

鲜牛乳及乳制品中4种雌激素含量的HPLC测定

谭欣同¹, 邓立刚^{2,3}, 赵善仓^{2,3}, 董燕婕^{2,3}, 王燕¹, 李增梅^{2,3*}

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摘要: 4 17 β - HPLC
QuEChERS C₁₈
230 nm 17 β -
S/N=3 0.03 mg/kg 0.026 mg/kg 0.03 mg/kg 0.012 mg/kg 0.024 mg/kg 0.021 mg/kg 0.025 mg/kg
0.01 mg/kg S/N=10 0.1 mg/kg 0.09 mg/kg 0.1 mg/kg 0.04 mg/kg 0.07 mg/kg 0.07 mg/kg
0.08 mg/kg 0.03 mg/kg 0.1 1.0 2.0 mg/kg 81%~97% RSD
3.2%~8.7%

关键词: ; ; ; QuEChERS; HPLC

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Determination on 4 Estrogens Content in Fresh Milk and Dairy Products by HPLC

TAN Xin-tong¹, DENG Li-gang^{2,3}, ZHAO Shan-cang^{2,3}, DONG Yan-jie^{2,3},
WANG Yan¹, LI Zeng-mei^{2,3*}

1. College of Food Science and Engineering/Shandong Agricultural University, Tai'an 271018, China

2. Institute of Agricultural Quality Standards and Testing Technology Research, Shandong Academy of Agricultural Sciences, Jinan 250100, China

3. Key Laboratory of Test Technology on Food Quality and Safety of Shandong Province, Jinan 250100, China

Abstract: A novel method for the determination of 4 estrogens 17 β -estradiol Estriol Estrone and Diethylstilbestrol in raw milk and milk powder has been developed by HPLC. The milk powder was dissolved in water and then it was extracted with acetonitrile by QuEChERS method and purified by NH₂ solid phase extraction cartridge after fats were removed by hexane. The analyte was separated on a C₁₈ column (Waters Atlantis dC₁₈, 4.6 \times 250 mm i.d 5 μ m) at UV 230 nm. The detection limits(S/N=3) of 17 β -estradiol, estriol, estrone and diethylstilbestrol in milk powder and fresh milk were 0.03 mg/kg, 0.026 mg/kg, 0.03 mg/kg, 0.012 mg/kg and 0.024 mg/kg, 0.021 mg/kg, 0.025 mg/kg, 0.01 mg/kg, respectively. The quantitation limit(S/N=10) in milk powder and raw milk were 0.1 mg/kg, 0.09 mg/kg, 0.1 mg/kg, 0.04 mg/kg and 0.07 mg/kg, 0.07 mg/kg, 0.08 mg/kg, 0.03 mg/kg, respectively. The recoveries were 81%~97% with the range of RSD from 3.2% to 8.7%.

Keywords: Fresh milk; milk powder; estrogen; QuEChERS; HPLC

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CAC() Maximum Residue Limits for Veterinary Drugs in Food
17 β -

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作者简介: (1990-), ,

. E-mail:tanxintong142536@163.com

***通讯作者:** Author for correspondence. E-mail:lizengmei78@163.com

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QuEChERS					
					[12] QuEChERS
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QuEChERS	SPE		HPLC		

1 材料与amp;方法

1.1 样品的采集

12

1.2 仪器与试剂

2.2.1 仪器	Waters2695		2489	waters	Heidolph L4000
	(Heidolph)	3K30		(sigma)	IKA-MS3
(IKA)		KQ-500E		()	0.22 μm
Pall)		100~1000 μL	(Eppendorf)		10~100
μL (Eppendorf)					
2.2.2 试剂	17β-			>99%	DR

2.2.3 标准溶液的配制	10.0 mg	100 mL
100 mg/kg;		10 mg/kg
	0.1 0.5 1.0 2.0 2.5	10.0 mg/kg

2.3 色谱条件

Waters Atlantis dC ₁₈	4.6×250 mm i.d 5 μm	230 nm	40
A (B)	0 7 min 70% 50% A	7 20 min	50% A 20
21 min 50% 70% A;	1.0 mL/min;		10 μL

2.4 样品处理

2.4.1 奶粉样品处理	10 g	0.01 g	100 mL	15 mL
	2 min	40 mL	2 min	10 min
5 g QuEChERS	4 g	1 g	8000 r/min	12 min
20 mL 50 mL		20 mL		
			40 °C	2 mL
	95:5, V/V			
2.4.2 生鲜牛乳样品处理		10 g	0.01 g	50 mL
20 mL	2 min	10 min	3 g QuEChERS	2.5 g
g	8000 r/min	12 min	10 mL	50 mL
				10 mL

