High Throughput Analysis of a Number of Common Drugs using a TSKgel ODS-140HTP, 2.3µm Column

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Introduction

- Reversed phase liquid chromatography (RPC) is an analytical technique widely used in the R&D and QC departments in the drug industry.
- An estimated $64 billion of pharmaceutical products are coming off-patent in the near future.
- The challenge for generic manufacturers is to develop validated chromatographic methods for some of these drugs (*viz.* lamotrigine, lansoprazole, levofloxacin, losartan potassium, desloratadine, orlistat and topiramate).
- 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.
- The availability of a column capable of separating a number of ingredients having a wide variety of hydrophobicities is useful.

Introduction Continued

- We used the following reversed phase columns to analyze a number of drugs: TSKgel ODS-140HTP, 2.3µm, 2.1mm ID x 5cm column and TSKgel ODS-140HTP, 2.3µm, 2.1mm ID x 10cm column.
- We report the separation of a number of drugs coming off patent in 2009 and in the coming years using a TSKgel ODS-140HTP column.
- Additional studies like forced degradation, determination of limit of detection and of quantitation, calibration curve, etc. are reported for lamotrigine.
- High throughput separation of these drugs using a conventional HPLC with an upper pressure limit of 41.4MPa is valuable in method development for the identification of ingredients, stability studies and quality control.

Materials and Methods: Chromatographic Conditions

LC System:	HP-1100 HPLC with Chemstation (ver B.03.01)
Columns:	TSKgel ODS-140HTP, 2.3mm, 2.1mm ID x 5cm TSKgel ODS-140HTP, 2.3mm, 2.1mm ID x 10cm
Mobile Phase:	as indicated in each chromatogram
Temperature:	40°C
Injection volume:	10µL
Detection:	UV@215 nm
Flow rate:	as noted

Material and Methods

- High purity Sigma-Aldrich brand chemicals were used for the preparation of stock solutions of drug substances.
- Stock solutions were prepared either in water or methanol as required and stored at -20°C.
- Working standards were prepared by dilution of the stock solutions in water.
- All the standards and the samples were filtered through a 0.45µm membrane filter prior to injection.

Material and Methods Continued

- The limit of detection (LOD) is one of the limit tests defined as the lowest concentration of the analyte in a sample that can be detected, but not necessarily be quantitated, under the stated experimental conditions mentioned.
- LOD is determined by the following USP method:
    - The standard deviation of the response (mAU) of the baseline is calculated during a blank injection.
    - The standard deviation is multiplied by a factor of 2 to provide an estimate of the limit of detection (mAU).
    - The limit of detection is subsequently confirmed by the analysis of the sample near that calculated limit.
    - For the determination of the limit of quantitation (LOQ), the standard deviation of the blank injection is multiplied by a factor of 10.

