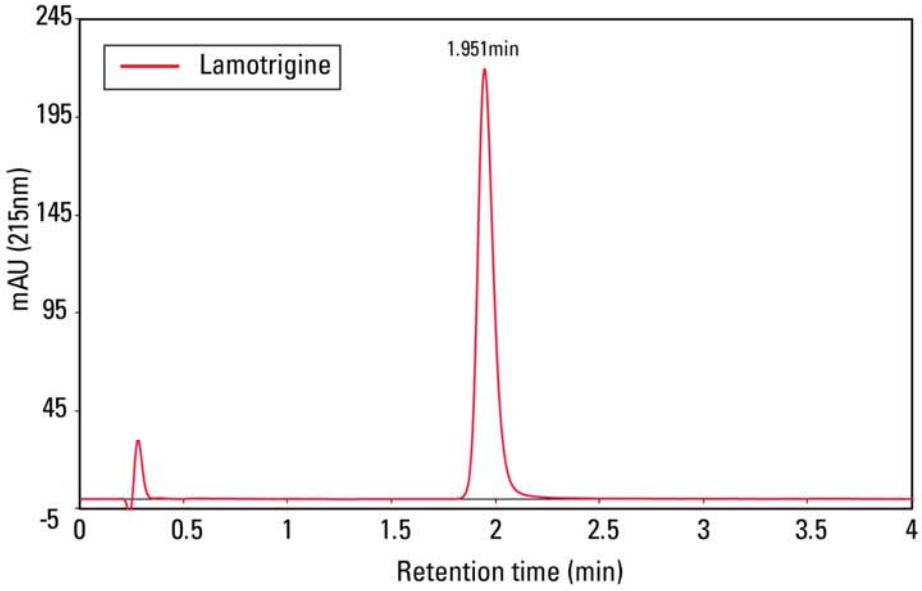
	TSKgel ODS-140HTP
Carbon Content	8%
Endcapped	Yes[1]
Particle Size (µm)	2.3
Pore Size (Å)	140
Preferred Sample Type	Hydrophobic
Bonded Phase Structure	Polymeric
Specific Surface Area (m^2/g)	-
*Asymmetry Factor (10%)	0.90 - 1.3
*Theoretical Plates	140,000 (plates/meter)

*N (Theoretical plates) and As (Asymmetry Factor) are based on naphthalene peak.
[1] Prepared by bonding the surface with a difunctional octadecylsilane reagent, followed by repeated endcapping with monofunctional trimethylsilane reagent.

Material and Methods

- High purity Sigma-Aldrich brand drug standards were used.
- All the standards and samples were filtered through a 0.45µm membrane.
- Working standards were prepared by dilution of the stock standard in water or 50% MeOH as necessary and were used to generate the calibration curve.
- The over-the-counter drug, alli was purchased from a local pharmacy. A total of 0.1145gm of the white, cube-shaped drug material was weighed from a single 60mg capsule. The material was dissolved in 50% MeOH in water, filtered through a 0.45µm membrane and stored at -20°C. The working standards were prepared by a 1:10 dilution in 50% MeOH and directly used for the chromatographic analysis.

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



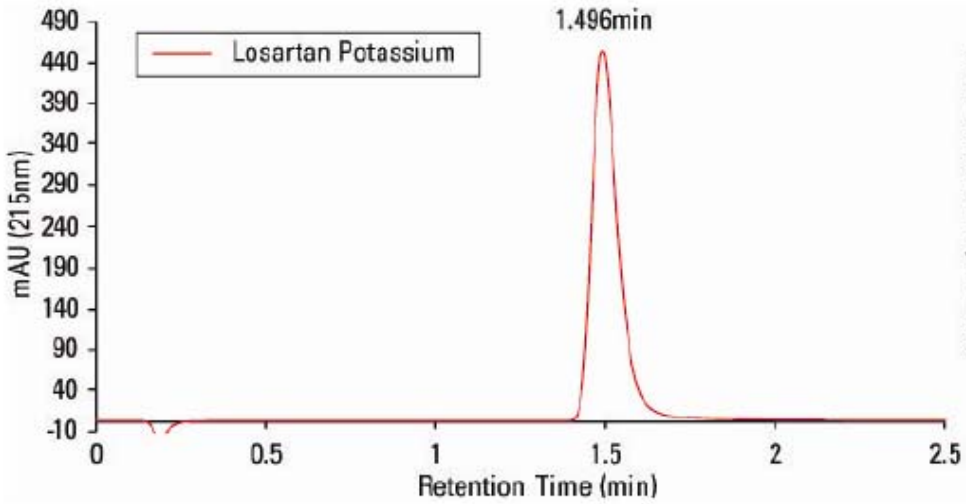
Equipment:	HP1100 series HPLC
Column:	TSKgel ODS-140HTP, 5cm
Mobile phase:	10% Acetonitrile with 0.15% TFA
Flow rate:	0.8mL/min
Detection:	UV@ 215nm
Temperature:	40°C
Injection vol.:	10µL
Sample:	lamotrigine 12.5µg/mL

