



Introduction

In clinical laboratories, the commonly used sample preparation techniques include immunoassay, liquid-liquid extraction (LLE) and solid phase extraction (SPE). QuEChERS (acronym for Quick, Easy, Cheap, Effective, Rugged and Safe) is a novel sample preparation technique that was originally reported by the scientists at the USDA in 2003 for the determination of multi-class pesticide residues in fruits and vegetables. Since then, QuEChERS has been adopted quickly and widely to determine various analytes of interest in different matrices, such as veterinary drugs in animal tissues, mycotoxins in grains, polycyclic aromatic hydrocarbons in seafood, bisphenol A in canned foods and beverages, and cannabinoids in medical marijuana and cannabis foods. In this study, QuEChERS methodology will be applied for the analysis of clinical therapeutic drugs, such as benzodiazepines and anabolic steroids in whole blood.

Method

Add 2 mL of extraction solvent with internal standards (optional) to a 15-mL centrifuge tube with pre-packed extraction salts, add 1 mL of whole blood to the centrifuge tube, shake and centrifuge. After centrifugation, the proteins, blood cells and some un-dissolved extraction salts remain in the bottom of the centrifuge tube, while the target analytes are extracted into the upper, clear solvent layer (shown in Figure 1). For sample cleanup, transfer 1 mL of the supernatant to a 2-mL dSPE centrifuge tube containing sorbents (e.g. PSA and C18). The matrix co-extractives, such as organic acids and lipids, are retained on the sorbents which results in a purified extract for LC-MS/MS analysis. The materials used for extraction and cleanup are listed in Table 1.

Table 1.
Materials used for the determination of benzodiazepines and steroids in whole blood

Materials	Benzodiazepines	Steroids
Extraction solvent	2 mL ACN w 0.4% formic acid	2 mL ACN
Extraction salts	400 mg MgSO ₄ and 100 mg NaOAc (UCT p/n: ECQUUS15CT)	400 mg MgSO ₄ and 100 mg NaCl (UCT p/n: ECQUUS1015CT)
dSPE sorbent	150 mg MgSO ₄ , 50 mg PSA, 50 mg C18 (UCT p/n: CUMPS18CT)	150 mg MgSO ₄ , 50 mg PSA (UCT p/n: CUMPS2CT)
HPLC column	Selectra® DA, 100x2.1mm, 3 μm (UCT p/n: SLDA100ID21-3UM)	Selectra® C18, 100x2.1mm, 3 μm (UCT p/n: SLC-18100ID21-3UM)