Properties of TSK-GEL ODS-140HTP Columns

	TSK-GEL 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	>14,000

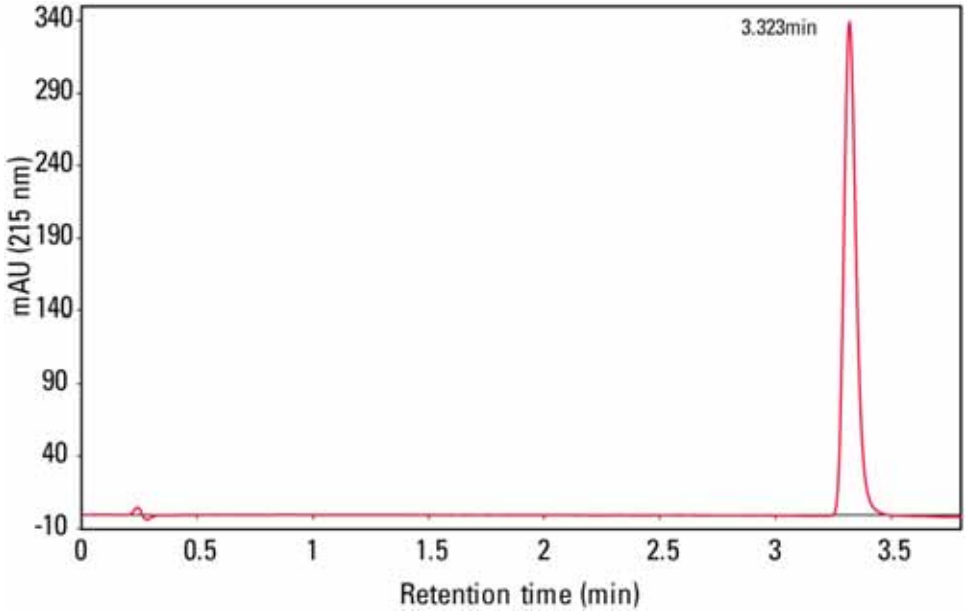
* N (Theoretical plates) and As (Asymmetry factor) are based on naphthalene peak.

[1] Prepared by bonding the surface with a difunctional octadecylsilane reagent, followed by repeated endcapping with monofunctional trimethylsilane reagent.

Off-patent Drug Schedule

Drug Standards	Generic Name	Class	Mechanism of Action	Disease	Degradation Products	Year going off patent
Levofloxacin LEV	Levaquine	Ofloxacin, quinolone	synthetic broad-spectrum antibacterial agent	Bacterial Infection	Decarboxy ofloxacin, 9-piperazino ofloxacin, des-methyl ofloxacin, ofloxacin-N-oxide	2011
Lamotrigine LTG	Lamictal™	Phenyl Triazine		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		2009
Lansoprazole LSP		Omeprazole	protein pump inhibitor		5 metabolites-acid degradation	2009
Losartan Potassium LOP	Cozaar™		angiotensin II receptor (AT1) antagonist.	Hypertension	Imidazole ring breaks down by photo-degradation or by UV	2010
Topiramate			anticonvulsant drug			

Figure 1: Analysis of Lamotrigine using a TSKgel ODS-140HTP, 2.3µm, 2.1mm ID x 5cm Column



Consecutive Injections	Area
1	1298.55
2	1295.98
3	1298.72
4	1304.17
5	1312.69
Average	1302.02
Std. Dev.	6.67
%RSD	0.51

Lamotrigine was eluted as a sharp peak using a linear gradient. The very low %RSD value over 5 consecutive injections shows the efficiency of the method.

