Determination of Hormones in Shrimp by Agilent 1290 Infinity LC with Agilent Poroshell 120 LC Column and Agilent Bond Elut QuEChERS for Sample Preparation

Application Note

Food

Authors

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Abstract

A method for the determination of hormones in shrimp was developed using an Agilent 1290 Infinity LC with an Agilent Poroshell 120 EC-C18 column. At the same time a new sample preparation method using QuEChERS was developed and optimized for extracting 13 hormones in shrimp. Results indicate that the sample preparation using QuEChERS and HPLC analysis using an Agilent Poroshell 120 EC-C18 column (3.0 mm × 100 mm, 2.7 µm) is suitable for determination of hormone compounds in shrimp. The 13 target compounds were well separated from each other and show no other interferences in the chromatogram. Method recoveries ranged from 91.6 to 107.2 % with relative standarddeviations (RSDs) between 0.15 and 3.5 %.

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Introduction

There are currently several hormones and hormone-like agents with marked ability to improve the rate of growth and efficiency of feed intake for animals. In some countries hormones are a common food additive and controlled use of certain compounds is even considered safe. However, longterm consumption of glucocorticoids can lead to hyperglycemia, osteoporosis, birth defects, and immune function decline. Other hormones such as estrogen, androgen, and progesterone are carcinogenic and can lead to breast cancer, ovarian cancer and cell carcinoma. Some other countries therefore strictly prohibit their application, especially the use of estrogens. A previous application note showed a new method for detecting hormones in crucian carp fish meat using an Agilent Bond Elut OPT SPE cartridge for sample preparation and an Agilent ZORBAX Eclipse Plus C18 column (4.6 mm × 250 mm, 5 µm) for HPLC analysis [1].

Although the current QuEChERS methodology has been designed for removing matrix interferences in food products of plant origin, such as polar organic acids, sugars, and lipids, it also has potential for other food matrices such as meat or seafood. Based upon the chemical properties of the compounds of interest and food matrices, some modifications of the original method might be necessary to obtain accurate and precise results. The purpose of this work is to extend the QuEChERS methodology to veterinary drug residues in seafood. Agilent Bond Elut QuEChERS EN buffered extraction kits (p/n 5982-5650) and dispersive-SPE 15 mL kits for drug residues in meat (p/n 5982-4956) were used for the analysis of 13 hormones (Table 1) in shrimp. The method was validated in terms of recovery and reproducibility.

A newly developed column, the Agilent Poroshell 120 EC-C18 that is packed with 2.7 µm superficially porous materials was used to separate these 13 common hormones. The new column has almost the same efficiency as sub-2-µm totally porous materials and can be used to provide a fast and high resolution analysis.

Table 1. Hormones Used in this Study

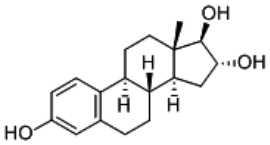
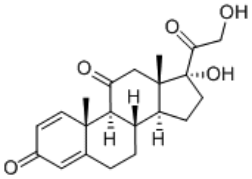
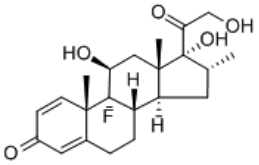
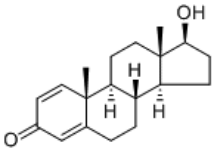
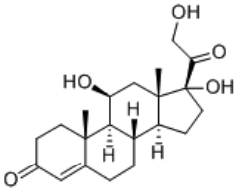
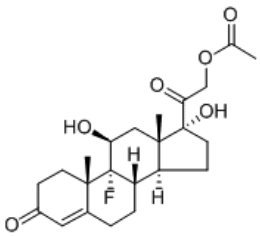
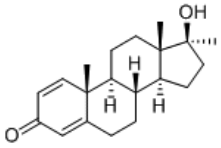
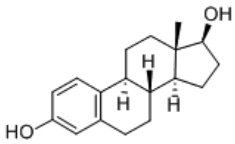
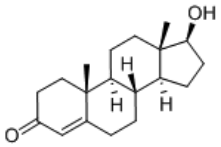
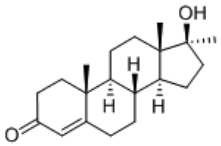
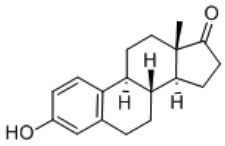
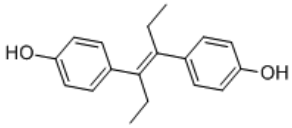
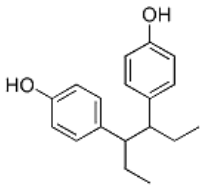
No.	Compound	CAS No.	Structure
1	Estriol	50-27-1	
2	Prednisone	53-03-2	
3	Dexamethasone	50-02-2	
4	Boldenone	846-48-0	
5	Hydrocortisone	50-23-7	

