Application Note

Comparison of compounds in Bourbon vanilla extract and vanilla flavor

Category	Food analysis
Matrix	Extract from Bourbon vanilla pods, vanilla flavors
Method	HPLC
Keywords	Vanillin, Bourbon vanilla, natural flavor,
Analytes	4-Hydroxybenzaldehyde, 4-Hydroxybenzoic acid, Coumarin, Ethylvanillin, Eugenol, Guaiacol, vanillic acid, vanillin
ID	**VFD0136N**, 06/15

Summary

This application note presents a gradient method using a core shell column for the determination of various substances in Bourbon vanilla extracts as well as in natural and artificial vanilla flavors. The aim is to compare the ingredients to get a prove of the authenticity of Bourbon vanilla. Applying the KNAUER AZURA Analytical system, a very short analysis time and therewith low eluent consumption could be reached. The high speed and reliability of the method make it well-suited for routine analyses in food control.

Introduction

Vanillin is one of the most popular flavoring agents used in various food products, beverages as well as in the pharma and perfume industry. With a high demand for the supply of vanilla pods and the continued increase in price, artificial vanilla flavoring agents of synthetic origin are nowadays available. It is a phenolic aldehyde, primarily obtained from the extracts of the pods of Vanilla planifolia, a species of vanilla orchids. It is also found in roasted coffee and Chinese red pine. Chemically, vanillin is 4-Hydroxy-3-methyl benzaldehyde.[1]

The high demand for vanillin far exceeds the supply from all sources of vanilla orchids, which is the only vanilla flavor allowed to use the name “Bourbon vanilla”. The high price of natural vanillin, compared with that of synthetic vanillin, and the poor availability are the reasons for the production of vanillin via chemical synthesis since the 1870s. These processes use coniferin, guaiacol or eugenol as a precurser.[2]

Biotechnological processes like fermentation that use ferulic acid and rice bran as precursors of vanillin are a relatively new. Biotechnologically produced vanillin is much more cost intensive than chemically synthesized vanillin, but the EU allows this product to use the designation “natural vanilla flavor” while chemically synthesized flavor has to use the name “vanilla flavor”. Some substances from the chemical or biotechnological processes are unwanted in food products due to negative health effects. This makes an analytical control indispensable. These molecules as well as the precursors used in the chemical synthesis are appropriate markers for the differentiation between synthetic vanilla flavor and Bourbon vanilla extract. Analysis of this kind is getting more and more attention caused by food scandals in the last years.

While an exact statement about the origin of vanilla flavor is only possible after complex analytical methods like isotopic analysis, a first statement about the origin of vanilla flavor is already possible by screening for marker substances using relatively easy, cost effective, and robust HPLC methods. Therefore, in this work ethanolic extracts of Bourbon vanilla pods are compared with synthetic vanilla flavors to find marker substances for the origin of the flavor.

