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Application Note

Simultaneous Analysis of Diuretics and Beta-Blockers by Mixed Mode SPE and UPLC-MS/MS for Anti-Doping Analysis

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Abstract

The goal of this study is to simultaneously identify banned diuretics and beta-blockers in urine samples to support anti-doping laboratories.

The use of mixed-mode SPE and the ACQUITY UPLC I-Class/Xevo TQ-S micro System enabled clean, fast, and efficient extraction and analysis of urinary diuretics and beta-blockers for anti-doping purposes. The Oasis MAX µELution plates enabled efficient and reproducible extraction of 20 beta-blockers and 20 diuretic compounds. Matrix effects were significantly decreased compared to diluted samples. The Xevo TQ-S micro had the speed and sensitivity to accurately identify all analytes in under four minutes, meeting WADA's strict ion ratio criteria even at 20% of the MRPL. The ability to perform rapid inter-scan polarity switching enabled the analysis in a single injection, saving both time and instrument wear compared to sequential injections of positive and negative ionizing compounds. This will enable rapid, accurate analysis of these compounds, while maintaining a cleaner UPLC-MS/MS system.

Benefits

Solid phase extraction and rapid polarity switching enable clean, fast, and efficient analysis of banned diuretics and beta-blockers.

Introduction

Diuretics and beta-blockers are both banned by the World Anti-Doping Agency (WADA).[1] Despite their chemical differences, many anti-doping laboratories prefer to analyze these two classes of molecules together for workflow considerations.[2-4] Beta-blocking agents are bases that ionize under positive electrospray ionization (ESI) and diuretics are mostly acids that ionize under negative ESI conditions. This often necessitates sequential analyses via LC-MS; one in positive mode and a second in negative mode. In addition, these chemical differences make simultaneous extraction a challenge, as any SPE sorbent must be able to accommodate a wide range of polarities and chemotypes.

Results and Discussion

The challenges described above have been solved using Oasis MAX µElution plates to cleanly and efficiently extract diuretics and beta-blockers from urine samples. This was followed by UPLC-MS/MS analysis on a Waters ACQUITY UPLC I-Class System and Xevo TQ-S micro Mass Spectrometer. Separation was achieved on a Waters ACQUITY UPLC CSH C_{18} Column (1.7 µm, 2.1 x 100 mm). Mobile phases consisted of 0.01% formic acid (MPA) and acetonitrile (MPB).

The compounds and their retention times are listed in Table 1. All compounds eluted within four minutes and the entire UPLC cycle was five minutes (Figure 1). Two to three MRM transitions were acquired for all compounds. The rapid polarity switching of the Xevo TQ-S micro enabled this fast, yet efficient chromatography, despite simultaneous analysis of 24 positive and 18 negative ionizing compounds.

	ESI positive			ESI negative	
	Name	**R.T.**		**Name**	**R.T.**
1	Sotalol	0.56	24	Acetazolamide	1.13
2	Atenolol	0.58	25	Chlorthiazide	1.22
3	Amiloride	0.57	26	Hydrochlorothiazide	1.31
4	Carteolol	0.93	27	Hydroflumethiazide	1.66
5	Pindolol	0.94	28	Dichlorphenamide	1.90
6	Nadolol	0.97	29	Chlorthalidone	1.94
7	Triamterine	1.01	30	Trichlormethiazide	2.22
8	Timolol	1.20	31	Methyclothiazide	2.33
9	Acebutolol	1.23	32	Metolazone	2.53
10	Metoprolol	1.24	33	Furosemide	2.67
	Metoprolol-d7 (IS)	1.24		Furosemide-d5 (IS)	2.66
11	Levobunolol	1.30	34	Indapamide	2.73
12	Esmolol	1.37	35	Benzthiazide	2.77
13	Celiprolol	1.47	36	Cyclothiazide	2.84
14	Oxprenolol	1.48	37	Bendroflumethiazide	2.94
15	Labetolol	1.57	38	Bumetanide	3.33
16	Bisoprolol	1.58	39	Probenecid	3.52
17	Metipranolol	1.68	40	Ethacrynic Acid	3.64
18	Propranolol	1.69			
19	Alprenolol	1.73			
20	Betaxolol	1.79			
21	Clopamide	2.21			
22	Carvedilol	2.08			
23	Canrenone	3.50			

Table 1. Names and retention times of beta-blockers and diuretics, sorted by ionization mode.