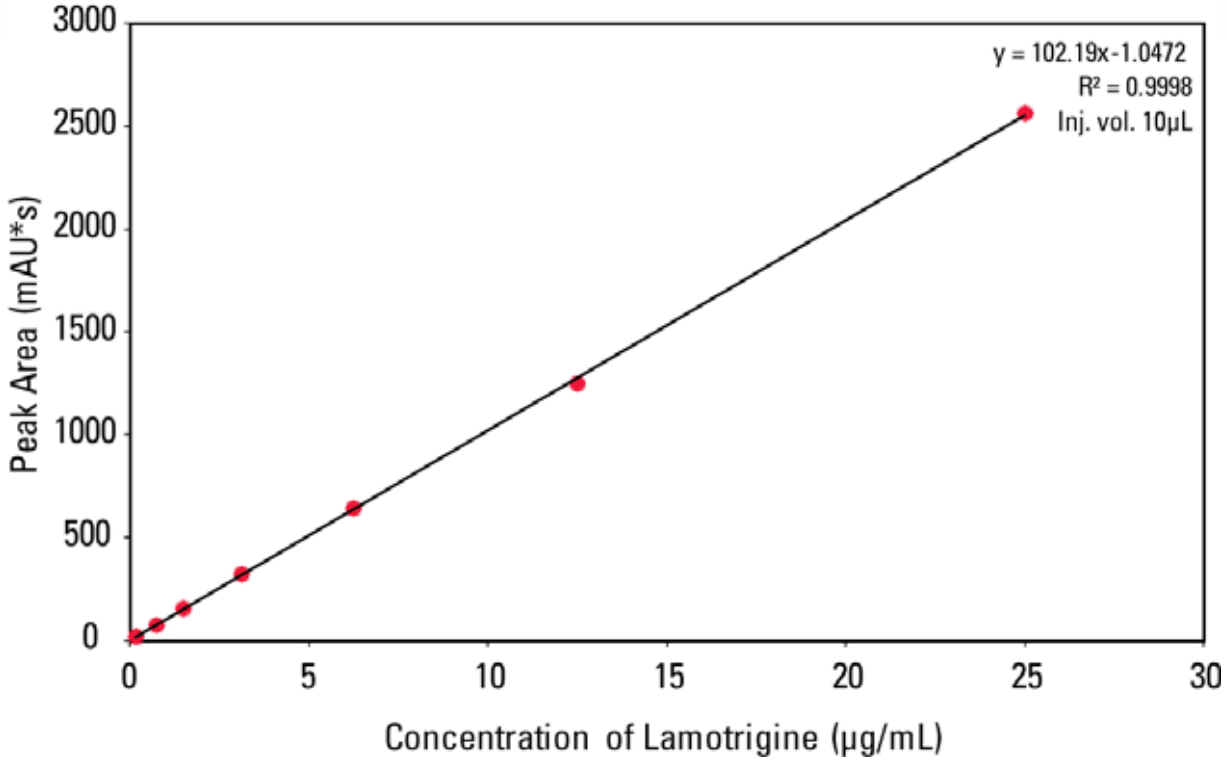
Mobile phase:

A: H_2O + 0.15% TFA
B: 100% ACN with 0.15% TFA

15 minute linear gradient from 4% A – 100% B

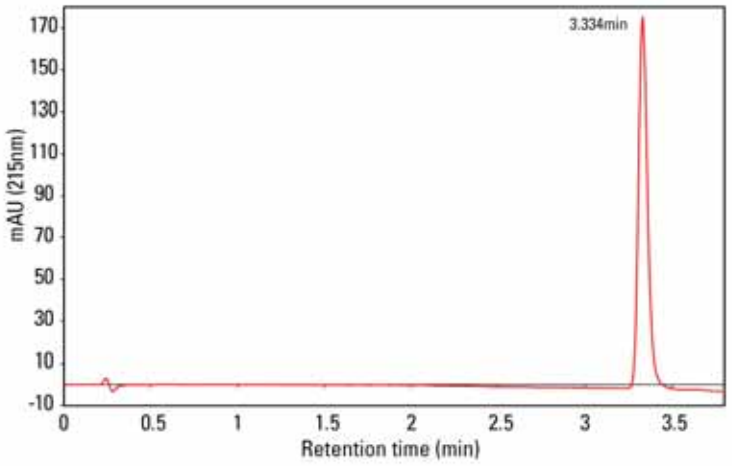
Flow rate: 0.8mL/min

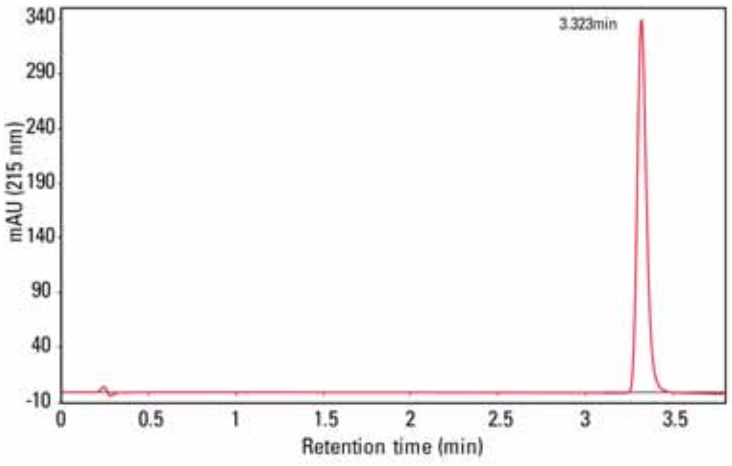
Figure 2: Calibration Curve of Lamotrigine using a TSKgel ODS-140HTP, 2.3µm, 2.1mm ID x 5cm Column



