



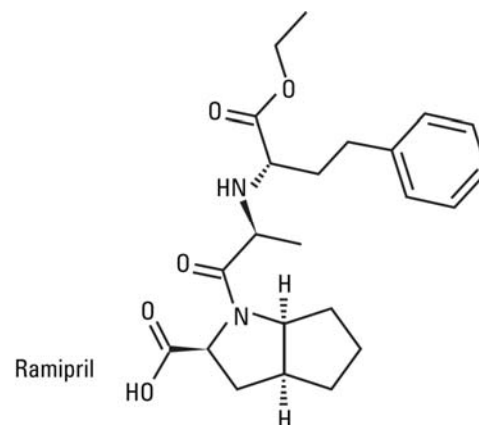
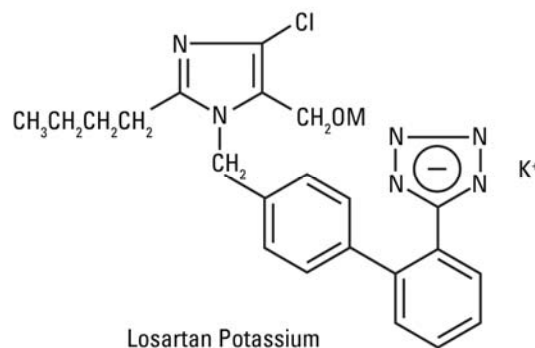
# High Throughput Separation of Hypertension Drug Standards by Reversed Phase Chromatography using TSK-GEL<sup>®</sup> ODS-140HTP, 2.3 $\mu$ m Columns

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# Introduction

- Losartan potassium, (2-butyl-4-chloro-1-{[2'-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl}-1*H*-imidazol-5-yl) methanol, is an angiotensin II receptor antagonist class compound. It is used to treat hypertension and available as prescription drug Cozaar®.
- Ramipril, (2*S*,3*aS*,6*aS*)-1-[(*S*)-2-[[(*S*)-1-(ethoxycarbonyl)-3-phenylpropyl]amino] propanoyl] octahydrocyclopenta[*b*]pyrrole-2-carboxylic acid, is an angiotensin-converting enzyme (ACE) inhibitor. It is used for the treatment of hypertension, heart failure, and nephropathia. It is a pro-drug that is transformed in the liver to its active metabolite ramiprilat.





# Objective

- To show the usefulness of the silica based TSKgel<sup>®</sup> ODS-140HTP, 2.3 $\mu$ m, 2.1mm ID x 5cm reversed phase column for high throughput analysis of the two common hypertension drug standards using a conventional HPLC system.

Table 1: Properties of TSK-GEL ODS-140HTP Columns

TSK-GEL ODS-140HTP	
Pore size (silica):	140Å
Endcapped:	yes
Particle size:	2.3 $\mu$ m
pH stability:	2.0 - 7.5
Functional group:	C18 (polymeric bonding chemistry)
% carbon:	8%



# 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 it as 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.



# Scope of Generic Drugs, Con't.

Table 2: Off-Patent Drug Schedule for Cozaar and Altace<sup>®</sup>

Generic or chemical name	Brand name	Class	Mechanism of action	Disease	Degradation products	Patent expiration
Losartan potassium LOP	Cozaar	Angiotensin II receptor (type AT <sub>1</sub> ) antagonist	Blocks the binding of antiotensin II to the AT <sub>1</sub> receptor	Hypertension	Imidazole ring breaks down by photodegradation or by UV	2010
Ramipril	Altace	2-aza-bicyclo [3.3.0]-octane-3-carboxylic acid derivative	Inhibits angiotensin-converting enzyme (ACE)	Cardiovascular, hypertension	NA	2009



# 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 lower retention times while maintaining or improving resolution from closely eluting impurities is very important for quality control analysis.
- Here we report the analysis of two hypertension drug standards (losartan potassium and ramipril) using a TSKgel ODS-140HTP, 2.3 $\mu$ m, 2.1mm ID x 5cm column.