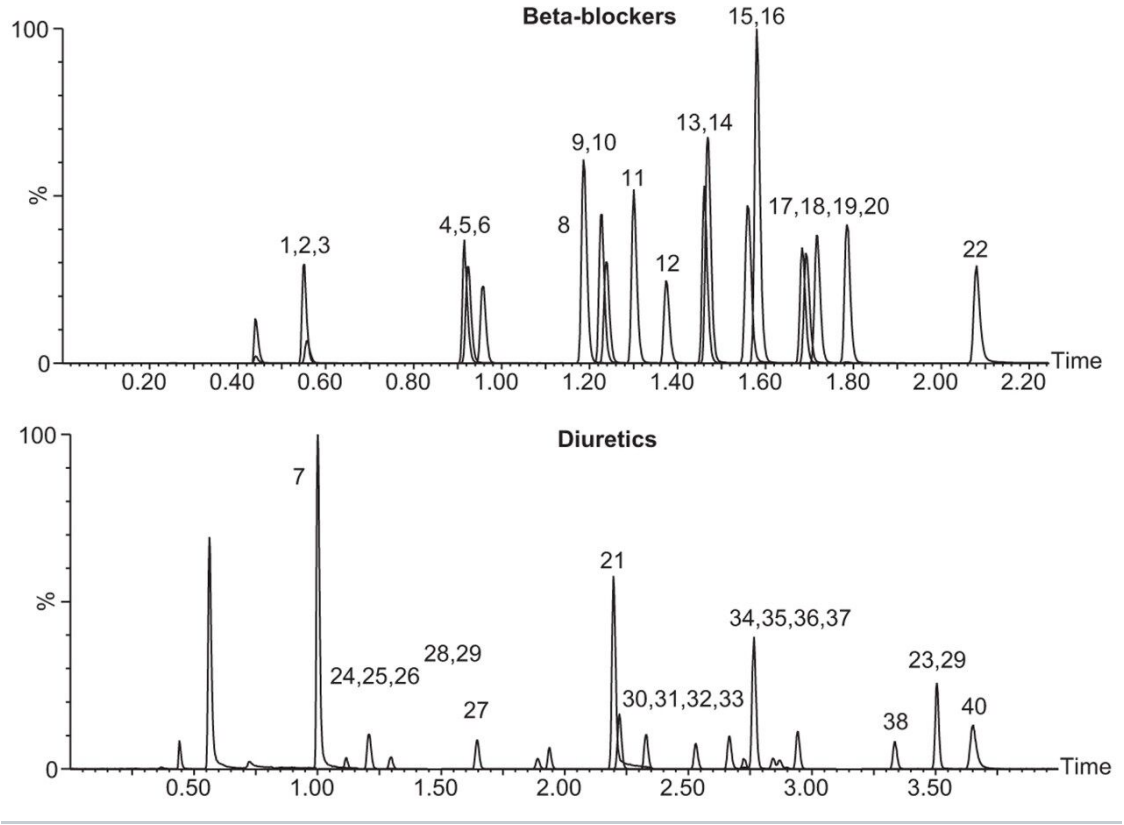

Figure 1. Chromatography of beta-blockers and diuretics. All compounds eluted within four minutes. Analyte labels are listed in Table 1.

Solid phase extraction using the Oasis MAX µElution Plate was performed as follows: 50 µL urine samples were pretreated 1:1 with 5% strong ammonia, then loaded onto the SPE plate, washed with 5:95 MeOH:H2O, eluted with 50 µL of 75:25 MeOH:ACN containing 2% formic acid, and diluted with 200 µL of water. Recovery was efficient for all compounds. Figure 2 shows the average recovery from 12 unique lots of urine. Amiloride, clopamide, and canrenone were grouped with the beta-blockers, as they are bases that ionize by positive ESI. Mean recovery for beta-blockers (and basic diuretics) was 85%, with all but one at 80% or higher; %RSDs were all <20%. Diuretic recovery ranged from 65–94%; all %RSDs <20% with the exception of acetazolamide. The use of the Oasis MAX µElution Plate substantially reduced matrix effects associated with simple sample dilution. As Figure 3 shows, matrix effects, particularly ion suppression, increased from negligible levels to up to over 60% for many of the beta blockers and from 20–40% up to 60–90% for many of the diuretics, even at a 1:10 dilution. Single-factor Anova analysis revealed that matrix effects were significantly increased for all compounds except for acebutolol, metolazone, bumetanide, probenecid, and ethacrynic acid.