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

Figure 3: Precision Study using a TSKgel ODS-140HTP, 2.3µm, 2.1mm ID x 5cm Column



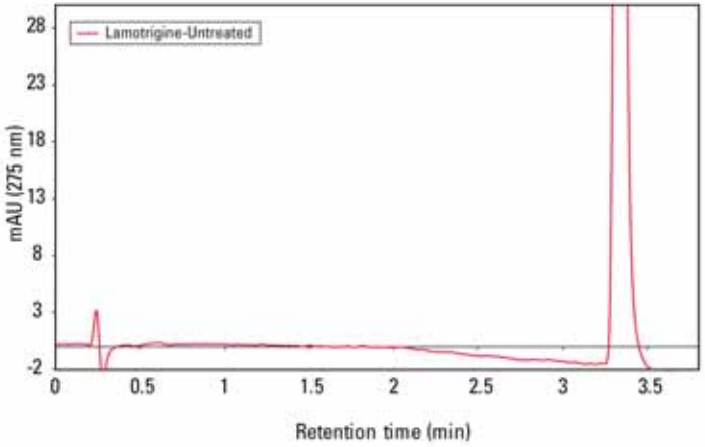


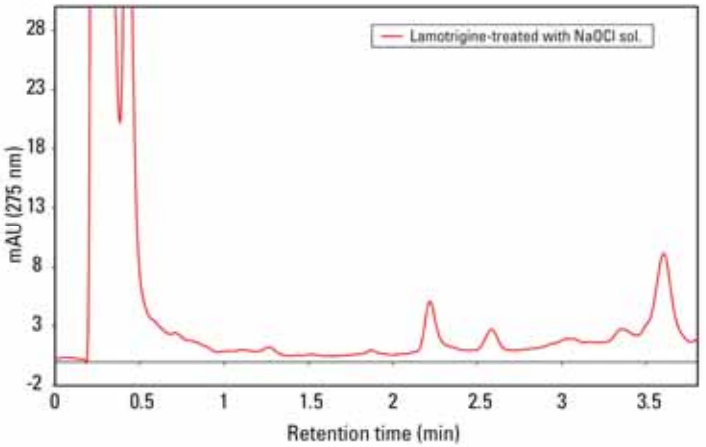
Day	Retention Time (min)
1	3.33
2	3.29
3	3.32
4	3.31
5	3.28
Average	3.31
Std. Dev.	0.02
%RSD	0.62

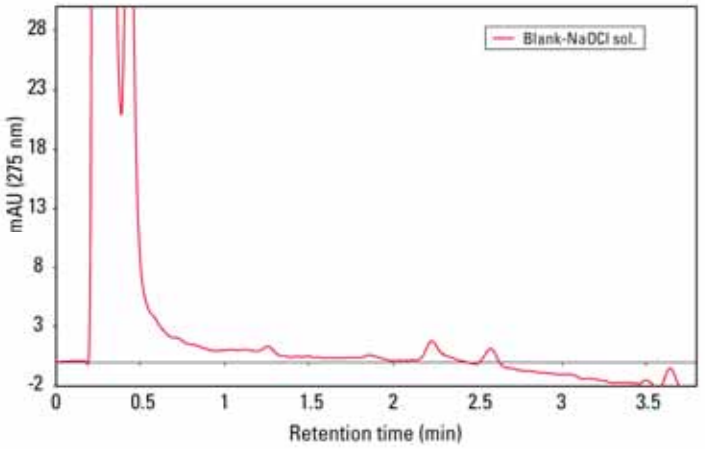
The analysis showed a considerable precision in retention time when analyzed at different times of the day, as shown in the chromatograms above.

