Analyze Barbiturates in Urine with Agilent 6430 LC/MS/MS and Poroshell 120 EC-C18

Application Note

Forensics and Toxicology

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Abstract

A fast, cost-effective, and highly sensitive method was developed for the determination of five barbiturates with four internal standards (ISTDs) in urine using an Agilent 6430 Triple Quadrupole LC/MS System and an Agilent Poroshell 120 EC-C18 column. The sample of urine was extracted using an Agilent SPEC-C18AR cartridge. Results indicate that the method effectively extracts the selected barbiturates from urine, resolving the target compounds and the ISTDs in 8.5 minutes. Though amobarbital and pentobarbital differ only in the position of a methyl group, and are, hence, difficult to separate, their resolution is sufficient for routine analysis.

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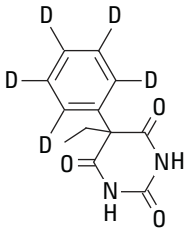
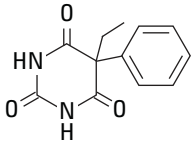
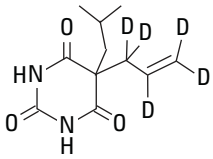
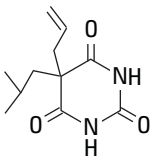
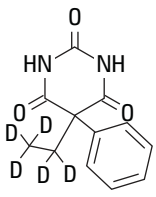
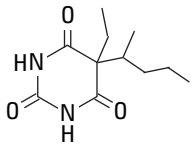
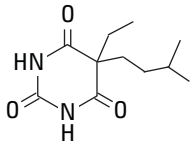
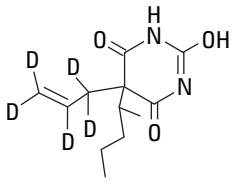
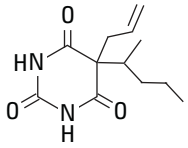
Materials and Methods

Table 1 lists the target compounds of barbiturates. All compounds were purchased from Cerilliant Corporation, Round Rock, TX, United States.

Sample preparation

1. Begin by centrifuging the urine sample at 2,800 rpm for 5 minutes.
2. Pipette 1 mL of centrifuged sample into a 13 × 100 mm borosilicate glass tube.
3. Add exactly 35 µL of the working deuterated internal standard (butalbital-D5, pentobarbital-D5, secobarbital-D5, and phenobarbital-D5).
4. Pipette 500 µL 0.1 M phosphate buffer into the sample. The phosphate buffer is prepared by adding 13.61 g KH_2PO_4 into 800 mL water, adjusting to pH 6.0 with KOH, then making the volume 1 L.
5. Use a vacuum chamber with the Agilent SPEC-C18AR cartridge for extraction. Condition the cartridge with 0.2 mL of MeOH and load the sample solution.
6. Wash the column with 0.5 mL water and dry for 1 minute.
7. Elute the cartridge with 1 mL 90:10 hexane:ethyl acetate mixture.
8. Collect the eluent and dry the sample under nitrogen gas at 35 °C.
9. Reconstitute with 0.5 mL 90:10 water:acetonitrile mixture.

Table 1. Compounds used in this study.

No.	Compound	CAS no.	Structure
1	Phenobarbital-d5	72793-46-5	
2	Phenobarbital	50-06-6	
3	Butalbital-d5	145243-96-5	
4	Butalbital	77-26-9	
5	Pentobarbital-d5	52944-66-8	
6	Pentobarbital	76-74-4	
7	Amobarbital	57-43-2	
8	Secobarbital-d5	130221-73-7	
9	Secobarbital	76-73-3	

The method was performed on the Agilent 1260 Infinity LC with a 6430 Triple Quadrupole LC/MS.

HPLC conditions

Column	Agilent Poroshell 120 EC-C18, 2.1 × 100 mm, 2.7 µm (p/n 695775-902)	
Sample prep	Agilent SPEC-C18CR, 3 mL, 15 mg (p/n A5321920)	
Eluent	A, 5 mM ammonium acetate; B, LC/MS grade acetonitrile	
Injection volume	20 µL	
Flow rate	0.4 mL/min	
Gradient[1]	Time (min)	% B
	0	10
	10	45
	10.5	90
	12	90
	12.5	10
Temperature	60 °C	

MS conditions

ESI	Drying gas 350 °C, 10 L/min
Nebulize	40 psi
Negative ionization mode	
Capillary	4,000 V, ΔEMV 400

[1] This Agilent 1260 Infinity LC did not have a low delay volume configuration. If running with low delay volume, use about 1 minute initial hold.

Table 2. Optimized MRM conditions.