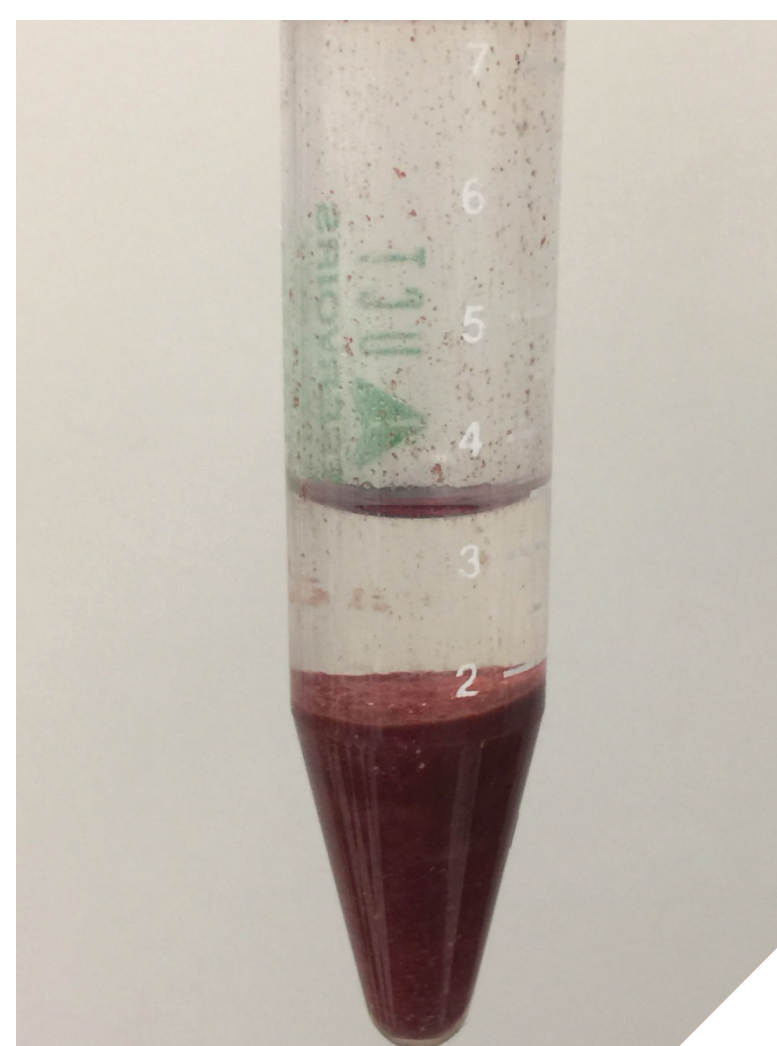


Figure 1.
Whole blood after QuEChERS extraction

Results

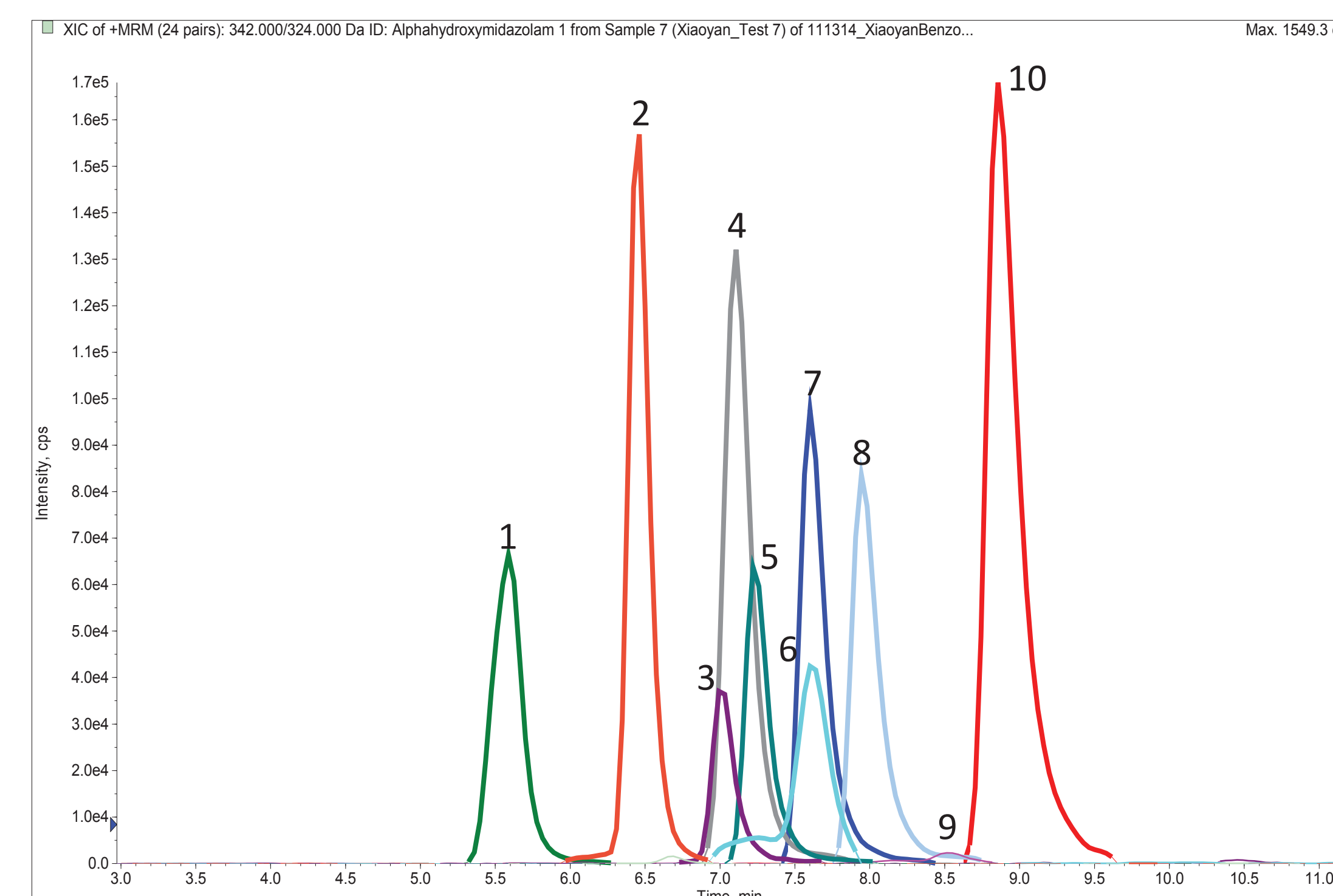
Matrix matched calibration curves were constructed for analyte quantification. The responses for 10 representative benzodiazepines and 12 steroids were linear with R² ranged from 0.9963 to 1.0000 over the concentration range of 10 - 500 ng/mL. The matrix effect was evaluated by comparing the slopes of the matrix matched calibration curves to those of the calibration curves of solvent standards. The matrix effect was found to be insignificant, from -22 to 18%, which indicated that the QuEChERS method effectively removed the matrix interferences that may cause significant ion suppression or enhancement. Excellent analyte recoveries (81.4- 105%) and relative standard deviations (RSD% ≤ 11%) were obtained (listed in Table 2).