# Material and Methods

All analyses were carried out using an Agilent 1200 HPLC system run by Chemstation (ver B.04.02) unless mentioned otherwise.

## Optimal chromatographic conditions:

- Columns:
  - TSKgel ODS-140HTP, 2.3 $\mu$ m, 2.1mm ID x 5cm
  - Hypersil GOLD<sup>®</sup> C18, 1.9 $\mu$ m, 2.1mm ID x 5cm
  - Luna<sup>®</sup> C18(2)-HST, 2.5 $\mu$ m, 2.0mm ID x 5cm
  - Acquity UPLC<sup>®</sup> BEH C18, 1.7 $\mu$ m, 2.1mm ID x 5cm
- Detection: 215nm (losartan potassium), 208nm (ramipril)
- Column temp: 50°C unless mentioned otherwise
- Flow rate: see respective chromatograms
- Injection volume: 2 $\mu$ L unless mentioned otherwise
- Mobile phases:
  - Isocratic condition: ACN (percentage as mentioned in the respective chromatograms) in H<sub>2</sub>O containing 0.15% TFA



## Material and Methods, Con't.

- High purity Sigma-Aldrich brand drug standards (losartan potassium and ramipril) were used for the preparation of stock standards.
- All the standards and samples were filtered through a 0.45 $\mu$ m membrane before injecting into the column.
- Working standards were prepared by dilution of the stock standard in water or methanol as necessary.



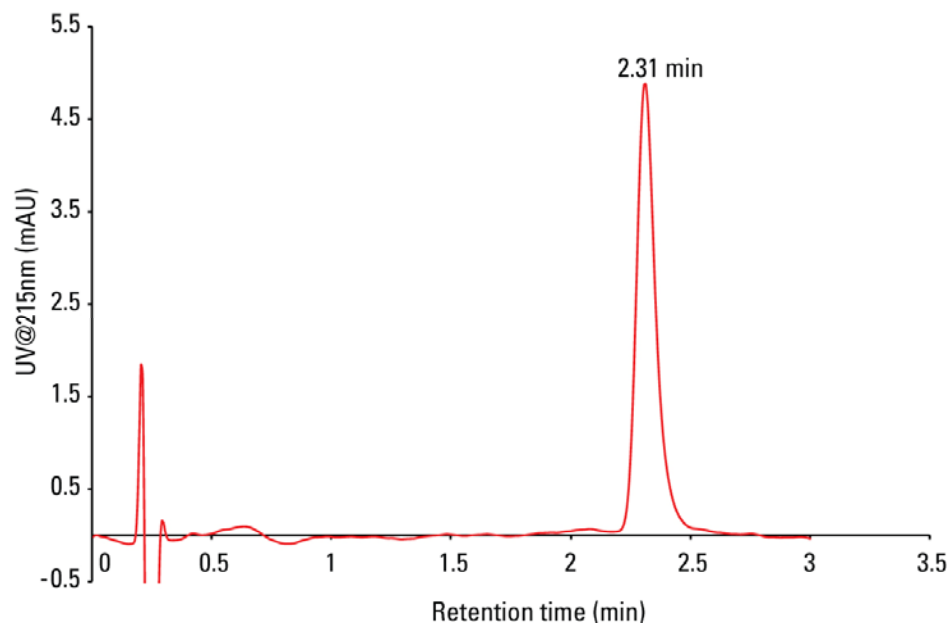


# Material and Methods: LOD and LOQ

- 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.