Table 1. Hormones Used in this Study (continued)

6	Fludrocortisone acetate	514-36-3	
7	Metandienone	72-63-9	
8	Estradiol	50-28-2	
9	Testosterone	58-22-0	
10	Methyltestosterone	58-18-4	
11	Estrone	53-16-7	
12	Diethylstilbestrol	56-53-1	
13	Hexestrol	84-16-2	

Experimental

Reagents and Chemicals

All reagents and solvents were HPLC or analytical grade. Hormone standards were purchased from NICPBP (National Institute for the Control of Pharmaceutical and Biological Products). Shrimp was purchased from a local market.

Stock solutions (1 mg/mL) were prepared in methanol and kept in the freezer (–20 °C). Working solutions were prepared using the stock solution diluted with methanol. The working solutions should be prepared every week and stored below 4 °C.

Equipment and Materials

Agilent Bond Elut QuEChERS EN Extraction kits, p/n 5982-5650, and Agilent Bond Elut QuEChERS dispersive-SPE kits for Drug Residues in Meat, 15 mL, p/n 5982-4956 (Agilent Technologies Inc., DE, USA). Flying Pigeon Centrifuge (Anting Science Instrument, Shanghai, P.R.China).

HPLC Method

Instrument	Agilent 1290 Infinity LC with DAD detector	
Column	Agilent Poroshell 120 EC-C18 3.0 x 100 mm 2.7 µm (p/n 695975-302)	
Flow rate	0.8 mL/min	
Injection volume	10 µL	
Column temperature	30 °C	
Detection wavelength	230 nm	
Mobile phase	Water-acetonitrile gradient	
Time (minutes)	% Water	% Acetonitrile
0	80	20
6	50	50
8	10	90

Sample Preparation

The sample preparation procedure includes sample homogenization, extraction/partitioning, and dispersive-SPE cleanup.

The shrimp purchased from a local grocery store, was washed and chopped into small pieces. The chopped shrimp was homogenized thoroughly with a food grinder and stored at 20 °C. A 5-g (±0.05 g) sample of homogenized shrimp was weighed in a 50 mL centrifuge tube. The tubes were centrifuged for 30 s to move the sample from the inside tube wall to the bottom of the tube. Samples were then fortified with appropriate QC spiking solutions when necessary. After vortexing the sample for 30 s, 5 mL of water were added. Tubes were then vortexed for 10 s to mix. A 10 mL volume of ACN was added to each tube. Tubes were capped and shaken by hand for 30 s. An Agilent Bond Elut QuEChERS EN extraction salt packet (p/n 5982-5650) was added to each tube. Sample tubes were capped tightly and shaken vigorously for 1 min. Tubes were centrifuged at 4,000 rpm for 5 min at 4 °C.

A 6 mL aliquot of the upper ACN layer was transferred into an Agilent Bond Elut QuEChERS dispersive-SPE 15 mL tube for drug residues in meat (p/n 5982-4956). This 15 mL dispersive-SPE tube contained 150 mg of C18 and 900 mg of anhydrous MgSO4. The tubes were tightly capped and vortexed for 1 min. The 15 mL tubes were centrifuged at 13,000 rpm for 3 min at 4 °C. A 4-mL volume of extract was transferred into another tube and dried by N_2 flow at 35 °C. Samples were reconstituted into 2 mL of 1:4 ACN/H_2O. After vortexing and sonicating for 10 min, the sample was filtered through a 0.22 µm Cellulose Acetate Spin Filter (p/n 5185-5990). The clear, filtered sample was transferred into an autosampler vial. The samples were capped and vortexed thoroughly in preparation for HPLC analysis.