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 eluted under isocratic conditions within 2 minutes with high intra-day and inter-day precision in retention time.

LOD and LOQ for Lamotrigine were found to be 180 and 1800ppm respectively.

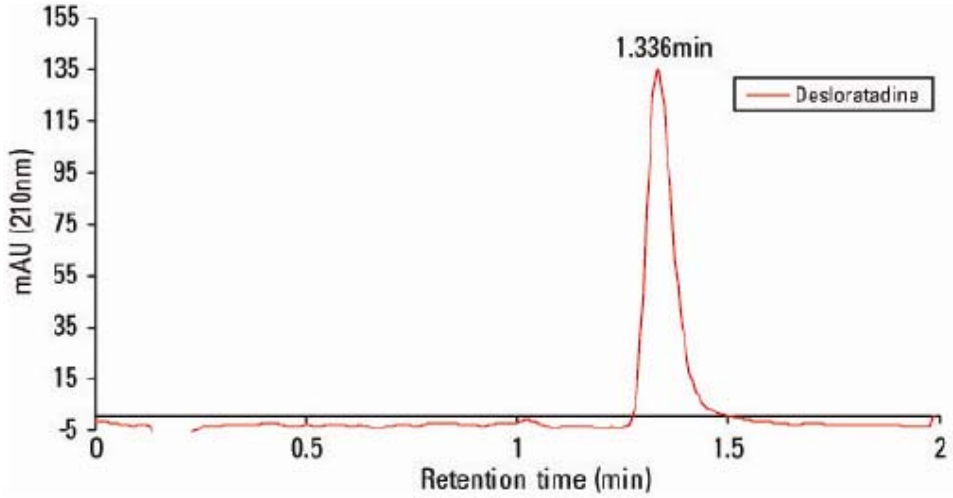
Figure 2: Isocratic elution of losartan potassium using a TSKgel ODS-140HTP column



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 eluted with low retention time (< 2 minutes).

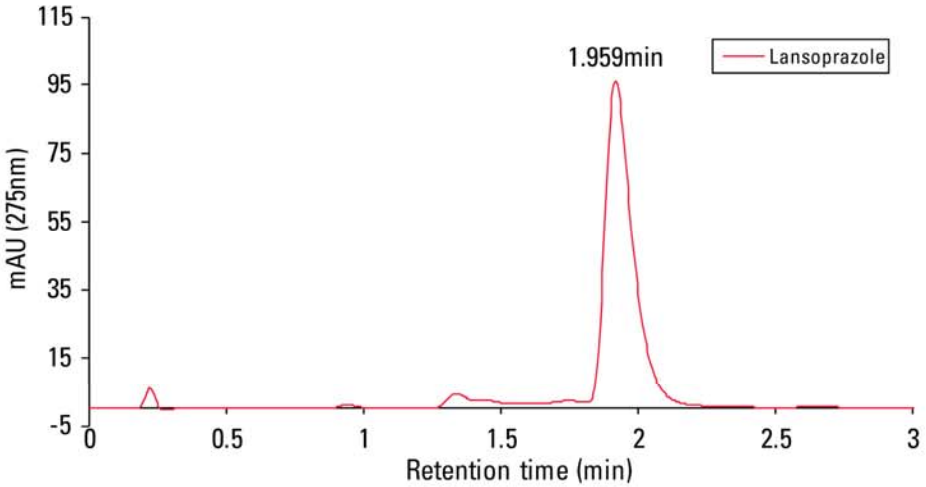
Figure 3: Isocratic elution of desloratadine using a TSKgel ODS-140HTP column



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 eluted with low retention time (< 2 minutes).