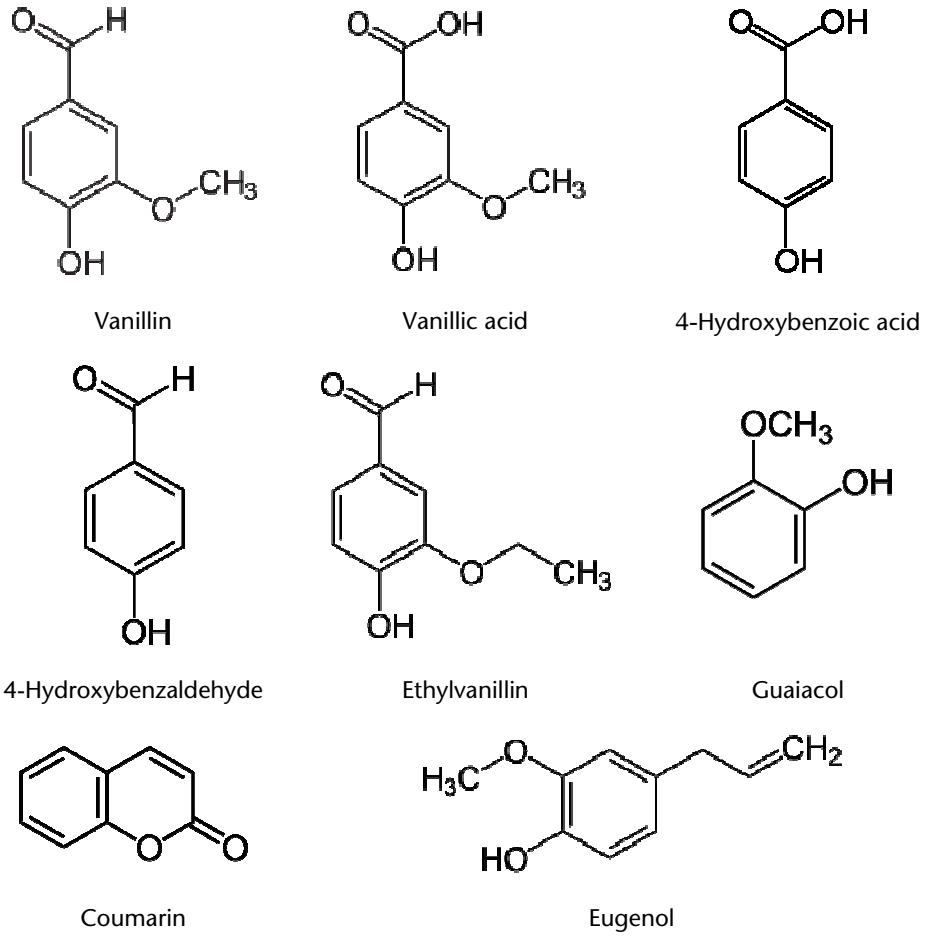

Fig. 1
Chemical structures of the analytes

Experimental sample preparation

Bourbon vanilla can be bought in supermarkets as they sell it as a baking supplement. Vanillin can be extracted easily from Bourbon vanilla pods using an ethanol as the solvent. To enhance the extraction process, the mixture is put in an ultrasonic bath and left to stay for extraction overnight. After filtration through a syringe filter and dilution with the mobile phase, the sample is ready for analysis by HPLC.

Experimental preparation of standard solution

Standards were solved and diluted in the mobile phase. 4-Hydroxybenzoic acid, Vanillic acid and 4-Hydroxybenzaldehyde were analyzed in addition to the vanillin as typically occurring substances in Bourbon vanilla extract. In addition, Guaiacol, Ethylvanillin, Coumarin and Eugenol were analyzed as markers for synthetic vanilla flavor or even unwanted additives.[3]

Method parameters

Column	BlueShell 80-2.6 C18A, 100 x 2 mm		
Eluent A	Water + 0.05 % TFA		
Eluent B	Acetonitrile/Water 80:20 (v/v) + 0.1 % TFA		
Gradient	**Time [min]**	**% A**	**% B**
	0.00	85	15
	0.50	65	35
	1.50	25	75
	1.70	0	100
	2.50	0	100
Flow rate	0.8 ml/min		
Injection volume	1 µl		
Column temperature	40 °C		
System pressure	approx. 600 bar		
Detection	UV at 280 nm, 260 nm, 230 nm and 3D data (50 Hz)		
Run time	5 min incl. column re-equilibration		

Results

Fig. 2 shows the overlay of the standard with a real sample of Bourbon vanilla extract. It is obvious that all substances could be separated in less than 2 minutes and elute in very sharp peaks. This makes the method really attractive for routine analyses because of its rapidity. The high resolution is achieved by the very low dead volume of the AZURA Analytical HPLC system and the high resolution core-shell column.

Furthermore, fig. 2 shows that in the sample of Bourbon vanilla extract the substances 4-hydroxybenzoic acid, Vanillic acid, 4-Hydroxybenzaldehyde and Vanillin could be found as expected. At the same time, the unwanted substances Guaiacol, Ethylvanillin, Coumarin and Eugenol were not found.