Results and Discussion

Separation

Figure 1 shows the standard hormone mixture separated on a Poroshell 120 EC-C18 column. All 13 compounds were well separated in 8 minutes in a 20% acetonitrile solution. Each compound was present at a 1 ppm level and excellent sensitivity was achieved at this level. To study the system suitability for the shrimp sample, the hormone mixture was spiked into the blank sample after the QuEChERS extraction method. The gradient method was adjusted so that the target compounds were separated from the matrix. Figure 2 shows the chromatograms of blank sample and 0.5 ppm spiked into the blank sample. The analysis time was extended to 10 minutes to allow matrix and nonrelevant sample components to be eluted. This is an excellent overall analysis time for this sample matrix and is a result of achieving high resolution quickly on the Poroshell 120 EC-C18 column.

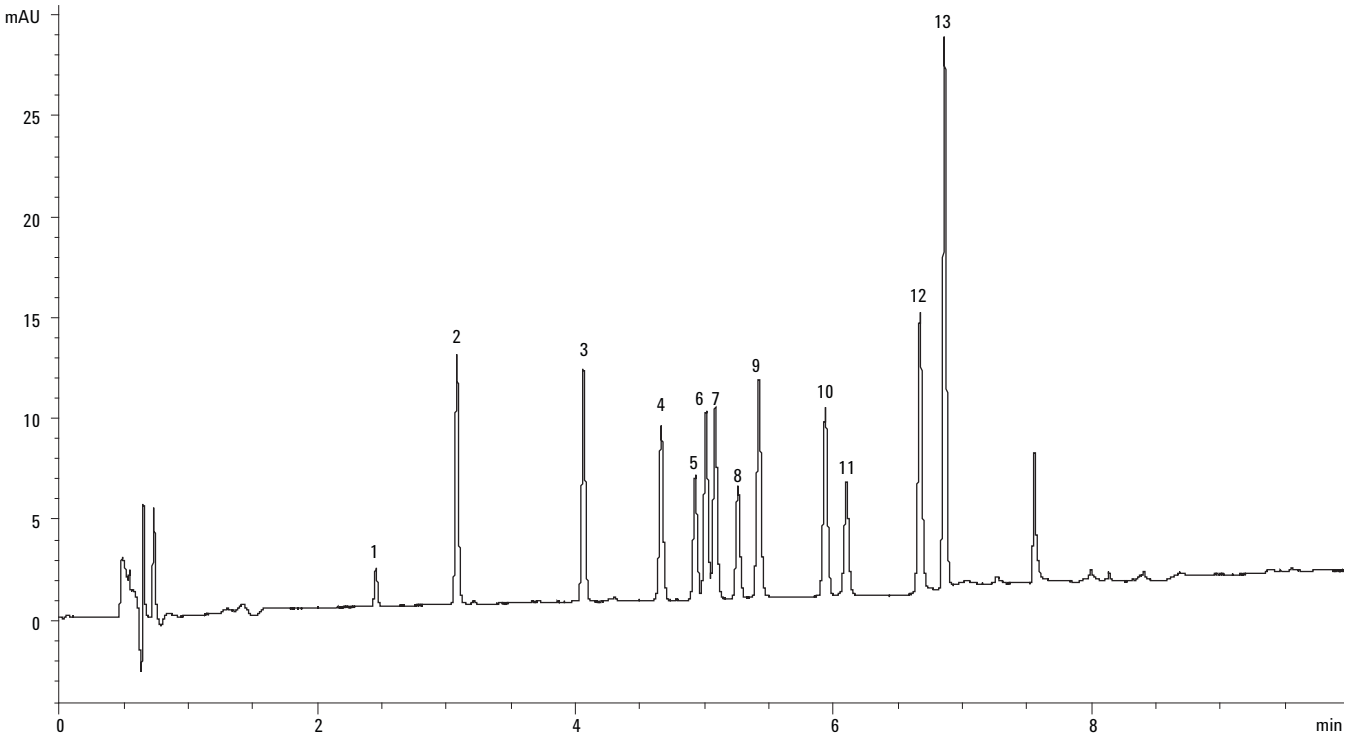


Figure 1. Chromatogram of 1 ppm standard compounds in 20% acetonitrile solution on the Agilent Poroshell 120 EC-C18, 3.0 x 100 mm, 2.7 µm column.

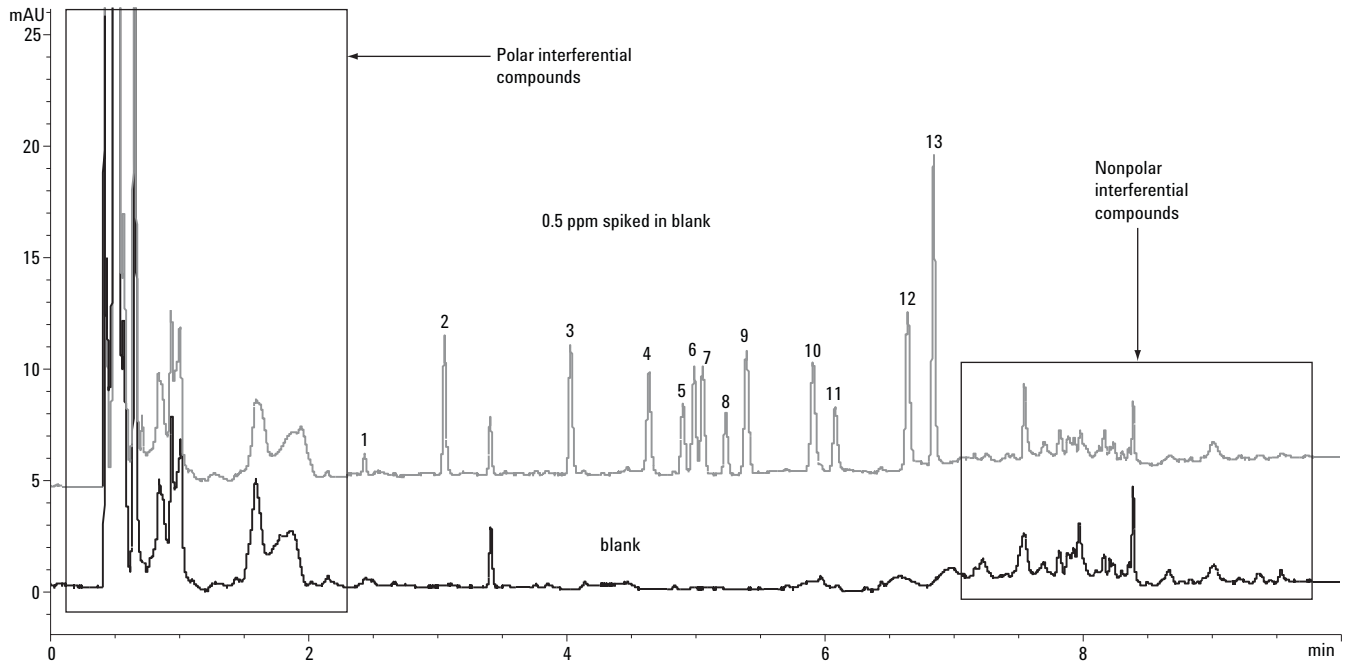


Figure 2. Chromatograms of blank sample and 0.5 ppm spiked in blank sample.

Linearity, Limits of Detection

The stock solution was diluted into 0.5 ppm, 1 ppm, 2 ppm, 5 ppm and 10 ppm standard solutions with blank solution as the calibration standard and they were analyzed by HPLC to make a calibration curve. Linear regressions were calculated for the hormones based on the peak areas and the solution concentrations. Limits of detection (LOD) were calculated with a signal-to-noise ratio of 3 based on the data at 0.5 ppm concentration. The linearity and LOD are shown in Table 2. The data showed good linearity in the range of 0.5–10 mg/kg.