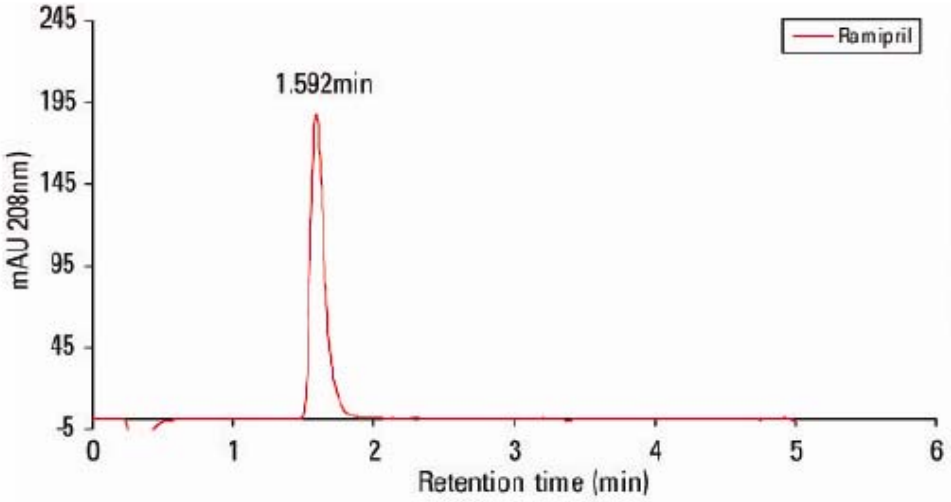
Figure 4: Isocratic elution of lansoprazole using a TSKgel ODS-140HTP column



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

Lansoprazole eluted with low retention time (< 2 minutes).

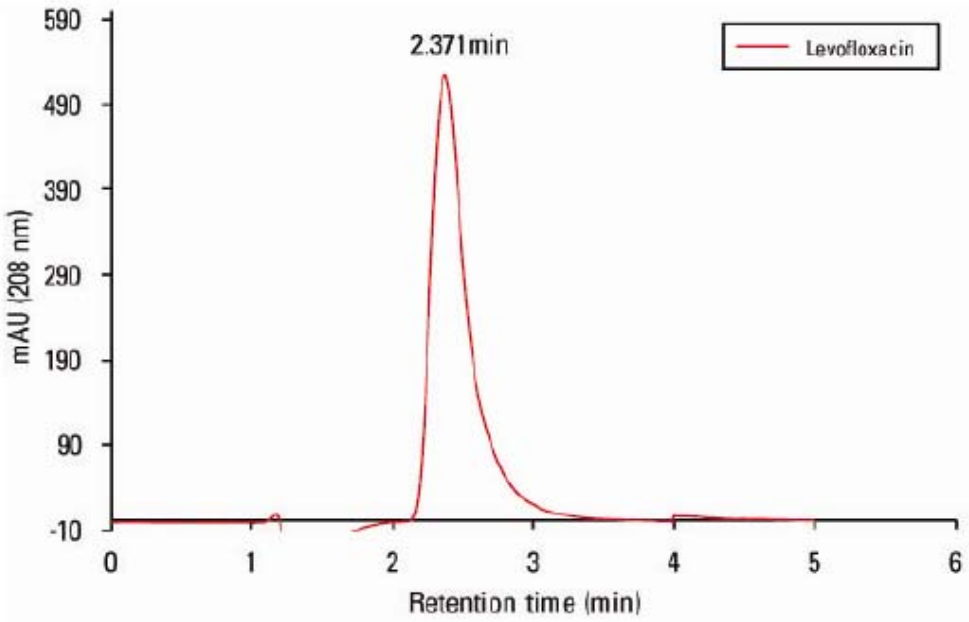
Figure 5: Isocratic elution of ramipril using a TSKgel ODS-140HTP column



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 eluted with low retention time (< 2 minutes).

