

Improved HPLC-UV Separation of Common Beta-2 Adrenergic Receptor Agonists

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Abstract

Beta-2 Adrenergic receptor agonists (BAs) are commonly prescribed medications used to treat Chronic Respiratory Diseases (CRDs). BAs have been amongst the top ten prescribed drugs for several years now. Here, we developed a method that reproducibly separated several types of BAs and BA impurities in under 14 minutes. We investigated the effect of pH on analysis. Further, we demonstrated the high pH stability and batch consistency of MaxPeak™ High Performance Surfaces (HPS) Technology.

Benefits

- Single method that offers baseline separation of a diverse panel of BAs and their impurities
 - Basic conditions used in this method reduce tailing by up to 65%
 - Method demonstrates the dependability and reproducibility of MaxPeak™ HPS Technology Columns and Systems in high pH conditions
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Introduction

Noncommunicable diseases account for 41 million deaths globally. Of that 41 million, 10% are because of chronic respiratory diseases (CRDs).¹ Asthma and chronic obstructive pulmonary disease (COPD) are recognized as CRDs.² While CRDs are not curable, maintenance medications such as Beta-2 Adrenergic receptor agonists (BAs) are available to help improve symptoms.³ Further, there are three classes of BAs: short-acting B2 agonists (SABAs), Long-acting B2 agonists (LABAs), and ultra-long-acting B2 agonists (ULABAs). Given the prevalence of CRDs it is no surprise that BAs, such as albuterol, have been on the top 10 list of most filled prescription medication for several years.⁴

Some current LC methods for BAs mention noticeable tailing when analyzing these compounds under acidic reversed phase conditions.^{5,6} However, there is evidence that high pH conditions may improve the overall chromatography for BAs.⁷ Further, there is a lack of full panel methods including a variety of BAs. Therefore, we developed a high pH reversed phase method for the separation of a BAs panel. The analysis of 6 BAs and some of their impurities is complete in under 14 minutes with reproducible, linear, and sensitive results. In addition, different column sorbent batches were compared using this method demonstrating the reliable and stable performance of Premier Columns and Systems with MaxPeak™ High Performance Surfaces (HPS) Technology, especially under high pH conditions.

Experimental

Method of Separation Sample Description

Various BAs were purchased to establish a full panel containing SABAs, LABAs, and ULABAs. Levalbuterol was sourced from Sigma Aldrich (Allentown, PA). Pirbuterol was purchased from Toronto Research Chemicals (Toronto, ON). Formoterol, Indacaterol, Olodaterol, and Vilanterol were obtained from Cayman Chemical (Ann Arbor, MI). Terbutaline was sourced from Santa Cruz Biotechnology (Dallas, TX).

Each standard was weighed into a 20 mL scintillation vial and diluted with Deionized Water: Acetonitrile (95:5). Salt factors and purities were taken into consideration. Stock concentrations varied from 0.5 mg/mL to 1 mg/mL, depending on standard solubility. Stocks were stored at 2 °C–8 °C and allowed to equilibrate to ambient temperature prior to analysis.

Stocks were diluted and combined to make a BAs Mix sample. Each Analyte was at a 100 µg/mL concentration.

Linearty and Impurities Sample Description

Linearity and Impurities standards were purchased from European Directorate for the Quality of Medicines & HealthCare (EDQM) (Strasbourg, FR). The standards purchased were the following: Formoterol, Formoterol Impurity I, Salbutamol, Salbutamol Impurity B, Salbutamol Impurity D, Salbutamol Impurity F, Salbutamol Impurity G, Terbutaline, and Terbutaline Impurity C.

Each standard was weighed into a 20 mL scintillation vial and diluted with Deionized Water: Acetonitrile (95:5). Salt factors and purities were taken into consideration. Stock concentrations varied from 0.1 mg/mL to 1 mg/mL, depending on standard solubility. Stocks were stored at 2 °C–8 °C and allowed to equilibrate to ambient temperature prior to analysis. Stocks were diluted and combined to make various curve points for each main analyte. For impurities analysis, analytes and their respective impurities were combined. Each impurity was at a 10 µg/mL concentration, while analytes were at a 100 µg/mL concentration.