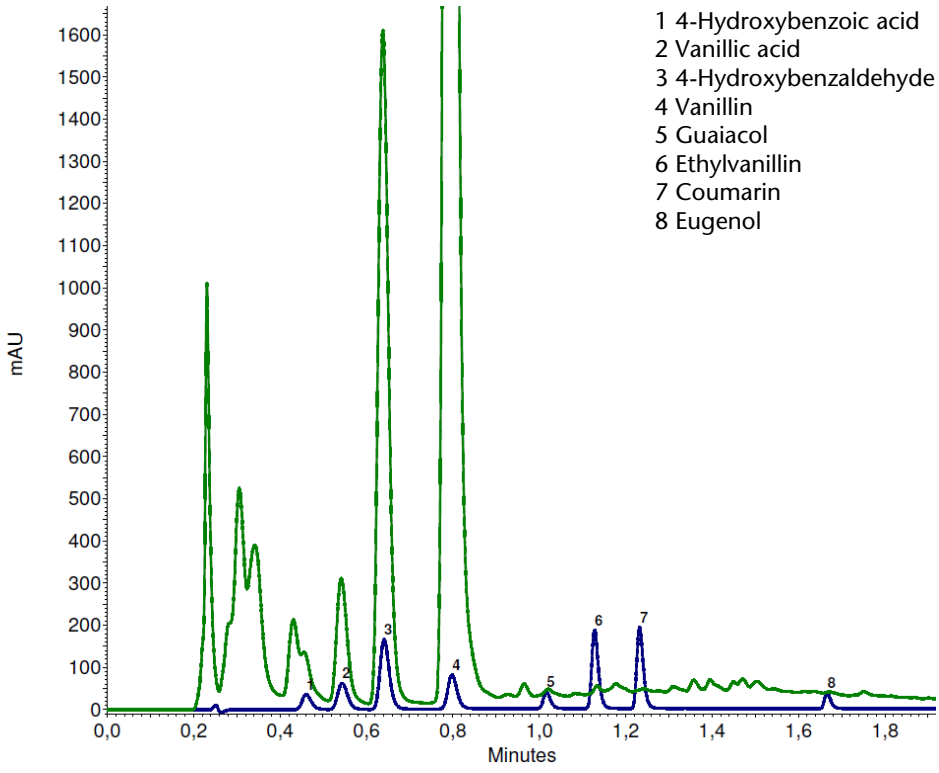

Fig. 2

Chromatograms at 280 nm, blue: Standard, green: Bourbon vanilla extract

At the same time, 3D data were acquired using the DAD 6.1 L diode array detector. This allows recording spectra of every single substance to get further information about the identity of occurring peaks. Fig. 3 shows the recorded spectra.

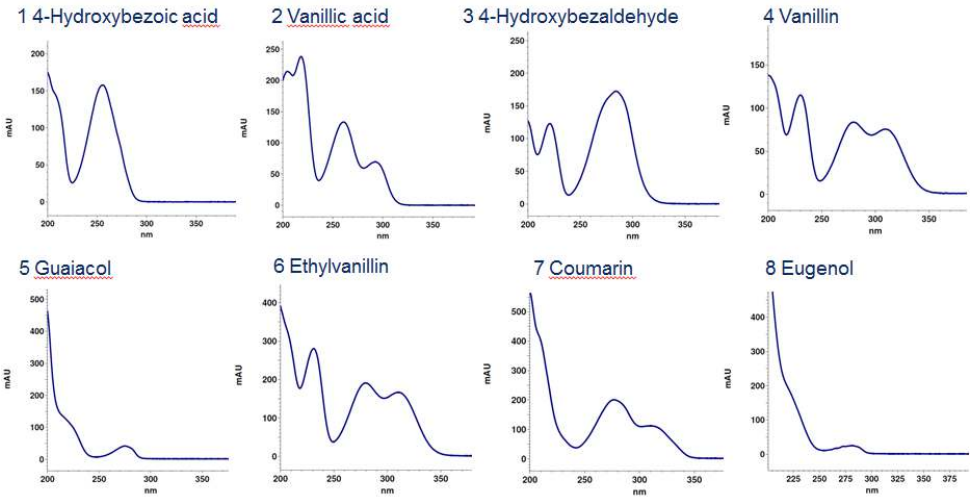

Fig. 3

Spectra of standards recorded with the DAD 6.1L

Based on these results, a commercially available „Bourbon vanilla extract" and a synthetic vanilla flavor were analyzed. In the Bourbon vanilla extract the typical markers for the vanilla pods could be found while the markers for synthesized flavor were not detected (see fig. 4).

