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Application Note: DA1003-A

Flare Mixed-Mode Column: β_2-Agonists and Amphetamines

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Introduction

Amphetamines are a class of chemicals that can act as stimulants, decongestants, and hallucinogens, where some compounds in this category are illicit substances.[1] All are primary or secondary amines giving these compounds higher pK_a values (9.3 – 9.8). At pH < 9.3 – 9.8 the molecules are increasingly protonated, generally reducing retention on C18 columns, but at pH > 9.3 – 9.8 they are increasingly deprotonated (neutral), which can facilitate a reversed-phase retention mechanism. Because of the lack of stability of most silica-based columns at elevated pH, there are relatively few reports of the separation of these compounds in their neutral form on silica-based C18 columns. Accordingly, it is often necessary to derivatize them.[2] Here we show their direct analysis at elevated pH using the Flare mixed-mode column.

Many β_2-agonists are used to treat asthma and other pulmonary diseases by relaxing smooth muscle tissue via action on the β_2-adrenergic receptor.[3] Some of them are used illegally to increase the muscle to fat ratios in livestock.[4] These chemicals are also amines – they are basic analytes. Similar to the amphetamines, it is advantageous to operate at elevated pH when separating them by a reversed-phase mechanism.

Separations of amphetamines and β_2-agonists were performed at pH 12 using the Flare Mixed-Mode column from Diamond Analytics. This column is the first functionalized, carbon-based phase.[5] As this column is diamond-based, it has stability under extreme pH conditions.[6]

Experimental

Analytes: Cimaterol, tulobuterol, mabuterol and mapenterol were purchased from Sigma-Aldrich. (St. Louis, MO). Phenylpropanolamine and methamphetamine were obtained from Restek (Bellefonte, PA).

Sample: Analyte mixtures were created in the mobile phase as ca. 1 mg/mL solutions.

Column: Diamond Analytics Flare Mixed-Mode (2.1 x 50 mm, 4 µm, 200Å)

System: Agilent 1290 UHPLC, binary pump, DAD, ChemStation software

Injection volume: 1.0 µL

Temperature: 35 °C

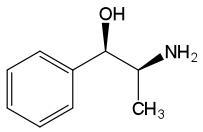
Flow rate: 0.2 mL/min

Detection: UV/Vis Diode Array Detector (254 nm)

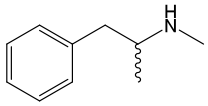
Needle wash: 1 min with methanol

Mobile Phase: 70:30 10 mM aqueous phosphate buffer (pH 12):acetonitrile, isocratic

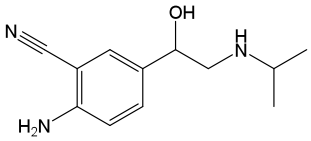
Phenylpropanolamine



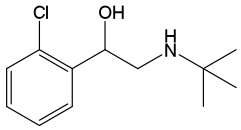
Methamphetamine



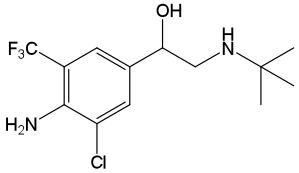
Cimaterol



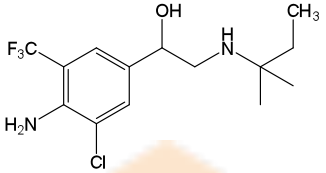
Tulobuterol



Mapenterol



Mapenterol



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Results and Discussion

Figure 1 shows baseline separation of four β_2-agonists in under three minutes. For the latter two compounds in the separation, efficiencies exceed 50,000 N/m and tailing factors are close to 1. Figure 2 shows the baseline separation of two amphetamines and three β_2-agonists in under three minutes. Efficiencies of the latter two compounds are again in excess of 50,000 N/m and tailing factors are ca. 1.

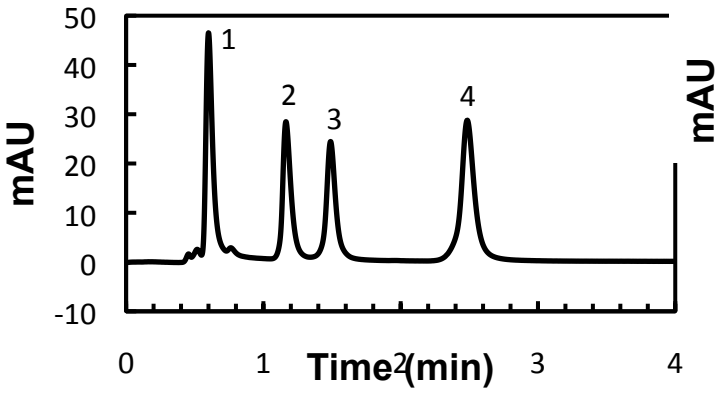

Figure 1. Separation of four β_2-agonists: (1) Cimaterol, (2) Tulobuterol, (3) Mabuterol, (4) Mapenterol.

Table 1. Retention of Various β_2-Agonists

		t_r	N/m	T_f
1.	Cimaterol	0.601	14960	1.74
2.	Tulobuterol	1.164	38060	1.40
3.	Mabuterol	1.489	52580	1.15
4.	Mapenterol	2.485	73220	1.05

Table 2. Retention of Amphetamines and β_2-Agonists

		t_r	N/m	T_f
1.	Phenylpropanolamine	0.521	15520	2.25
2.	Methamphetamine	0.760	25060	N/A
3.	Tulobuterol	1.168	37620	1.36
4.	Mabuterol	1.488	52580	1.15
5.	Mapenterol	2.485	73400	1.06

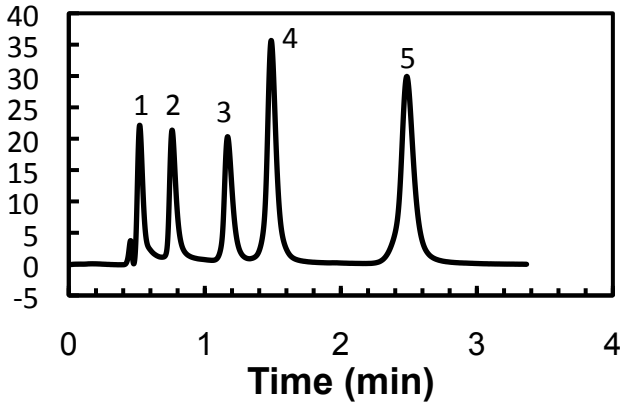

Figure 2. Separation of β_2-agonists and amphetamines: (1) Propanolamine, (2) Methamphetamine, (3) tulobuterol, (4) Mabuterol, (5) Mapenterol

References

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