# Figure 1: Isocratic elution of losartan potassium standard using a TSKgel ODS-140HTP column

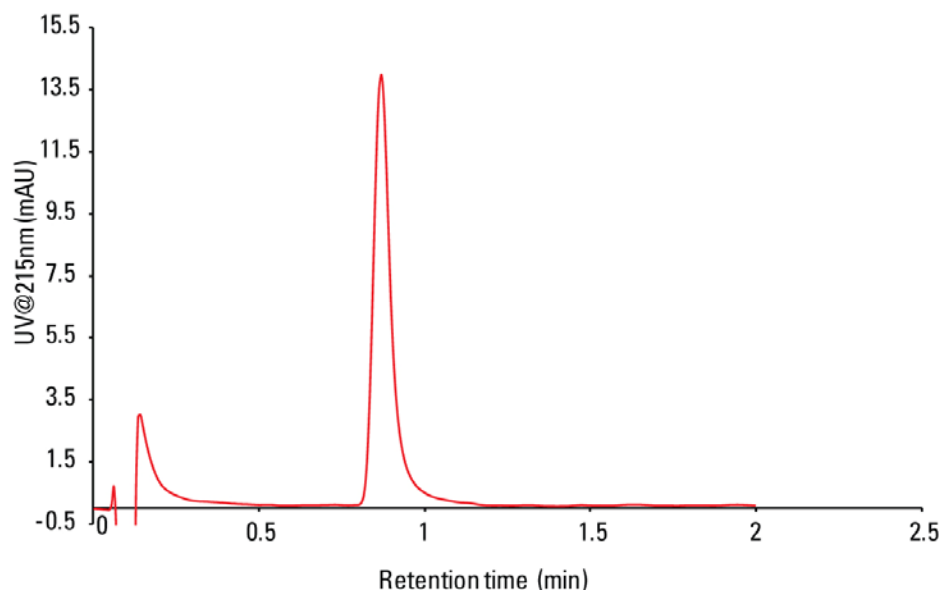


Column: TSKgel ODS-140HTP, 5cm  
Mobile phase: 20% ACN in H<sub>2</sub>O with 0.15% TFA  
Flow rate: 0.6mL/min  
Detection: UV@215nm  
Temperature: 50°C  
Injection vol.: 2µL  
Sample: losartan potassium, 2µg/mL