Table 2. Linearity and LOD of Hormones by HPLC

No.	Compound	Regression equation	Correlation coefficient	LOD (mg/kg)
1	Estriol	Y = 3.331x+0.306	0.9997	0.22
2	Prednisone	Y = 22.182x+0.0212	0.9999	0.023
3	Dexamethasone	Y = 22.622x+0.0523	0.9999	0.0086
4	Boldenone	Y = 18.829x+1.955	0.9998	0.031
5	Hydrocortisone	Y = 14.085x+0.0388	0.9999	0.044
6	Fludrocortisone acetate	Y = 21.152x+0.0591	0.9999	0.029
7	Metandienone	Y = 22.052x+0.0325	0.9999	0.022
8	Estradiol	Y = 12.377x+0.0108	0.9999	0.039
9	Testosterone	Y = 25.370x+0.0748	0.9999	0.019
10	Methyltestosterone	Y = 23.149x+0.651	0.9999	0.033
11	Estrone	Y = 13.641x+0.690	0.9999	0.057
12	Diethylstilbestrol	Y = 31.526x+0.347	0.9999	0.023
13	Hexestrol	Y = 51.174x+0.224	0.9999	0.012

Note: x- Concentration (ng/uL); y- Area

Recovery and Repeatability

The precision of the method was determined in terms of the recovery of spiked hormone standards in homogenized shrimp at 0.5, and 10 mg/kg. The analysis was repeated six times for each level. The chromatograms of the blank and the spiked standard (0.5 mg/kg and 10 mg/kg) samples are shown in Figure 3. The data in Table 3 demonstrate excellent recovery and reproducibility for the QuEChERS method developed for hormone determination in shrimp.

Table 3. Recoveries and RSDs of Hormones in Shrimp

Compound	Spiked level (mg/kg)	Recovery (%)	RSD (n = 6, %)
Estriol	0.5	107.2	3.5
	10	98.2	0.98
Prednisone	0.5	97.6	2.3
	10	101.7	0.58
Dexamethasone	0.5	101.8	0.96
	10	96.1	1.2
Boldenone	0.5	98.9	1.5
	10	96.2	1.8
Hydrocortisone	0.5	103.5	1.5
	10	92.3	0.23
Fludrocortisone acetate	0.5	104.3	1.9
	10	91.8	0.17
Metandienone	0.5	100.0	1.4
	10	95.6	0.25
Estradiol	0.5	99.4	1.3
	10	97.8	0.54
Testosterone	0.5	98.0	0.85
	10	98.2	0.15
Methyltestosterone	0.5	97.1	0.99
	10	92.1	0.63
Estrone	0.5	103.4	1.2
	10	92.5	0.68
Diethylstilbestrol	0.5	100.9	1.9
	10	97.3	0.79
Hexestrol	0.5	98.5	1.6
	10	91.6	0.81