Analysis of the sample on 5 different days over a span of 2 months showed a very consistent elution profile. %RSD of the retention times obtained over this period are shown in the table.

Figure 4: Oxidation of Lamotrigine by NaOCl Solution using a TSKgel ODS-140HTP, 2.3µm, 2.1mm ID x 5cm Column

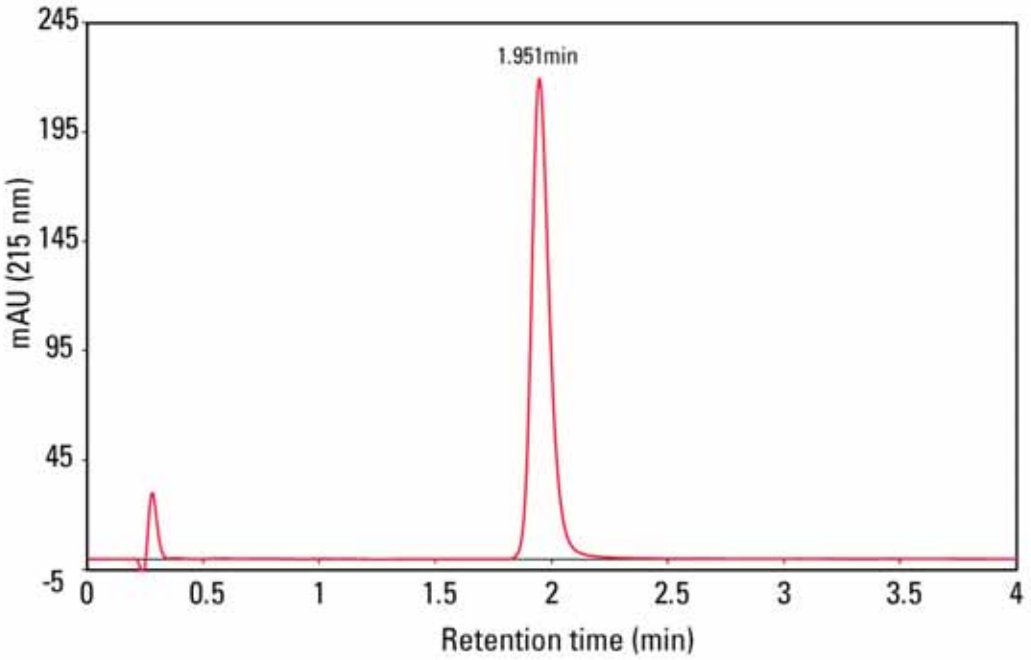






- **Lamotrigine is known to form two different *N*-chloro products by NaOCl solution (6%) identified by Xray crystallography. Ref: DMD 35:1050–1056, 2007**
- **Lamotrigine (25µg/mL; 750µL) in mobile phase A treated with 750µL of 6% NaOCl solution for 1min – Final concentration of Lamotrigine is 12.5µg/mL.**
- **Upon NaOCl treatment the lamotrigine peak disappeared.**
- **Further study is in progress to isolate and identify the degradation products.**

Figure 5: Isocratic Elution of Lamotrigine using a TSKgel ODS-140HTP, 2.3µm, 2.1mm ID x 5cm Column