LC Conditions

LC system:	Arc™ Premier LC System
Detection:	Waters™ 2998 Photodiode Array Detector, 276 nm
Column(s):	XBridge™ Premier BEH™ C ₁₈ , 130 Å, 4.6 x 100 mm, 2.5 µm
Column temperature:	40 °C
Sample temperature:	Ambient
Injection volume:	10 µL
Flow rate:	1.0 mL/min
Mobile phase A:	Deionized Water with 10 mM Ammonium Formate, pH 10, adjusted using Ammonium Hydroxide

Mobile phase B:

Acetonitrile

Gradient Table

Time (min)	Flow (mL/min)	%A	%B	Curve
Initial	1.0	95.0	5.0	Initial
14.00	1.0	35.0	65.0	6
14.10	1.0	5.0	95.0	6
16.10	1.0	5.0	95.0	6
16.20	1.0	95.0	5.0	6
21.00	1.0	95.0	5.0	6

Data Management

Chromatography software:

Empower™ 3.7.0

Results and Discussion

Method of Separation Results

This method was reproducible in the retention and separation of BAs. Over the course of 12 injections of the BAs Mix sample, the %RSD for area, retention time, and tailing for each analyte was $\leq 1\%$ (Tables 1, 2, and 3). Below, an overlay chromatogram of the 12 injections provides a clear picture of the method's performance (Figure 1).

Area	Pirbuterol	Terbutaline	Levalbuterol	Formoterol	Olodaterol	Vilanterol
Mean	95294.445	273621.326	148624.581	113654.921	202025.069	463010.139
Std. dev.	451.838	1024.773	582.086	437.674	759.486	2185.978
% RSD	0.474	0.375	0.392	0.385	0.376	0.472

Table 1. Table containing data for the area across 12 injections for the BAs Mix sample.

Retention time	Pirbuterol	Terbutaline	Levalbuterol	Formoterol	Olodaterol	Vilanterol
Mean	2.368	3.444	3.933	6.675	8.123	11.239
Std. dev.	0.001	0.001	0.001	0.001	0.001	0.007
% RSD	0.044	0.026	0.034	0.011	0.017	0.064

Table 2. Table containing data for the retention time across 12 injections for the BAs Mix sample.

USP tailing	Pirbuterol	Terbutaline	Levalbuterol	Formoterol	Olodaterol	Vilanterol
Mean	1.312	1.293	1.357	1.146	1.248	1.537
Std. dev.	0.016	0.001	0.001	0.002	0.001	0.009
% RSD	1.190	0.076	0.085	0.149	0.045	0.618

Table 3. Table containing data for the tailing across 12 injections for the BAs Mix sample.