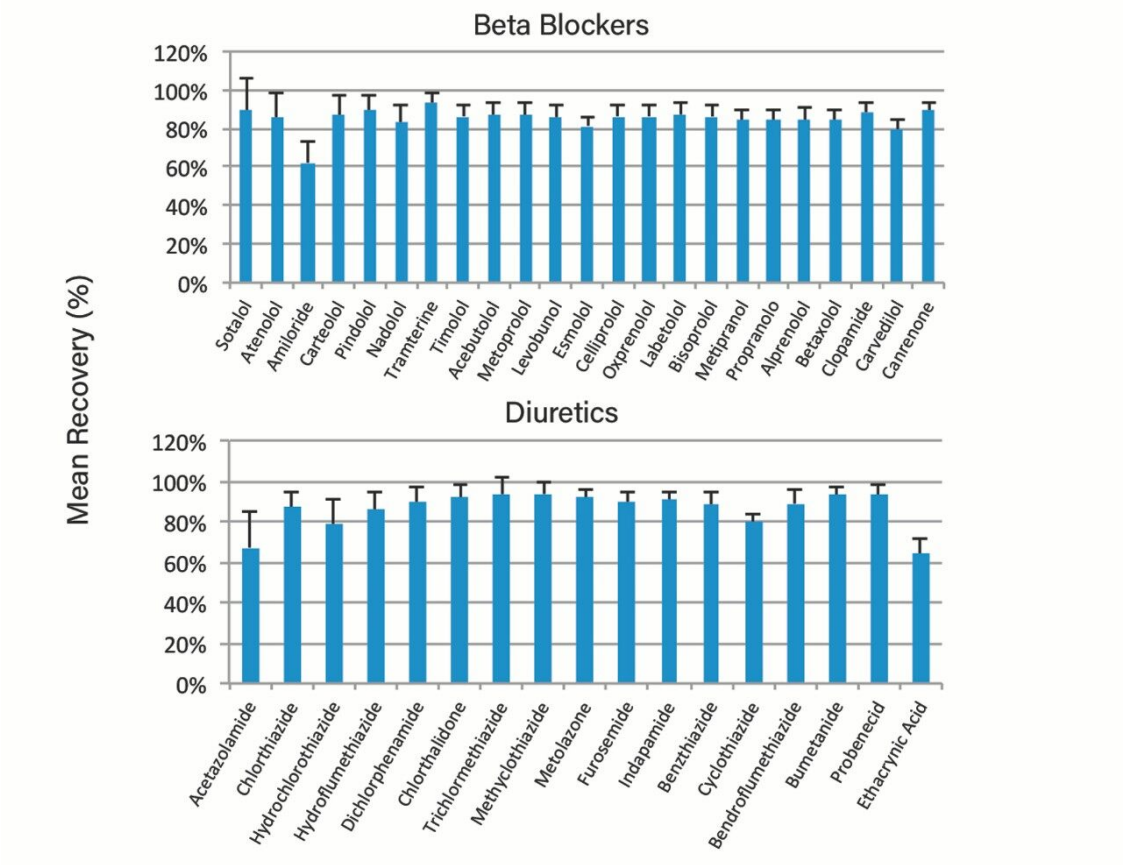

Figure 2. Mean recovery of beta-blockers and diuretics from 12 unique lots of urine matrix. Beta-blocker recovery averaged 85% and all %RSD's <20%. Diuretic (negative ESI) recovery ranged from 64-94%. All %RSD's <20% with the exception of acetazolamide (26%). Bars and error bars represent mean +/- S.D. (N=12).