No.	Compound	Ion pair qualitative and quantitative analyses	RT (min)
1	Phenobarbital-d5	236.1 → 42.1; 236.1 → 193.1	4.825
2	Phenobarbital	231.1 → 42.1; 231.1 → 188.2	4.871
3	Butalbital-d5	228.1 → 42.1; 228.1 → 185.1	6.105
4	Butalbital	223.1 → 42.1; 223.1 → 180.1	6.150
5	Pentobarbital-d5	230.2 → 42.1; 230.2 → 187.1	7.348
6	Pentobarbital	225.1 → 42.1; 225.1 → 182.2	7.375
7	Amobarbital	225.1 → 42.0; 225.1 → 182.1	7.485
8	Secobarbital-d5	242.2 → 42.1; 242.2 → 199.2	8.118
9	Secobarbital	237.1 → 163; 237.1 → 194.1	8.155

Results and Discussion

Separation

The superficially porous particles of Poroshell 120 have nearly identical efficiency as sub-2 µm totally porous materials and can, therefore, be used to provide similarly fast and high resolution analyses at a lower pressure. A separation of the nine barbiturates in 8.5 minutes was achieved on the column with a gradient method (Figure 1). Reasonable resolution was achieved between the standard components, except for pentobarbital and amobarbital. They are the isomers with the same product ions, which could not be identified by MS. However, they still have some separation on Poroshell 120 EC-C18 and the resolution for amobarbital and pentobarbital is sufficient for routine analysis.

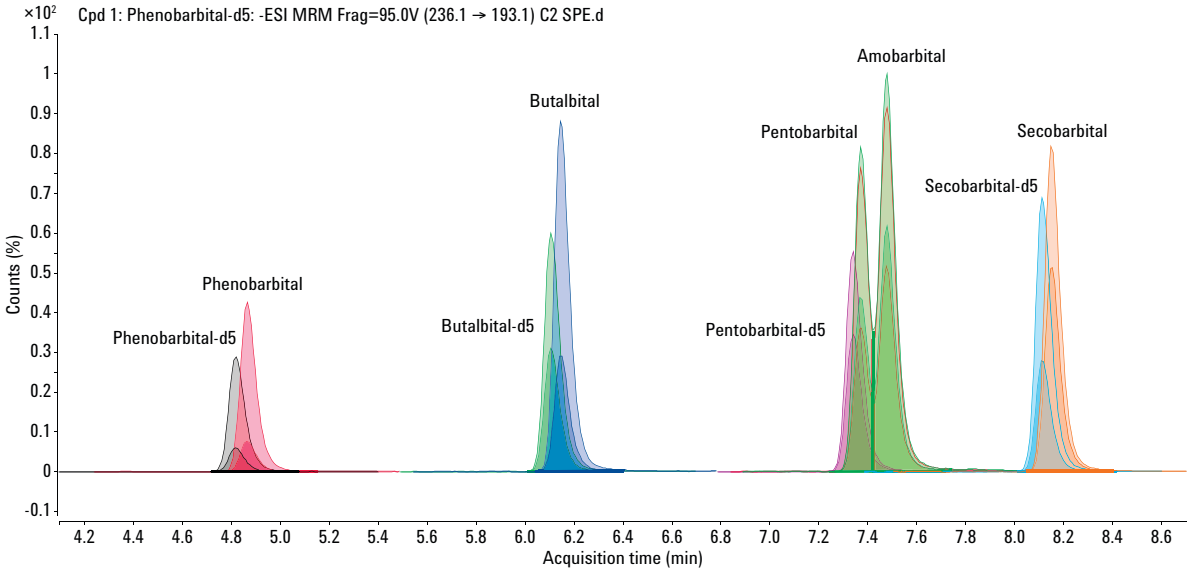


Figure 1. MRM chromatograms of barbiturates and internal standards using an Agilent Poroshell 120 EC-C18 column.

Linearity and recovery

The stock standards solution, containing phenobarbital, butalbital, pentobarbital, amobarbital, and secobarbital, was diluted to a series of linear solutions of 3,000, 1,500, and 150 ng/mL. In each solution, the ISTDs of phenobarbital-d5, butalbital-d5, pentobarbital-d5, and secobarbital-d5, were made up to a concentration of 1,000 ng/mL. The calibration curves resulting from these standard injections on the Poroshell 120 EC-C18 column using the 6430 Triple Quadrupole LC/MS System are shown in Figure 2. The method showed excellent linearity, being very close to 1.0 (from 0.9995 to 0.99998).