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

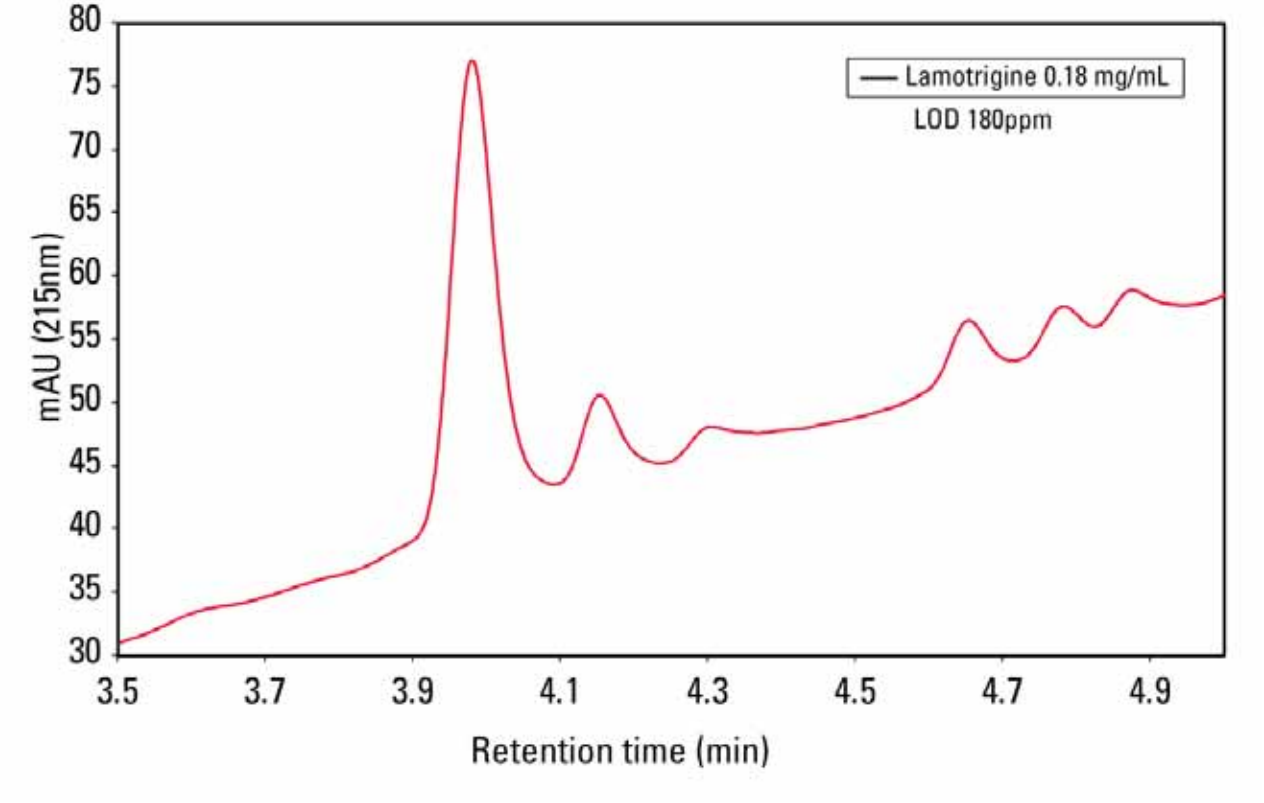
Isocratic conditions:

90% A: H_2O + 0.15% TFA
10% B: 100% ACN with 0.15% TFA

Flow rate: 0.8mL/min

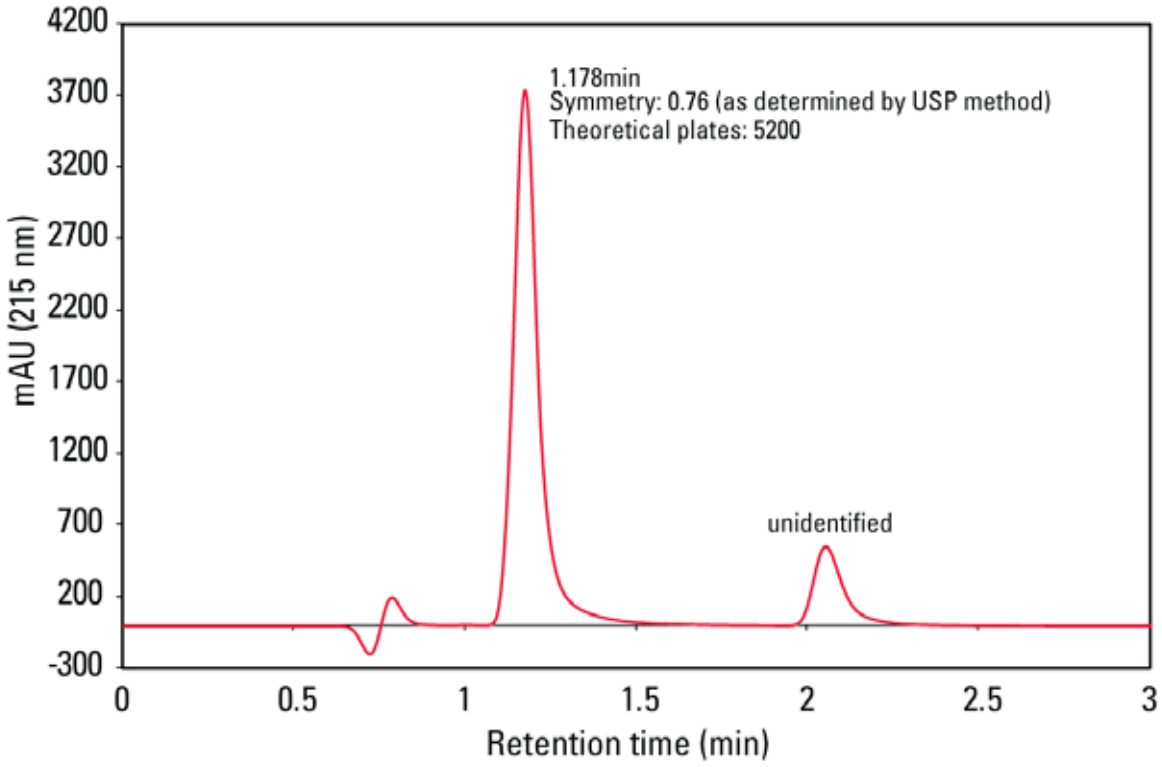
Lamotrigine could be separated with low retention time (< 2 minutes), good symmetry and high theoretical plate values.

Figure 6: Determination of Limit of Detection (LOD) and Limit of Quantitation (LOQ) of Lamotrigine using a TSKgel ODS-140HTP, 2.3µm, 2.1mm ID x 5cm Column