2.4.3 净化 40 °C 2 mL 95:5, V/V
 10 mL 95:5, V/V
 15 mL 40 °C 1 mL 2 mL/min 0.22 μm

3 结果与分析

3.1 色谱条件的优化

Waters Atlantis dC₁₈ 4.6×250 mm i.d 5 μm Waters SunFire™C₁₈ (4.6×150 mm i.d 5 μm) Waters Atlantis dC₁₈ 4.6×150 mm i.d 5 μm 4
 150 mm C₁₈
 10 mmol/L 20% 30%
 50:50 V/V 2.2 17β- 230 nm 280 nm
 nm 220 nm 260 nm 230 nm 280 nm
 230 nm 280 nm 230 nm
 4
 (1)

3.2 样品前处理的优化

QuEChERS^[12] QuEChERS 4 g 1 g
 81% 97% 88%
 (2)

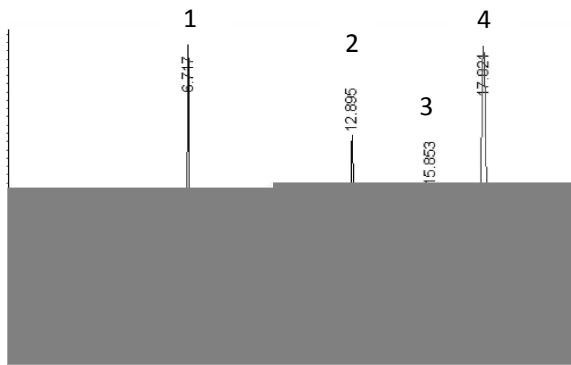


图 1 4 种雌激素标准品液相色谱图 (5 mg/kg)
 Fig.1 HPLC of 4 estrogen standards (5 mg/kg)
 1: Estriol; 2: 17β-estradiol; 3: Estrone; 4: Diethylstilbestrol

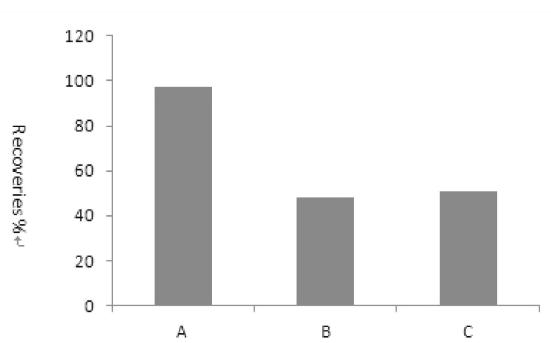


图 2 三种不同提取方法的回收率比较
 Fig.2 Comparison among recovery rates of 3 different methods
 A: QuEChERS 提取方法; B: 甲醇提取; C: 乙腈提取
 A: QuEChERS extracts; B: Methanol extracts; C: Acetonitrile extracts

V/V - 90:10 V/V

- 90:10 V/V - 95:5
 - 95:5 V/V - 95:5 V/V

10 mL~20 mL
 mL 4

15 mL 97% 5
 15 mL

3.3 线性范围和检出限

0.5~10.0 mg/kg
 X(ng) R 0.999 3 Y($\mu\text{V}\cdot\text{sec}$)
 (LOD),10 (S/N=10) S/N=3
 1 (LOQ)

表 1 线性方程、线性相关系数及 4 种雌激素在奶粉中和生鲜牛乳中的检出限和定量限

Table1 The liner equation, correlation coefficient, LOD and LOQ of 4 estrogens in milk powder and fresh milk

Estrogen	Liner equation	Correlation coefficient	LOD mg/kg	LOQ mg/kg
17 β -	y=28391x+4106.9	0.9992	0.01/0.012	0.04/0.03
	y=9787.2x+132.65	0.9999	0.024/0.03	0.07/0.1
	y=10697x+120.33	0.9999	0.021/0.026	0.07/0.09
	y=9643.2x-473.4	0.9999	0.025/0.03	0.08/0.1