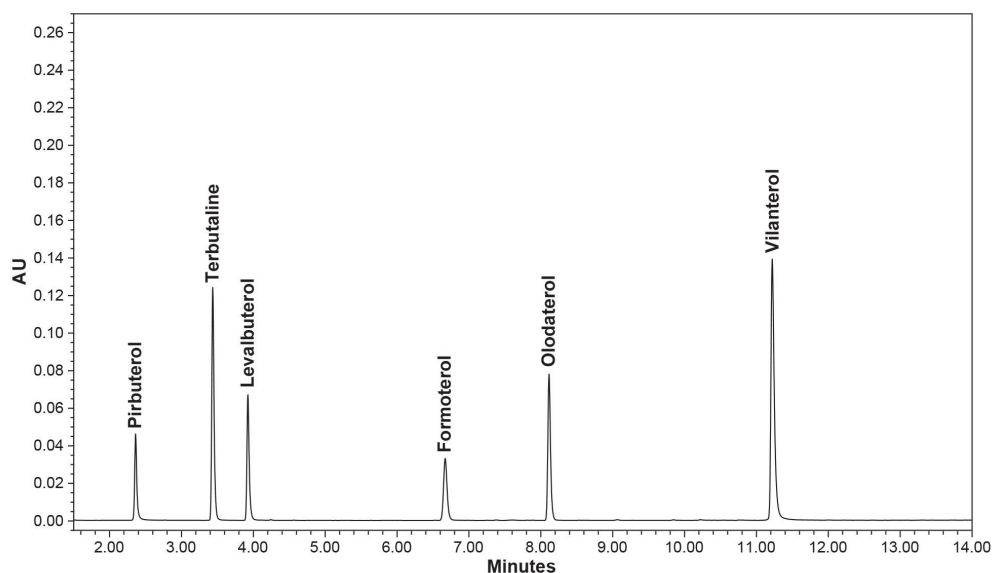


Figure 1. Overlay chromatogram of 12 injections for the BAs Mix sample.

Effects of pH

It is well known that pH has a notable effect on most reversed phase applications.^{7,8} To investigate its effect on BAs we analyzed the same BAs Mix sample using a basic and acidic mobile phase A. The basic mobile phase used was deionized water with 10 mM ammonium formate, pH 10, adjusted using ammonium hydroxide. The acid mobile phase was deionized water with 10 mM ammonium formate, pH 3, adjusted using formic acid.

Under basic conditions, tailing improves amongst the analytes, and baseline resolution is achieved for levalbuterol and terbutaline, the critical pair in the acidic conditions. There is up to a 65% decrease in tailing when switching to the basic method. Below is a data table and chromatogram representative of the performance of both methods (Figure 2a and 2b, Tables 4 and 5).

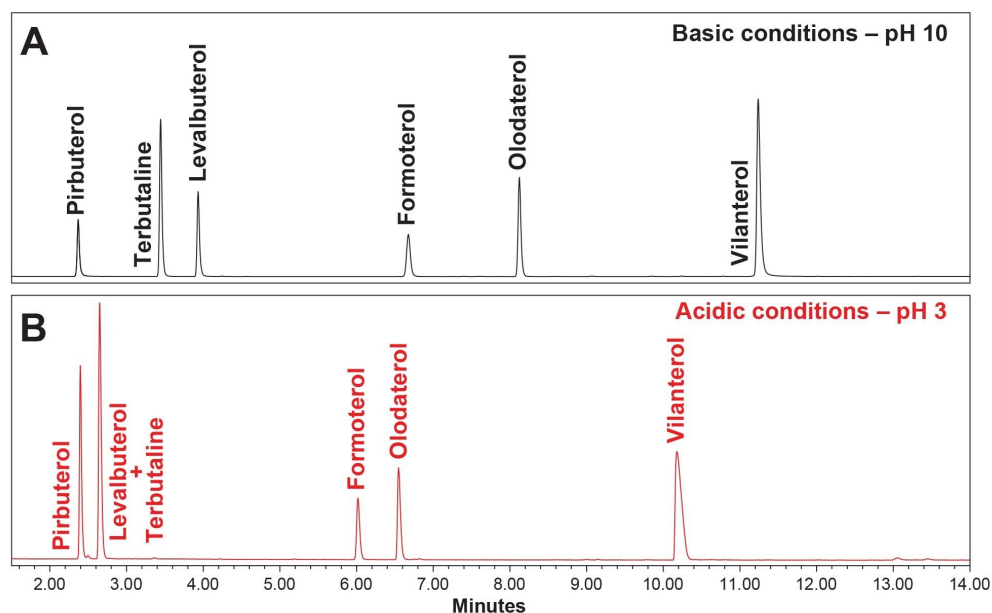


Figure 2a. A representative chromatogram detailing the performance of the basic method conditions for the BAs Mix sample.

Figure 2b. A representative chromatogram detailing the performance of the acidic method conditions for the BAs Mix sample.

USP tailing	Pirbuterol	Terbutaline	Levalbuterol	Formoterol	Olodaterol	Vilanterol
Base	1.312	1.293	1.357	1.146	1.248	1.537
Acid	1.389	Coelution		1.308	1.500	2.331

Table 4. Data detailing the improvements in the tailing between the two methods.