This method was applied to 6 real whole blood samples screening for benzodiazepines, no target analytes were detected above the limit of quantitation of 10 ng/mL.

Table 2.
Accuracy and precision of benzodiazepines and steroids in spiked whole blood

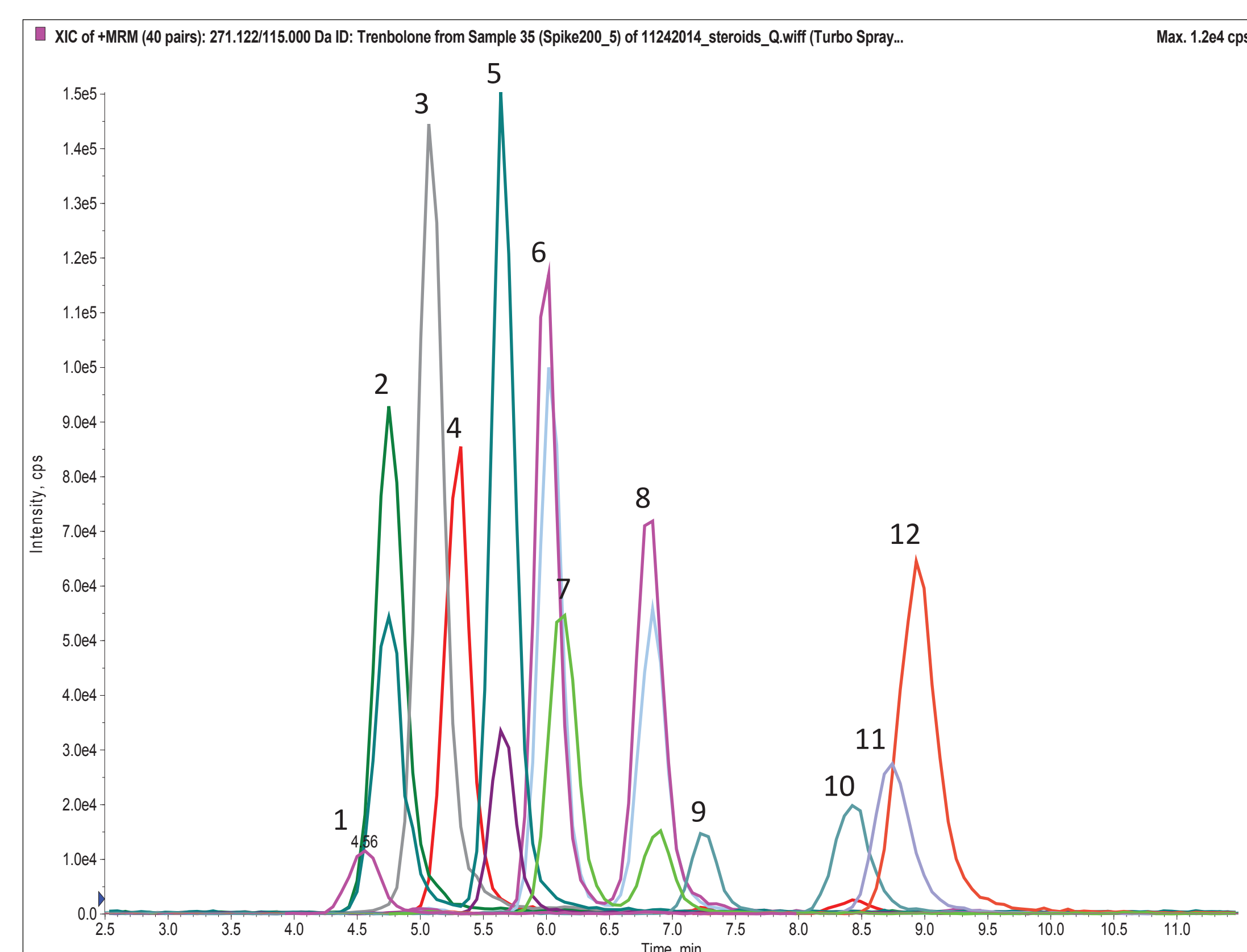
Benzodiazepines	Spiked at 10 ng/mL		Spiked at 50 ng/mL		Spiked at 200 ng/mL	
	Recovery%	RSD% (n=6)	Recovery%	RSD% (n=6)	Recovery%	RSD% (n=6)
7-aminoclonazepam	88.6	7.5	96.9	2.1	99.7	3.8
Alpha-Hydroxy-Alprazolam	101.2	3.4	91.0	2.0	90.3	2.7
Alprazolam	92.3	10.7	90.2	4.0	86.5	3.5
Clonazepam	96.4	3.6	105.0	3.2	103.0	2.0
Diazepam	85.5	3.3	103.0	2.7	100.4	1.9
Lorazepam	96.9	5.1	93.7	4.1	91.6	2.7
Midazolam	96.7	2.7	101.6	2.7	100.6	1.9
Nordiazepam	88.4	3.9	99.7	2.5	97.8	2.3
Oxazepam	86.5	1.9	93.8	2.4	92.6	1.7
Temazepam	96.7	2.7	101.6	2.7	100.6	1.9
Steroids	Spiked at 10 ng/mL		Spiked at 50 ng/mL		Spiked at 200 ng/mL	
	Recovery%	RSD% (n=6)	Recovery%	RSD% (n=6)	Recovery%	RSD% (n=6)
17-hydroxyprogesterone	89.6	6.6	99.2	5.7	99.3	3.2
Androstenedione	93.5	9.2	95.7	3.3	94.3	1.5
Boldenone	91.2	8.2	101.6	2.9	99.4	1.4
Methandienone	94.7	6.5	97.2	3.3	96.1	3.0
Methenolone	98.2	4.5	96.0	4.7	95.3	3.9