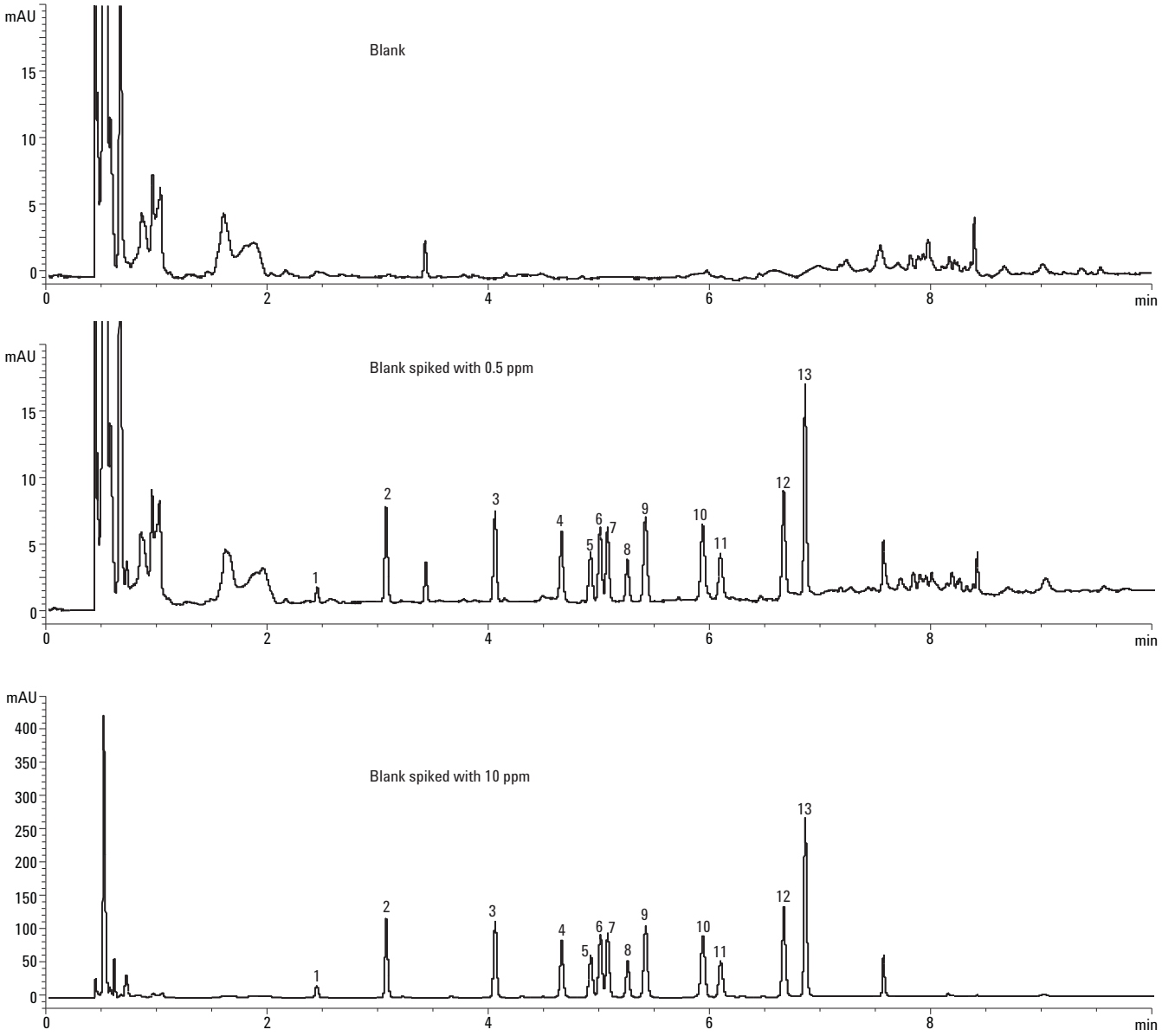


Figure 3. Chromatograms of shrimp sample blank and shrimp sample spiked with 0.5 ppm and 10 ppm standard mixture.

Conclusions

The Agilent Bond Elut QuEChERS Buffered Extraction EN kit and the Agilent dispersive-SPE kit for drug residues in meat provide a simple, fast and effective method for the purification of hormones in shrimp. Compared to the other sample pretreatment methods, such as LLE and SPE, the QuEChERS method is easier to handle, faster, labor-saving, and cheaper. The recovery and reproducibility, based on matrix spiked standards, were acceptable for multiresidue hormone determination in shrimp. The Agilent 1290 Infinity LC with the Agilent Poroshell 120 column resolved the 13 compounds in 10 min and all the compounds were well separated from the matrix. The method developed is suitable for the determination of hormones in shrimp at low mg/kg levels.

Reference

1. Chen-Hao Zhai and Yun Zou, "Determination of Hormones in Fish (Carassius Carassius) by Bond Elut OPT Solid Phase Extraction with High Performance Liquid Chromatography" Agilent Technologies publication, 5990-3845EN (2009).

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