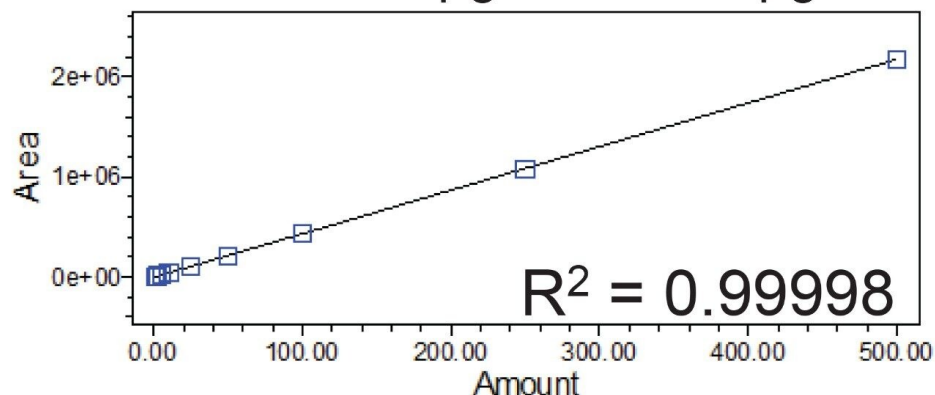
USP resolution	Pirbuterol	Terbutaline	Levalbuterol	Formoterol	Olodaterol	Vilanterol
Base		18.945	8.319	36.289	17.897	39.807
Acid		Coelution		48.687	7.131	30.855

Table 5. Data detailing the improvements in the resolution between the two methods.

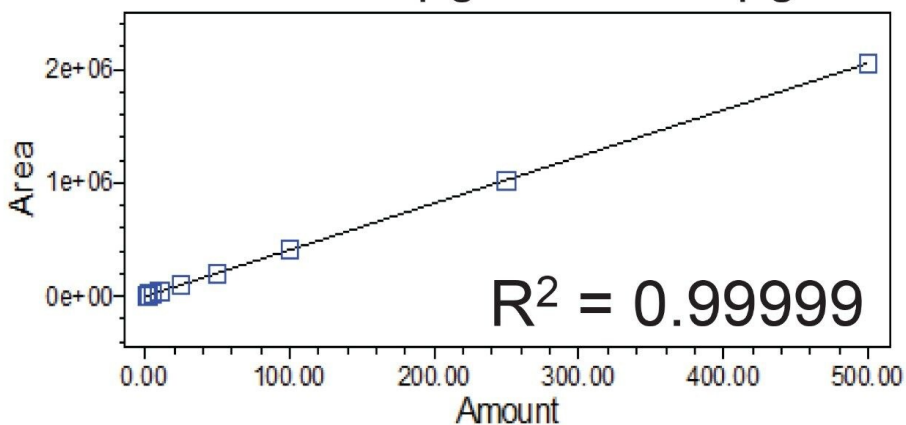
Linearty Results

Each of the EDQM analytes were investigated for their linearity. The linearity for each analyte was calculated using a linear fit through the calibration features in Empower 3. Below, the quantitative capability of this method is clear, suggesting its use in quality control testing (Figures 3a through 3c).