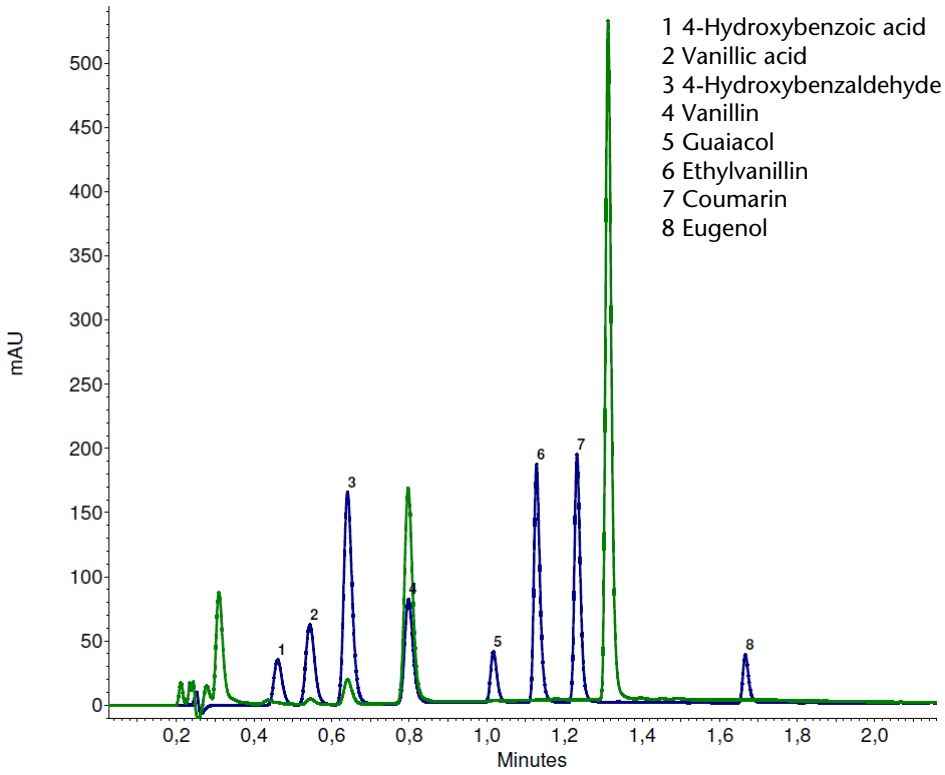

Fig. 4

Chromatograms at 280 nm, blue: Standard, green: Bourbon vanilla flavor

Additionally, the peak eluting at about 1.3 min could be identified as a substance, that may be analog to 4-Hydroxybenzaldehyde because the spectra are matching. For a proof of this thesis, further analysis would be needed like the coupling of the HPLC to a mass spectrometer.

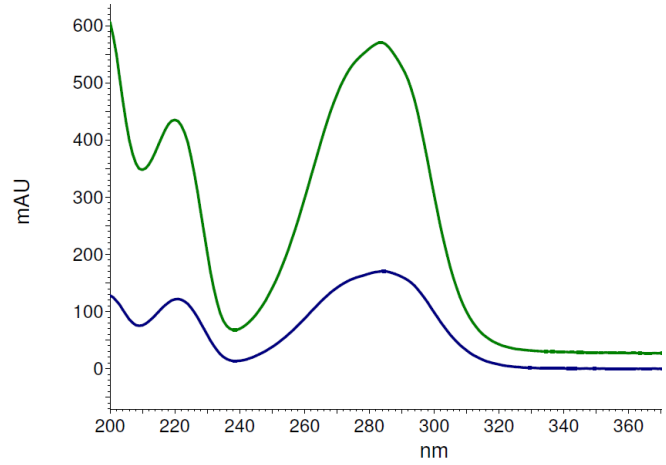

Fig. 5

DAD spectra, blue: 4-Hydroxy-benzaldehyde, green: unknown peak from Bourbon vanilla flavor at ca. 1.3 min

For the synthetic vanilla flavor, results were completely different. Markers for the natural extract were not detected in significant amounts while the relative amount of vanillin itself was much higher.