Norethandrolone	94.0	6.7	98.5	5.1	99.8	4.0
Nandrolone	96.4	9.6	92.3	1.1	89.8	1.6
Progesterone	101.6	5.0	95.5	1.3	94.8	4.0
Stanozolol	85.1	5.9	92.1	3.4	91.3	2.2
Testosterone	92.4	6.3	95.0	3.4	95.1	2.4
Trenbolone	81.4	9.0	93.2	6.9	95.0	3.0
Epitestosterone	89.8	5.4	97.6	4.4	99.3	2.8

Chromatogram of a whole blood sample spiked with 200 ng/mL benzos



Peak list: 1. 7-aminoclonazepam; 2. Midazolam; 3. Lorazepam; 4. Oxazepam; 5. Clonazepam; 6. Alpha-Hydroxy-Alprazolam; 7. Nordiazepam; 8. Temazepam; 9. Alprazolam; 10. Diazepam

Chromatogram of a whole blood sample spiked with 200 ng/mL steroids



Peak list: 1. Trenbolone; 2. Boldenone; 3. Androstenedione; 4. Nandrolone; 5. Methandienone; 6. Testosterone; 7. 17-hydroxyprogesterone; 8. Epitestosterone; 9. Methenolone; 10. Norethandrolone; 11. Stanozolol; 12. Progesterone

Conclusion

A novel sample preparation technique, QuEChERS, has been demonstrated to be simple, fast, and effective for the quantitative determination of benzodiazepines and anabolic steroids in whole blood. This provides clinical laboratories a successful alternative sample preparation method for monitoring therapeutic or other drugs in whole blood.