%RSD	RT	k	Area	AF	Plates
(5 consecutive injections)	0.34	0.37	2.1	1.5	1.8



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

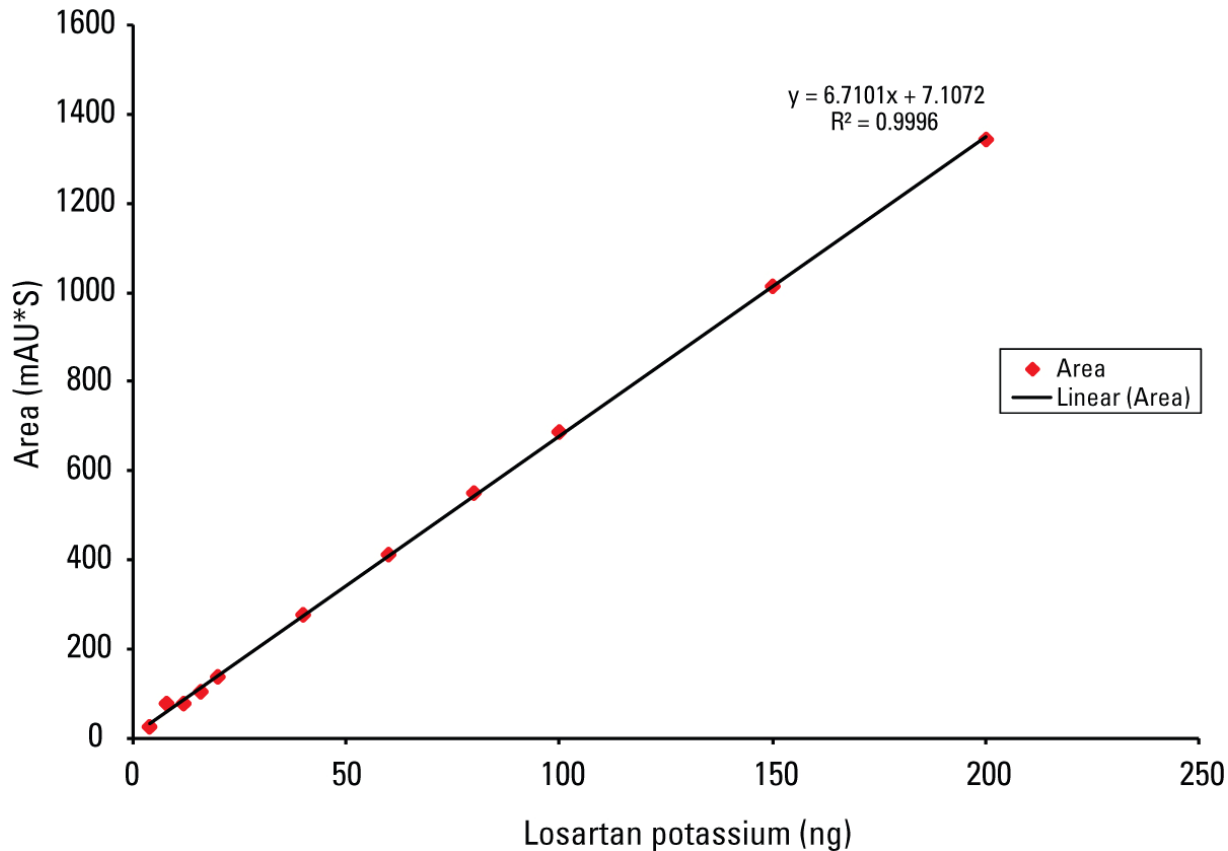


Column: TSKgel ODS-140HTP, 5cm  
Mobile phase: 20% ACN in H<sub>2</sub>O with 0.15% TFA  
Flow rate: 1.5mL/min  
Detection: UV@215nm  
Temperature: 50°C  
Injection vol.: 10µL  
Sample: losartan potassium, 2µg/mL

**Losartan potassium could be eluted within 1 minute with some compromise with tailing.**



# Figure 3: Linearity of losartan potassium



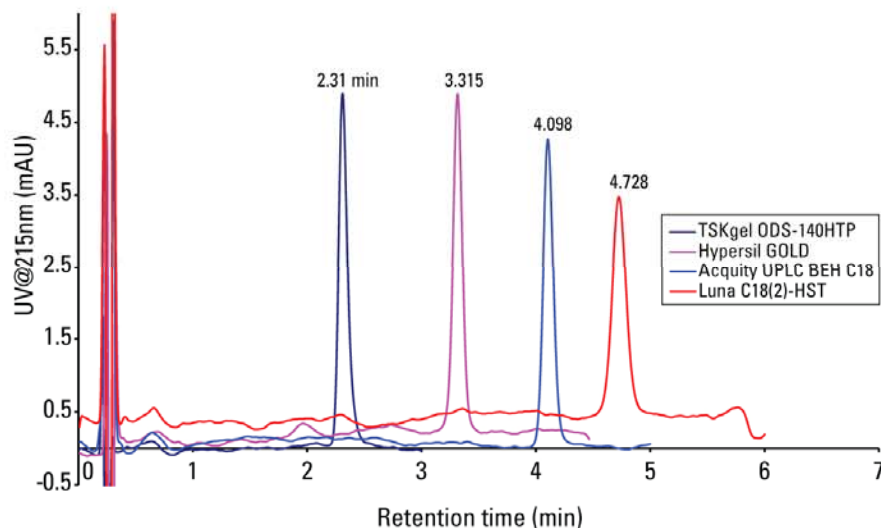
**Losartan potassium was found to be linear in the concentration range of 4-200ng.**