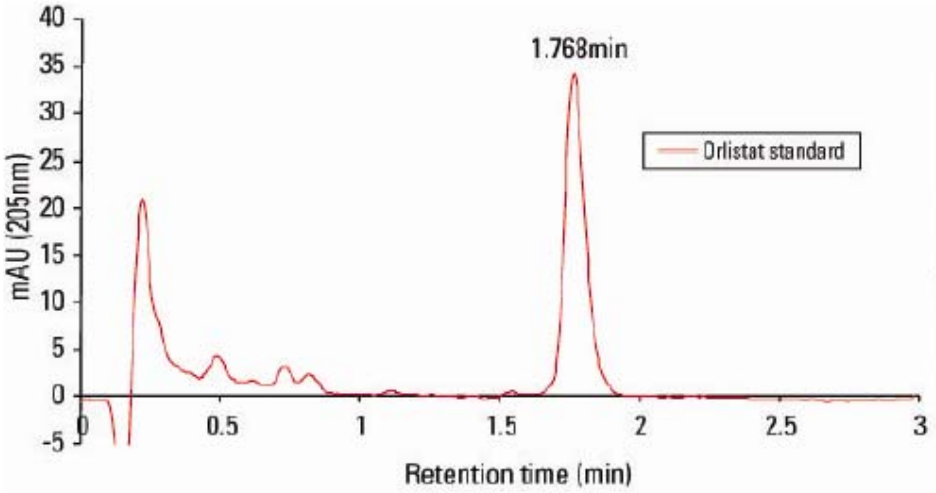
Figure 6: Isocratic elution of levofloxacin using a TSKgel ODS-140HTP column



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 eluted with low retention time – a further study to reduce tailing is in progress.

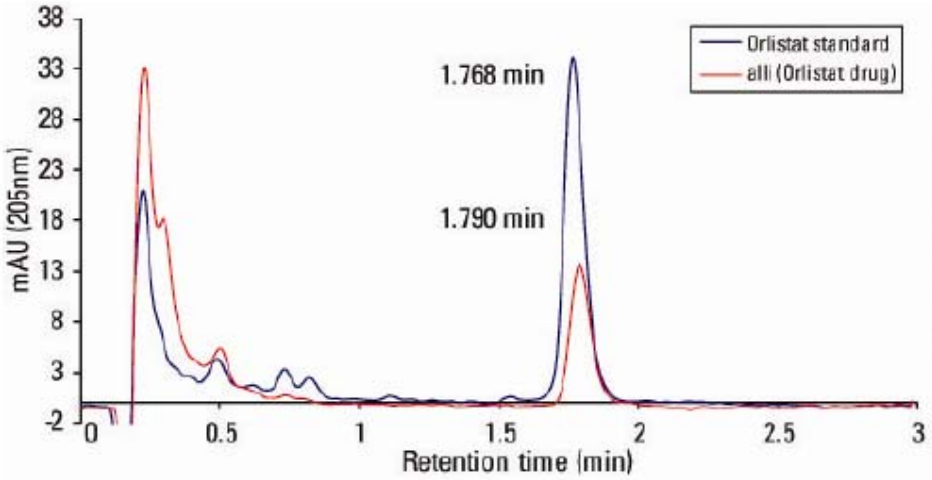
Figure 7: Isocratic elution of orlistat using a TSKgel ODS-140HTP column



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 eluted with low retention time (< 2 minutes).

Figure 8: 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 orlistat standard peak eluted at 1.768 minute, while the orlistat sample from alli eluted at 1.790 minute.**
- **This study shows that the column can be used for the method development of these generic drugs.**

Conclusions

This study demonstrates that TSK-GEL ODS-140HTP columns are very useful for:

- **high throughput separation using conventional HPLC**
- **the separation of drugs comprised of a wide variety of hydrophobicities**
- **method development for generic drugs coming off of patent protection**
- **efficient method development for quality control purposes**
- **reducing cost and organic waste by decreasing run times**