A. Formoterol 1 $\mu\text{g/mL}$ to 500 $\mu\text{g/mL}$



B. Salbutamol 1 $\mu\text{g/mL}$ to 500 $\mu\text{g/mL}$



C. Terbutaline 5 $\mu\text{g/mL}$ to 500 $\mu\text{g/mL}$

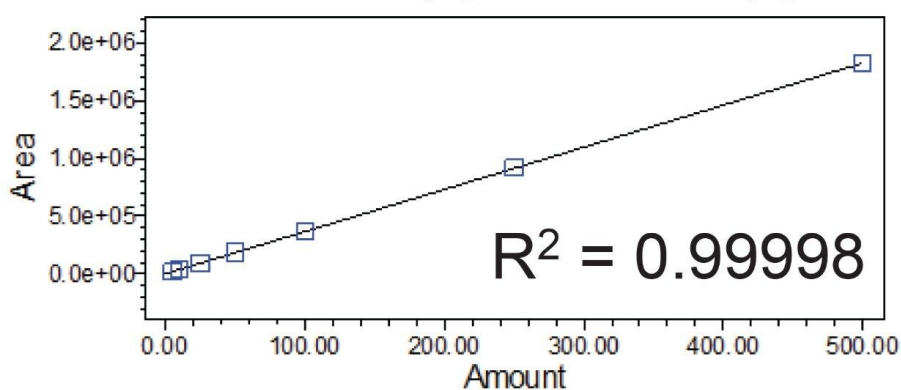


Figure 3a. The calibration curve for Formoterol spanning from the concentrations of 1 $\mu\text{g/mL}$ to 500 $\mu\text{g/mL}$.

Figure 3b. The calibration curve for Salbutamol spanning from the concentrations of 1 $\mu\text{g/mL}$ to 500 $\mu\text{g/mL}$.

Figure 3c. The calibration curve for Terbutaline spanning from the concentrations of 5 $\mu\text{g/mL}$ to 500 $\mu\text{g/mL}$.

Impurities Analysis Results

This method was also investigated for its impurities analysis suitability. The EDQM analytes and their respective impurities were combined so that impurities were at 10% of the main analyte concentration. Below, this method demonstrated its ability to separate analytes from their impurities with baseline resolution and good sensitivity (Figures 4a and 4b).

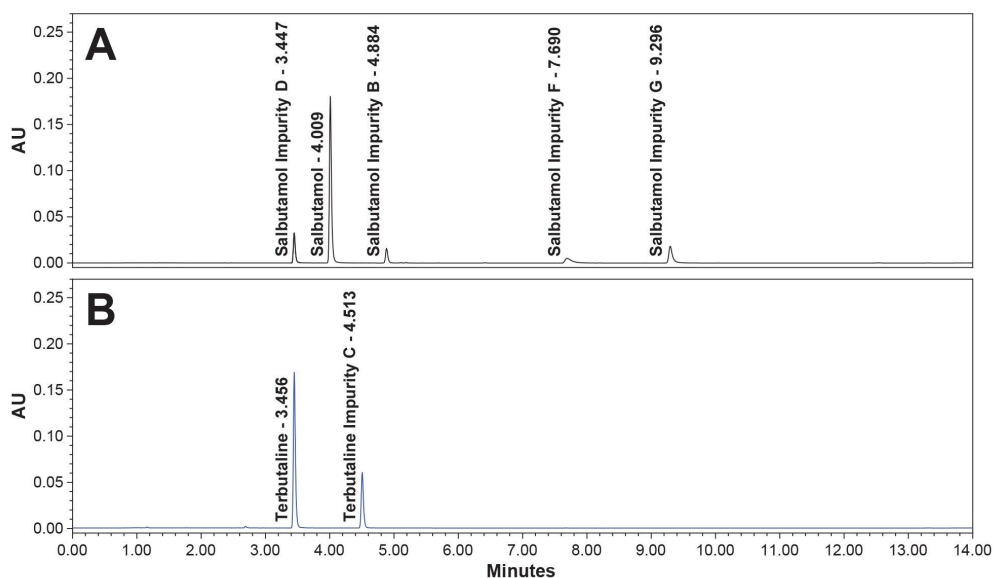


Figure 4a. Representative chromatogram for the separation of Salbutamol (100 $\mu\text{g/mL}$) and its related impurities (10 $\mu\text{g/mL}$).

Figure 4b. Representative chromatogram for the separation of Terbutaline (100 $\mu\text{g/mL}$) and its related impurity (10 $\mu\text{g/mL}$).