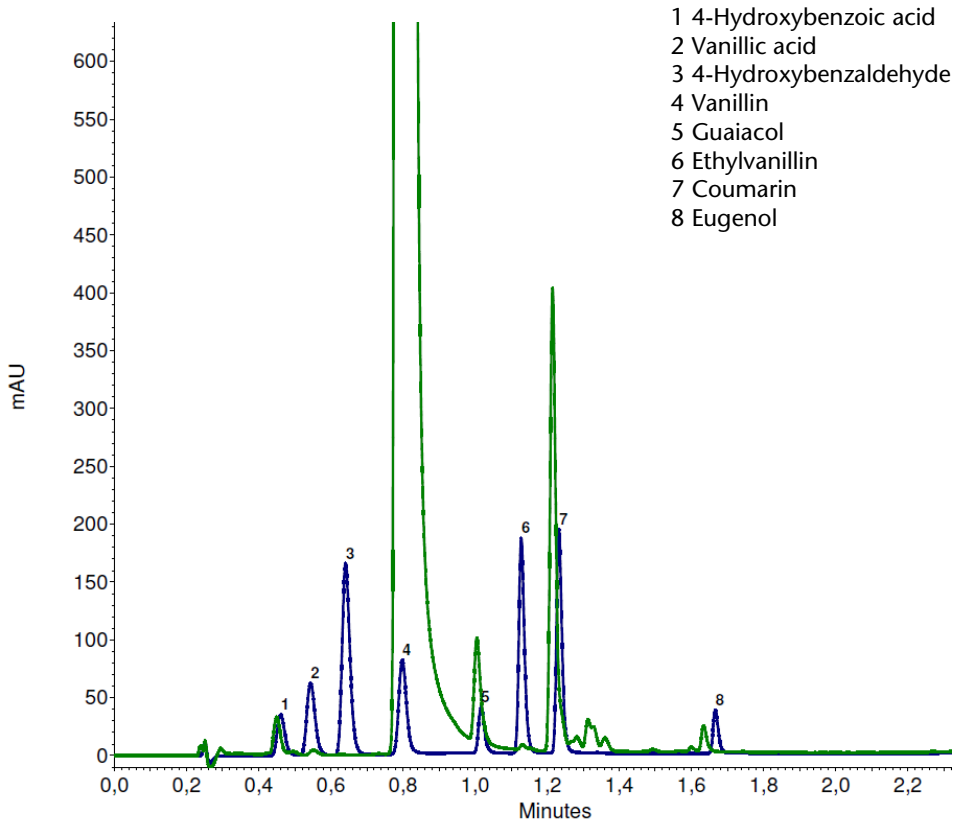

Fig. 6

Chromatograms at 280 nm, blue: standard, green: artificial vanilla flavor

The peak eluting at about 1.2 min grabs special attention because it elutes at a time very close to Coumarin, what is a really unwanted substance in food products because it has adverse health effects. The German Federal Institute for Risk Assessment (Bundesinstitut für Risikobewertung BfR) defines a daily tolerable intake (TDI) of 0.1 mg per kg body weight that can be consumed a whole life long without negative health effect.4 Coumarin can be found in significant amounts in tonka beans which also have a strong vanilla flavor. They are used as a different source for vanilla flavor. Again the DAD spectra of Coumarin and the peak from synthetic vanilla flavor were compared. From fig. 7 A it becomes obvious that the substance is in all likelihood not Coumarin, because the spectra differ at several points.

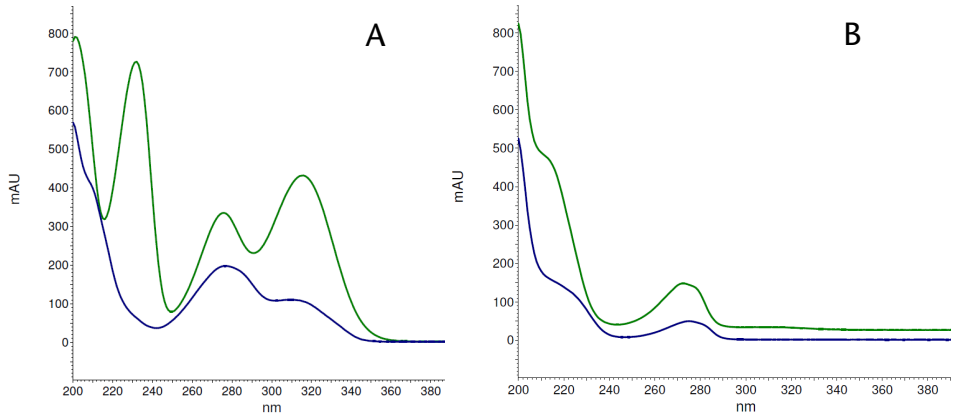

Fig. 7
DAD spectra

A: blue: Coumarin, green: Peak from artificial vanilla flavor at ca. 1.2 min

B: blue: Guaiacol, green: Peak from artificial vanilla flavor at ca. 1.0 min

Fig. 7 B shows an overlay of the spectra from Guaiacol and the sample at 1.0 min. The similarity makes it likely to be the substance Guaiacol, which is used as a precursor in the vanillin synthesis process. This is today's established process for the synthesis of vanillin and therefore the occurrence of this substance in the sample is not surprising. At the same time we can see from these results that Guaiacol is a good marker for chemically synthesized vanillin.