**LOD is 0.1µg/mL = 0.1mg/L = 0.1ppm = 100ppb.**

**LOQ (calculated) is 1ppm.**



# Figure 4: Competitive column study for the separation of losartan potassium



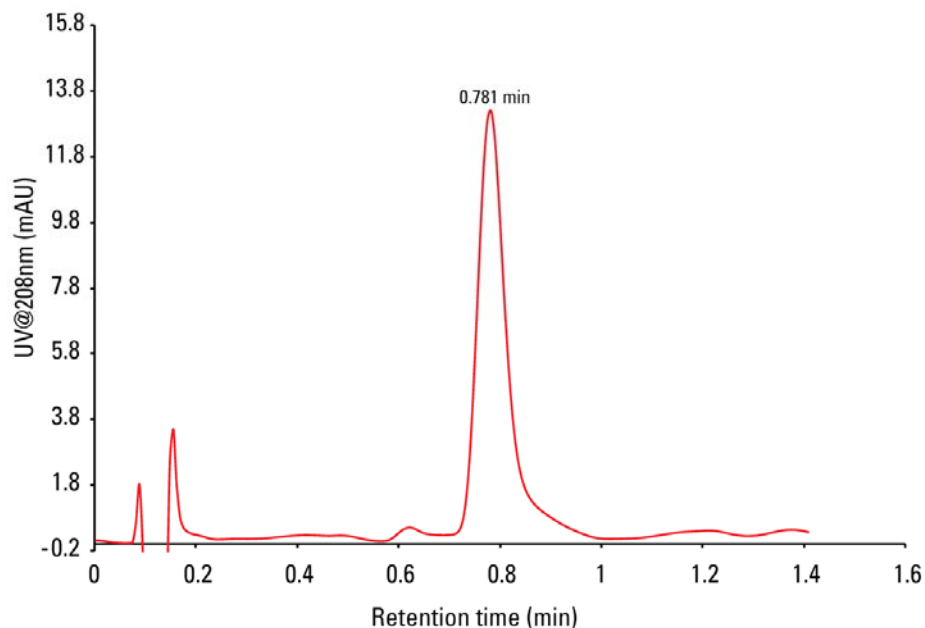
Columns: TSKgel ODS-140HTP, 5cm  
Hypersil Gold C18, 2.1mm ID x 5cm  
Luna C18(2)-HST, 2.0mm ID x 5cm  
Acquity UPLC BEH C18, 2.1mm ID x 5cm

Mobile phase: 20% ACN in H<sub>2</sub>O with 0.15% TFA  
Flow rate: 0.6mL/min  
Detection: UV@215nm  
Temperature: 50°C  
Injection vol.: 2µL  
Sample: losartan potassium, 2µg/mL