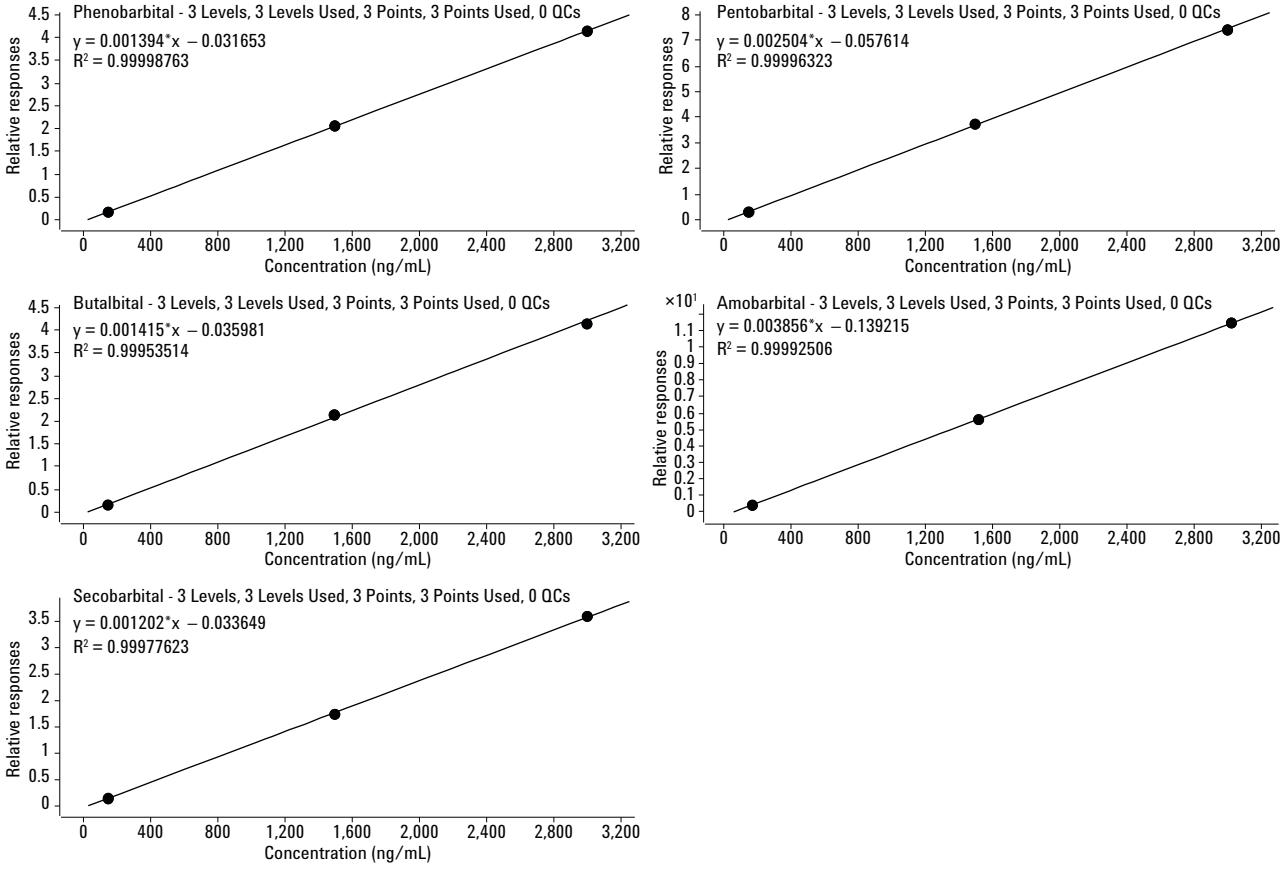


Figure 2. Calibration curves of standards on an Agilent Poroshell 120 EC-C18 column.

The standards, at a concentration of 150 ng/mL, were spiked into the urine sample blank and processed with the SPE procedure. The recoveries were calculated and are shown in Table 3.

Sample analysis

Two samples of urine were analyzed followed by the method described above. Concentrations of five barbiturates could be calculated according to the calibration curves in Figure 2, and the data are listed in Table 4.

Conclusions

A method was developed for the extraction and separation of barbiturates using an Agilent SPEC-C18AR for sample extraction and an Agilent Poroshell 120 EC-C18 column for separation. The sample preparation method effectively extracted the selected barbiturates from urine, with sufficient recoveries and precision. The column provided good selectivity and good resolution for these compounds. The method developed on the Agilent 6430 Triple Quadrupole LC/MS System was suitable for quantitative analysis of these compounds in urine, especially at low concentration levels.

Table 3. Recoveries of barbiturates from a urine sample with SPE.

Compounds	Phenobarbital	Butalbital	Amobarbital	Pentobarbital	Secobarbital
Recovery % (150 ng/mL)	60.6	87.0	125.8	92.7	97.8

Table 4. Concentrations of the target barbiturates in urine samples.

	Phenobarbital	Butalbital	Pentobarbital	Amobarbital	Secobarbital
Sample 1 (ng/mL)	224.5	218.2	226.8	213.4	216.4
Sample 2 (ng/mL)	1,044.6	1,045.4	1005.8	972.1	1,013.9

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