Conclusion

The presented HPLC method with diode array detection is well-suited for fast and easy screening the origin of vanilla flavors in food products. Using comparable easy-to-handle, cost-effective and robust equipment this difficult problem could be solved. The substances 4-Hydroxybenzoic acid, Vanillic acid and 4-Hydroxybenzaldehyde as well as their relative concentrations to Vanillin could be used as markers for natural Bourbon vanilla flavor while Guaiacol, Ethylvanillin, Coumarin and Eugenol could be used as markers for synthetic vanilla flavor. Especially Guaiacol the chemical precursor for today's most widely used vanillin synthesis process could be detected in artificial vanilla flavor.

References

1 Krishna Veni et al, J Adv Sci Res, 2013, 4(1): 48-51: Analysis of Vanillin In Food Products By High Performance Thin Layer Chromatography

2 JAGERDEO ET AL.: JOURNAL OF AOAC INTERNATIONAL VOL. 83, NO. 1, 2000 Liquid Chromatographic Determination of Vanillin and Related Aromatic Compounds

3 AUTHENTICITY OF VANILLA AND VANILLA EXTRACTS, Elke Anklam, JOINT RESEARCH CENTRE EUROPEAN COMMISSION, Environment Institute Food & Drug Unit, 1993, EUR 15561 EN

4 Neue Erkenntnisse zu Cumarin in Zimt, Stellungnahme Nr. 036/2012 des BfR vom 27. September 2012, http://www.bfr.bund.de/cm/343/neue-erkenntnisse-zu-cumarin-in-zimt.pdf"

Physical properties of recommended column

To obtain ultra-high performance results comparable with sub-2 µm columns, without the disadvantage of high backpressure, the new BlueShell columns are your first choice. BlueShell columns are packed with special core-shell particles, developed to provide improved speed, higher resolution, and reduced eluent consumption, all while keeping moderate HPLC backpressures. BlueShell C18 A is a polar endcapped C18 phase with alternative selectivity; designed for use with 100 % aqueous eluents for analysis of very polar compounds, basic pharmaceutical ingredients, water soluble vitamins, catecholamines as well as organic acids.

Stationary phase	BlueShell 80 - 2.6 C18 A
USP code	L1
Pore size	80 Å
Pore volume	0.8 ml/g
Particle size	2.6 µm
Form	spherical
Surface area	130 m^2/g
% C	9
Endcapping	Yes, polar
Dimensions	150 x 2 mm
Order number	15BD184SHA

Recommended instrumentation

This application was carried out using a binary high pressure gradient HPLC Plus system equipped with degasser, autosampler, column thermostat, and diode array detector. Other configurations are also available. Please contact KNAUER to configure a system that's perfect for your needs.

Description	Order No.
AZURA P 6.1L Quaternary analytical HPLC pump with degasser 10 ml pump head	APH34EA
AZURA Detector DAD 6.1L 190 - 1000 nm, diode array detector	ADC11
AZURA Column Thermostat CT 2.1 for constant temperatures and reproducible results	A05852
Autosampler 3950 standard version of a fast and very versatile analytical HPLC autosampler, 1000 bar, 0.1-5000 µl injection volume	A50070
Standard KNAUER LightGuide UV Flow Cell Cartridge 10 mm path length, 1/16", 2 µl volume, 100 bar, biocompatible	AMC19
AZURA Eluent tray E 2.1L Eluent tray for up to 6 x 1000 ml bottles (delivery without bottles) or 4 x 2.5 l bottles or 2 x 5 l bottles or 1 x 10 l bottle	AZC00
OpenLAB CDS EZChrom Edition Basic Workstation license, includes System Suitability, Fraction Collector Control and Software Maintenance Agreement	A2600-1
OpenLAB CDS EZChrom Edition Option 3D option for DAD	A2611-1

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