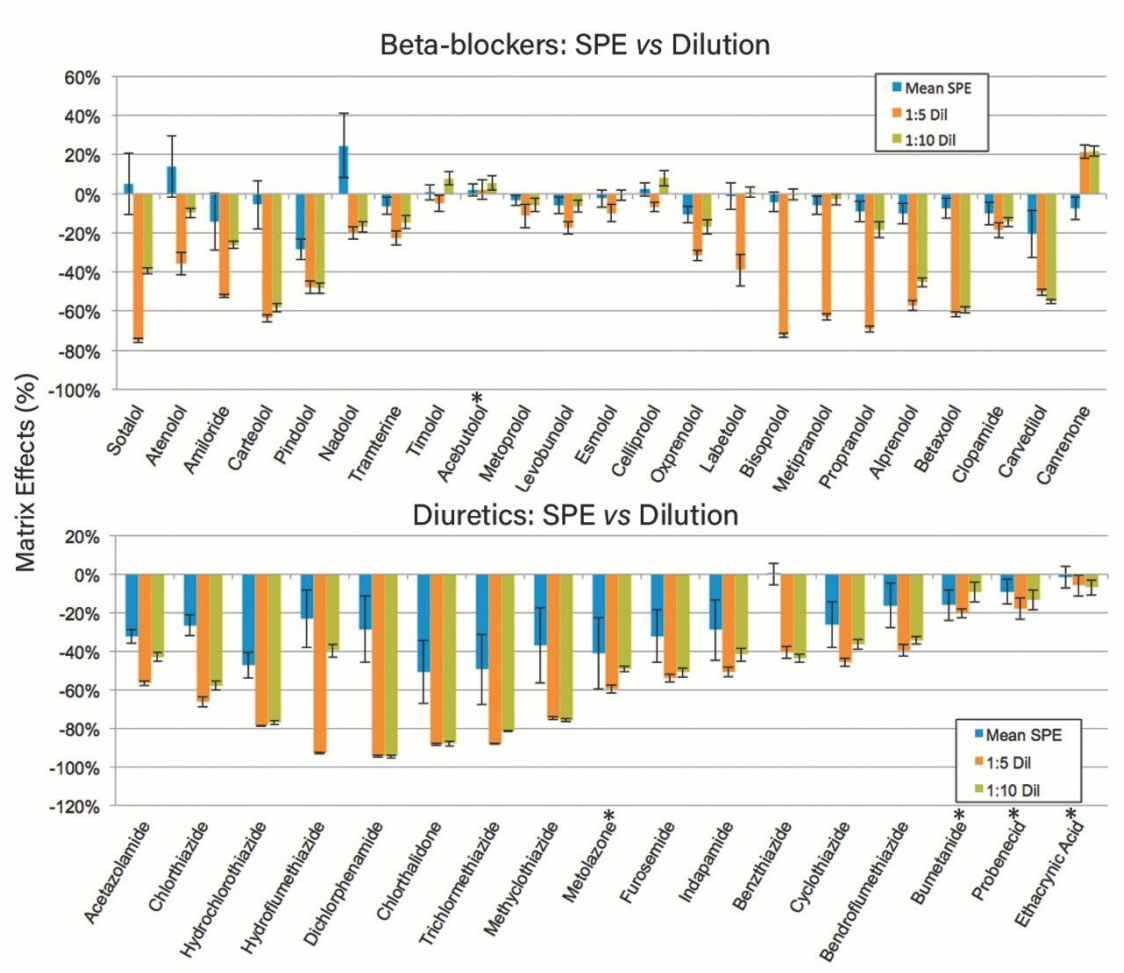

Figure 3. Matrix effects for beta-blockers and diuretics. Mean matrix effects from 12 lots of urine were compared to matrix effects from 1:5 and 1:10 dilution of pooled blank urine in 97:2:1 H_2 O:ACN:formic acid. Even at 1:10 dilution, ion suppression was significantly increased in the diluted samples compared to those prepared by SPE with the MAX µElution plate (N=12 for mean SPE matrix effects and N=4 for the diluted samples). Asterisks indicate compounds in which matrix effects were NOT different between SPE prepared and diluted samples.

All compounds were readily detectable, even at 20% of WADA's Minimum Required Performance Level (MRPL) of 100 ng/mL for beta-blockers and 200 ng/mL for the diuretic compounds.[5] Retention time tolerances were well within WADA requirements as well.[5] Despite the speed of the analysis and the need for polarity switching, WADA ion ratio criteria for confirmation were met for all compounds, even at 20% of the MRPL.[6]

Conclusion

The use of mixed-mode SPE and the ACQUITY UPLC I-Class/Xevo TQ-S micro System enabled clean, fast, and efficient extraction and analysis of urinary diuretics and beta-blockers for anti-doping purposes. The Oasis MAX µELution plates enabled efficient and reproducible extraction of 20 beta-blockers and 20 diuretic compounds. Matrix effects were significantly decreased compared to diluted samples. The Xevo TQ-S micro had the speed and sensitivity to accurately identify all analytes in under four minutes, meeting WADA's strict ion ratio criteria even at 20% of the MRPL. The ability to perform rapid inter-scan polarity switching enabled the analysis in a single injection, saving both time and instrument wear compared to sequential injections of positive and negative ionizing compounds. This will enable rapid, accurate analysis of these compounds, while maintaining a cleaner UPLC-MS/MS system.

References

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