3.4 奶粉与生鲜牛乳样品的添加回收实验

0.1 mg/kg 1.0 mg/kg 2.0 mg/kg 4
 5 3.2%~8.7% (3-6) 2 81.3%~97.4%

表 2 生鲜牛乳和奶粉中雌激素的添加回收实验

Table 2 The average recovery and RSD in fresh milk and powder

Estrogen	mg/kg		%	
	Spiked	Average recovery	RSD	
17 β -	0.1	91.2/88.2	6.3/6.4	
	1.0	97.4/96.7	4.5/6.2	
	2.0	94.5/91.8	3.2/4.3	
	0.1	89.2/81.3	7.2/8.7	
	1.0	94.3/87.6	5.4/4.3	
	2.0	93.4/89.9	4.6/4.0	
	0.1	89.5/82.6	4.4/5.3	
	1.0	93.4/82.5	4.9/5.9	
	2.0	89.5/88.8	3.2/6.2	
	0.1	88.9/84.7	7.1/6.3	
	1.0	89.4/92.7	4.3/4.5	
	2.0	87.2/90.6	5.5/3.6	

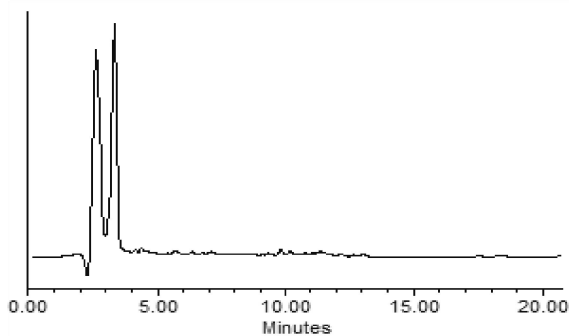


图 3 空白样品色谱图

Fig.3 The chromatography of blank samples

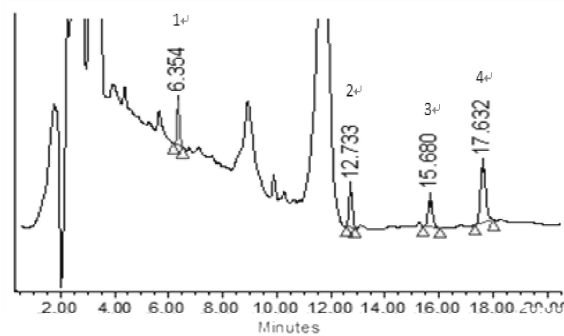


图 4 空白样品添加激素后色谱图 (0.1 mg/kg)

Fig.4 The chromatography of blank milk powder samples after adding 4 estrogens (0.1 mg/kg)

1:雌三醇; 2:17 β -雌二醇; 3:雌酮; 4:己烯雌酚
 1:Estriol; 2:17 β -estradiol; 3:Estrone; 4:Diethylstilbestrol

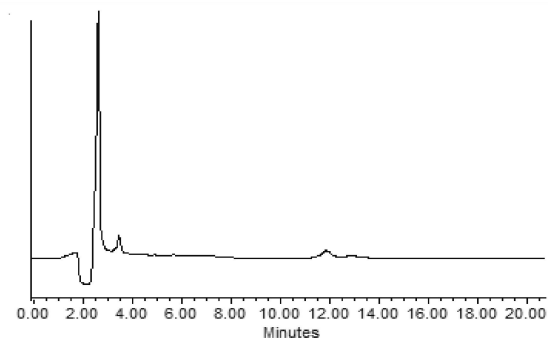


图 5 鲜牛乳空白样品色谱图

Fig.5 The chromatography of blank fresh milk sample

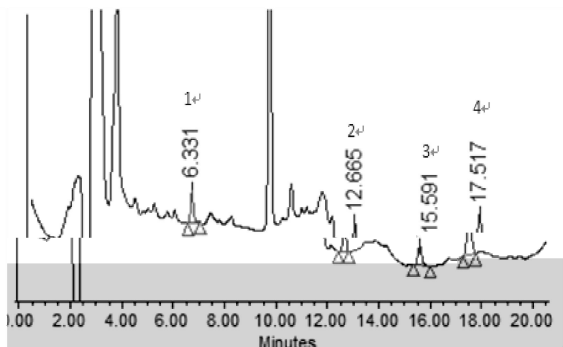


图 6 鲜牛乳添加激素样品色谱图(0.1 mg/kg)

Fig.6 The chromatography of blank fresh milk samples after adding 4 estrogens (0.1 mg/kg)

1:雌三醇; 2:17β-雌二醇; 3:雌酮; 4:己烯雌酚
1:Estriol; 2:17β-estradiol; 3:Estrone; 4:Diethylstilbestrol

3.5 实际样品的测定

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0.045 mg/kg

4 结论

QuEChERS

SPE

HPLC

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