**The analysis time was the shortest with the TSKgel ODS-140HTP column.**



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

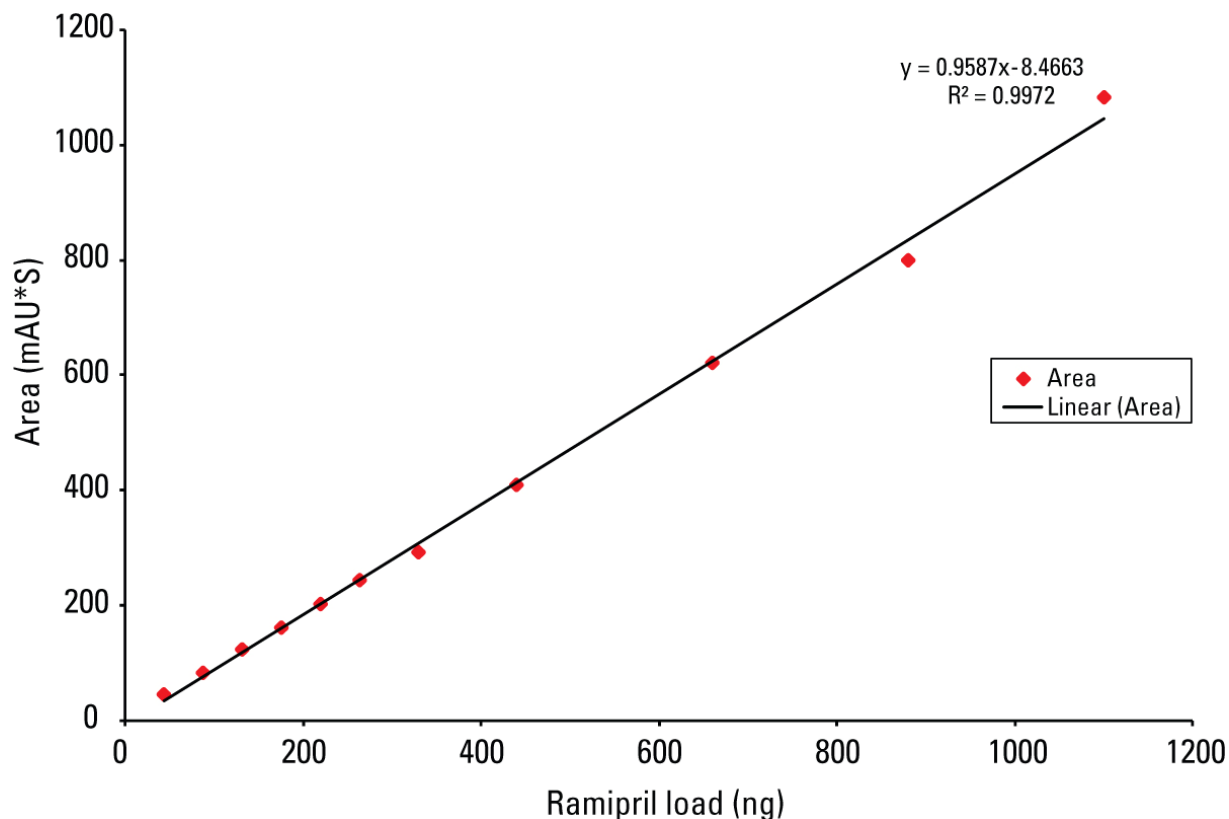


Column: TSKgel ODS-140HTP, 5cm  
Mobile phase: 25% ACN in H<sub>2</sub>O with 0.15% TFA  
Flow rate: 1.2mL/min  
Detection: UV@208nm  
Temperature: 50°C  
Injection vol.: 2µL  
Sample: ramipril, 22µg/mL

**Ramipril retention time less than 1 minute.**



# Figure 6: Linearity of ramipril



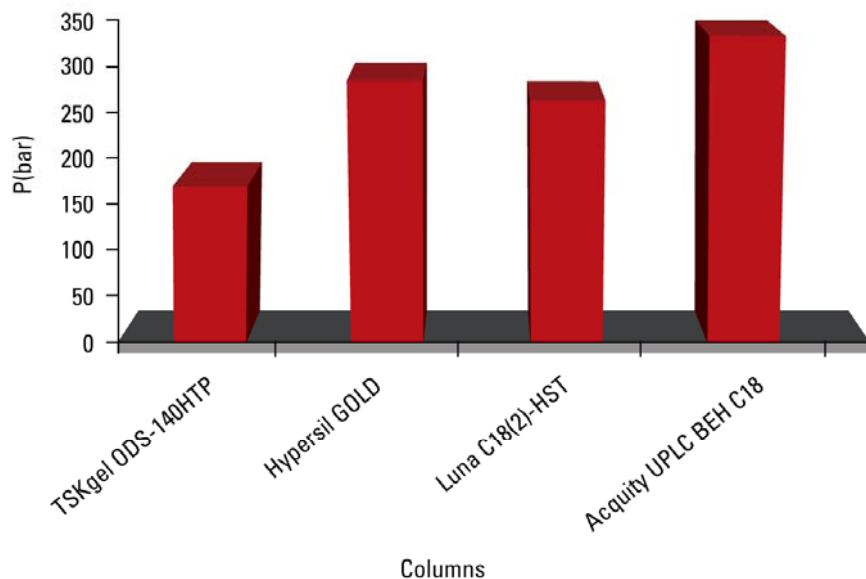
**Ramipril was found to be linear in the concentration range of 44-1100ng.**