Column Batch Reproducibility

To prove the consistency of column batches for the XBridge™ Premier BEH C₁₈ Column, three different manufacturing lots were investigated. Each column was examined across 12 injections of the BAs Mix sample. The performance across batches is undoubtedly equivalent (Figures 5a through 5c, Tables 6 and 7). The data provides a clear image of the reliability of XBridge™ Premier BEH C₁₈ Columns.

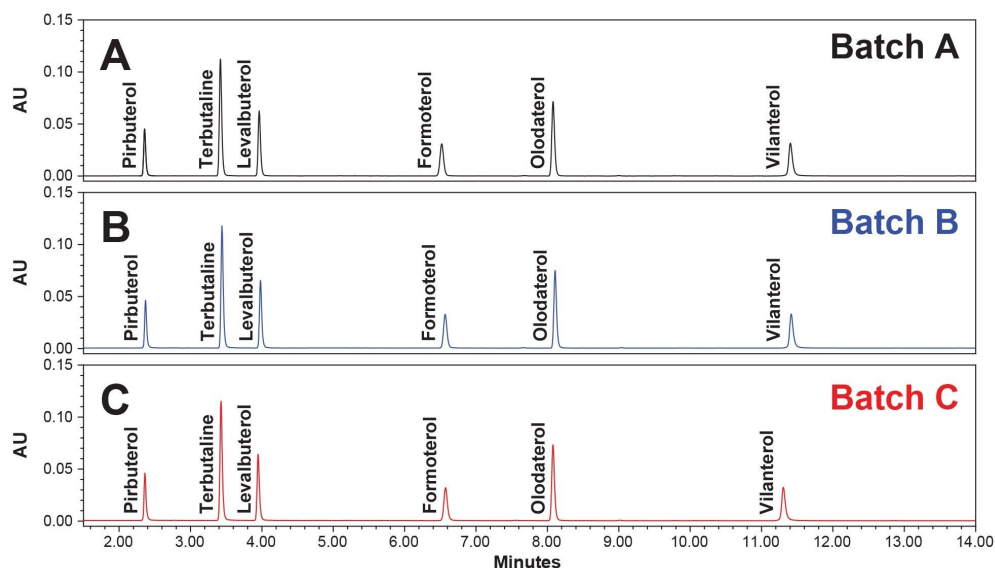


Figure 5a. Representative chromatogram for the separation of the BAs mix for column batch A.

Figure 5b. Representative chromatogram for the separation of the BAs mix for column batch B.

Figure 5c. Representative chromatogram for the separation of the BAs mix for column batch C.

Avg. retention time	Pirbuterol	Terbutaline	Levalbuterol	Formoterol	Olodaterol	Vilanterol
Batch A	2.359	3.419	3.962	6.522	8.080	11.397
Batch B	2.377	3.446	3.986	6.574	8.114	11.416
Batch C	2.372	3.435	3.954	6.583	8.085	11.299

Table 6. Table containing average retention time data for the column batch reproducibility across 12 injections for the BAs Mix sample.

Avg. tailing	Pirbuterol	Terbutaline	Levalbuterol	Formoterol	Olodaterol	Vilanterol
Batch A	1.249	1.237	1.243	1.088	1.193	1.236
Batch B	1.277	1.258	1.271	1.119	1.202	1.274
Batch C	1.315	1.255	1.277	1.144	1.205	1.281

Table 7. Table containing average tailing data for the column batch reproducibility across 12 injections for the BAs Mix sample.

High pH System and Column Reproducibility

To demonstrate the resilience of Premier Columns and Systems with MaxPeak™ HPS Technology under high pH conditions, a fresh XBridge™ Premier BEH C₁₈ Column was installed on the Arc™ Premier LC System. This column underwent 300 injections of the BAs Mix sample equating to over 100 hours of run time. The average retention time and standard deviation over the course of 300 injections is detailed below (Table 8).