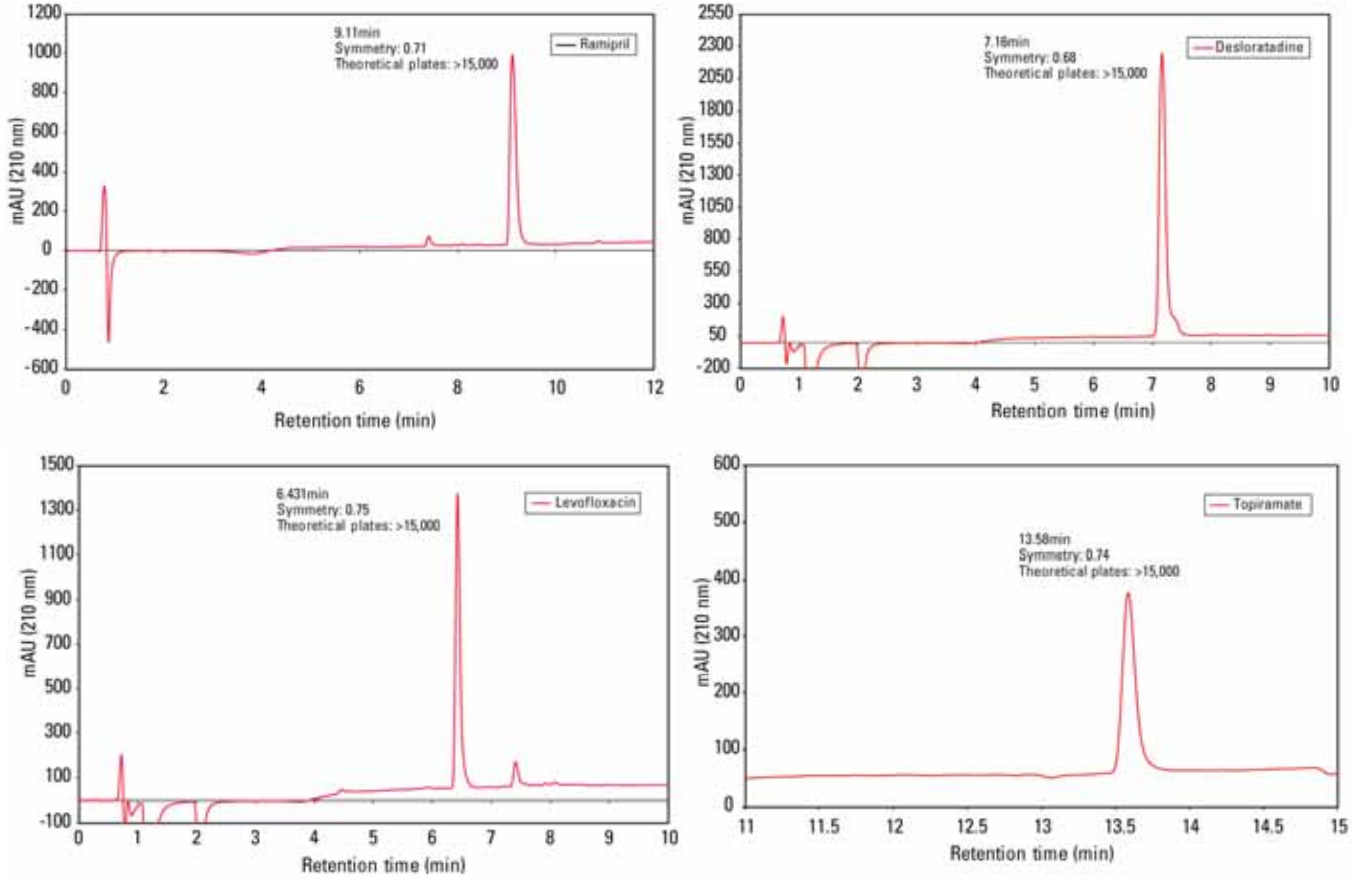
Limit of detection of lamotrigine was found to be 180ppm and LOQ 1800ppm.

Figure 7: Analysis of Losartan Potassium using a TSKgel ODS-140HTP, 2.3µm, 2.1mm ID x 5cm Column



Losartan Potassium could be separated within 2 minutes with high symmetry.

Figure 8: Analysis of Ramipril, Desloratadine, Levofloxacine, and Topiramate using a TSKgel ODS- 140HTP, 2.3μm, 2.1mm ID x 10cm Column



Mobile phase:

A: H_2O + 0.15% TFA
B: 100% ACN with 0.15% TFA

15 minute linear gradient from 4% A – 100% B

Flow rate:

0.4mL/min for Ramipril, Desloratadine, Levofloxacin

0.3mL/min for Topiramate

Preliminary studies show that a TSKgel ODS-140HTP, 10cm column could be used to separate these drugs. Further study in improving the profile in relation to retention time and symmetry is in progress.

Conclusions

In this study a TSKgel ODS-140HTP column was very useful for

(a) the analyses of several drugs with a wide variety of hydrophobicities which will soon come off patent,

(b) efficient method development,

(c) and in reducing cost and organic waste by decreasing the run times.