**LOD is  $0.0011\mu\text{g/mL} = 0.0011\text{mg/L} = 0.0011\text{ppm} = 1.1\text{ppb}$ .**

**LOQ (calculated) is 11ppb.**



# Figure 7: Comparative chart of pressure (bar) observed under identical chromatographic conditions



Columns: TSKgel ODS-140HTP, 2.1mm ID x 5cm  
Hypersil Gold C18, 2.1mm ID x 5cm  
Luna C18(2)-HST, 2.0mm ID x 5cm  
Acquity UPLC BEH C18, 2.1mm ID x 5cm

Mobile phase: 25% ACN in H<sub>2</sub>O with 0.15% TFA  
Detection: UV@208nm  
Temperature: 50°C  
Injection vol.: 2µL  
Sample: ramipril, 22µg/mL

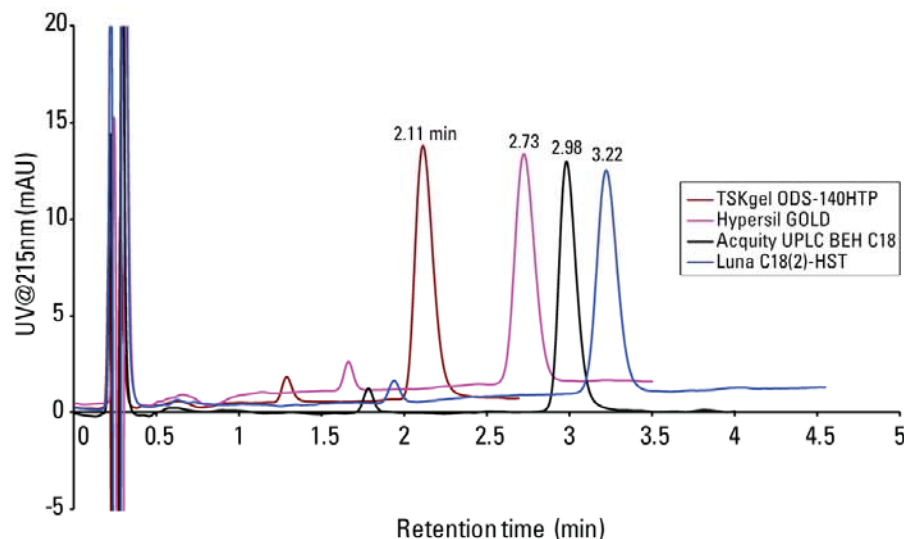
	u(cm/s)	P(bar)
TSKgel ODS-140HTP	0.416	186
Hypersil GOLD	0.377	302
Luna C18(2)-HST	0.416	282
Acquity UPLC BEH C18	0.377	350