Retention time	Pirbuterol	Terbutaline	Levalbuterol	Formoterol	Olodaterol	Vilanterol
Mean	2.355	3.451	3.955	6.652	8.134	11.387
Std. dev.	0.006	0.003	0.015	0.024	0.005	0.078
% RSD	0.243	0.098	0.388	0.358	0.056	0.688

Table 8. Table containing average data for the column and system high pH stability across 300 injections for the BAs Mix sample.

Conclusion

Due to the prevalence and mortality associated with CRDs, the quality and efficacy of maintenance drugs such as BAs is critical. To support this, we developed a method that can separate a variety of BAs and their impurities. Results were reproducible and linear. The basic conditions of this method provided reduction in tailing by up to 65% and improved baseline separation for some critical pairs. Further, this method demonstrated the dependability and reproducibility of MaxPeak™ HPS Technology when exposed to basic pH conditions for an extended period of run time. Finally, it was shown that the XBridge™ Premier BEH C₁₈ Column reliably performs

across different sorbent lots.

References

1. WHO. Noncommunicable diseases [Internet]. www.who.int. World Health Organization: WHO; 2022. Available from: <https://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases#:~:text=Cardiovascular%20diseases%20account%20for%20most> <<https://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases#:~:text=Cardiovascular%20diseases%20account%20for%20most>>
2. WHO. Chronic respiratory diseases. Who.int. World Health Organization: WHO; 2019. Available from: https://www.who.int/health-topics/chronic-respiratory-diseases#tab=tab_1 <https://www.who.int/health-topics/chronic-respiratory-diseases#tab=tab_1>
3. Abosamak NR, Shahin MH. Beta 2 Receptor Agonists/Antagonists. PubMed. *Treasure Island (FL): StatPearls Publishing*; 2023. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK559069/?report=classic> <<https://www.ncbi.nlm.nih.gov/books/NBK559069/?report=classic>>
4. Wirth J. The 50 most-filled prescriptions of 2022. The Checkup. SingleCare Administrators; 2022. Available from: <https://www.singlecare.com/blog/most-prescribed-drugs-2022/#:~:text=Prescription%20drug%20use%20in%202022%20vs.%20previous%20years> <<https://www.singlecare.com/blog/most-prescribed-drugs-2022/#:~:text=Prescription%20drug%20use%20in%202022%20vs.%20previous%20years>>
5. Zhang L, Jia Q, Liao G, Qian Y, Qiu J. A Fast Method for the Simultaneous Analysis of 26 Beta-Agonists in Swine Muscle with a Multi-Functional Filter by Ultra-High Performance Liquid Chromatography-Tandem Mass Spectrometry. *Separations*. 2022 May 1;9(5):121. Available from: <https://www.mdpi.com/2297-8739/9/5/121> <<https://www.mdpi.com/2297-8739/9/5/121>>
6. Yan K, Zhang H, Hui W, Zhu H, Li X, Zhong F, *et al*. Rapid screening of toxic salbutamol, ractopamine, and clenbuterol in pork sample by high-performance liquid chromatography—UV method. *Journal of Food and Drug Analysis*. 2016 Apr 1;24(2):277–83. Available from: <https://www.sciencedirect.com/science/article/pii/S1021949816000107?via%3Dihub> <

<https://www.sciencedirect.com/science/article/pii/S1021949816000107?via%3Dihub>

7. Alkhateeb FL, Wilson I, Maziarz M, Rainville P. Ultra high-performance liquid chromatography method development for separation of formoterol, budesonide, and related substances using an analytical quality by design approach. *Journal of Pharmaceutical and Biomedical Analysis*. 2021 Jan 30;193:113729. Available from: <https://www.sciencedirect.com/science/article/pii/S0731708520316150?viewFullText=true> <
<https://www.sciencedirect.com/science/article/pii/S0731708520316150?viewFullText=true>>
8. Dolan J. Back to Basics: The Role of pH in Retention and Selectivity. *Chromatography Online*. LCGC; 2017. Available from: <https://www.chromatographyonline.com/view/back-basics-role-ph-retention-and-selectivity> <
<https://www.chromatographyonline.com/view/back-basics-role-ph-retention-and-selectivity>>

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