**The TSKgel ODS-140HTP column showed the lowest back pressure.**





# Figure 8: Elution profile of losartan potassium using competitive columns

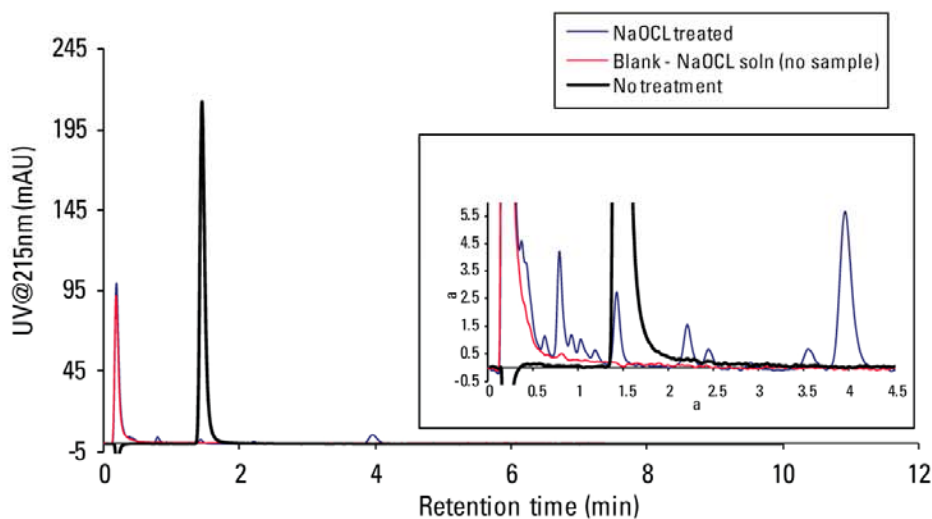


Columns: TSKgel ODS-140HTP, 5cm  
Hypersil Gold C18, 2.1mm ID x 5cm  
Luna C18(2)-HST, 2.0mm ID x 5cm  
Acquity UPLC BEH C18, 2.1mm ID x 5cm

Mobile phase: 25% ACN in H<sub>2</sub>O with 0.15% TFA  
Flow rate: 0.6mL/min  
Detection: UV@208nm  
Temperature: 50°C  
Injection vol.: 2µL  
Sample: ramipril, 22µg/mL

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

# Figure 9: Separation of the forced degradation products of losartan potassium using a TSKgel ODS-140HTP column

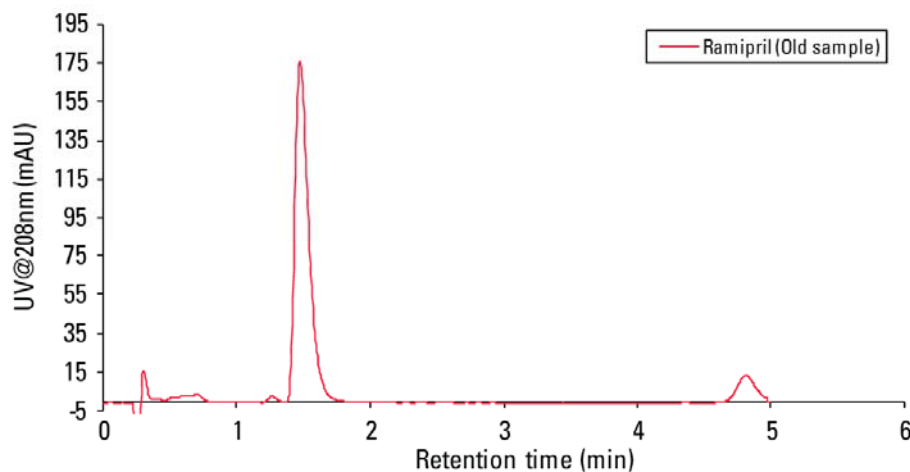


Equipment: Agilent 1100 series HPLC  
 Column: TSKgel ODS-140HTP, 5cm  
 Mobile phase: 20% ACN in H<sub>2</sub>O with 0.15% TFA  
 Flow rate: 1.2mL/min  
 Detection: UV@215nm  
 Temperature: 40°C  
 Injection vol.: 10µL  
 Sample: losartan potassium, 14µg/mL

**The new peaks obtained from forced degradation did not interfere with the drug standard peak.**



# Figure 10: Separation of the degradation products of ramipril upon storage using a TSKgel ODS-140HTP column



Equipment: Agilent 1100 series HPLC  
Column: TSKgel ODS-140HTP, 5cm  
Mobile phase: 25% ACN in H<sub>2</sub>O with 0.15% TFA  
Flow rate: 0.8mL/min  
Detection: UV@208nm  
Temperature: 50°C  
Injection vol.: 10mL  
Sample: ramipril, 11.8µg/mL

**Long term storage of ramipril at room temperature produced a degradation product that eluted at 4.9 minutes.**



# Conclusions

- The TSKgel ODS-140HTP column was successfully used for the analysis of two common hypertension drugs.
- Both of the drugs eluted with short retention times (< 1 minute).
- This data also shows that generic manufacturers can use this column under these conditions for the analysis of these two 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 an active pharmaceutical ingredient (API) from the product
- TSK-GEL ODS-140HTP columns yielded the shortest retention times in comparison to other competitive columns tested under identical chromatographic conditions.