

Extraction of Vanillin From a Cola Soft Drink Using SOLA with Analysis by GC-MS

Anila I Khan, Thermo Fisher Scientific, Runcorn, Cheshire, UK

Key Words

- SOLA Cartridges and Plates
- TraceGOLD TG-WaxMS A
- Vanillin
- Soft Drinks
- SPE
- Capillary GC

Abstract

Vanillin is a widely used flavor compound in food and beverages. In this application note, extraction and quantification of vanillin from cola is demonstrated using a revolutionary new solid phase extraction product, SOLA and an acid-deactivated polyethylene glycol phase GC column.

Introduction

Vanillin is an aromatic compound and is widely used as a flavor additive in food and beverages. This synthetic compound is normally made from guaiacol. This is used in the replacement of natural vanilla which is becoming scarce and expensive. As the demand for vanilla flavoring increases, the supply of natural vanilla is decreasing, therefore the food and beverage industries are now increasingly using artificial vanilla to flavor their food and drinks.

In this application note, the extraction of vanillin is demonstrated using Thermo Scientific SOLA products, which is revolutionary new Solid Phase Extraction (SPE) product range. This first in class SPE product range introduces next generation, innovative technological advancements, giving unparalleled performance characteristics compared to conventional SPE, Phospholipid and Protein precipitation products.

This includes:

- Higher levels of reproducibility
- Higher levels of extract cleanliness
- Reduced solvent requirements
- Increased sensitivity

SOLA™ products have a significant advantage for the analyst when processing compounds in complex matrices particularly in food and flavor industries where higher analysis speed and lower sample detection limits are critical.

The increased performance from SOLA products provides higher confidence in analytical results and lowers cost without compromising ease of use or requiring complex method development.

The pH of 5% vanillin in water is 4.3 and the pKa of the phenol group in vanillin is 7.38¹. SOLA cartridges are used for the extraction of vanillin from cola as the sample is overall neutral which can be retained on the SOLA cartridges. For obtaining a symmetrical peak shape of vanillin, a Thermo Scientific TraceGOLD TG-WaxMS A GC column is used for analysis. The TraceGOLD™ TG-WaxMS A GC column is an acid-deactivated polyethylene glycol phase that allows for the analysis of



acidic compounds. This modified polar phase column resists oxidative damage to the stationary phase yielding excellent peak shapes for acidic compounds. Another advantage of the TraceGOLD TG-WaxMS A GC column is that it can also be used for mass spectrometry applications.

Experimental Details

Chemicals and Reagents	Part Number
Fisher Scientific HPLC grade water	W/0106/17
Fisher Scientific HPLC grade methanol	M/4056/17
Fisher Scientific Analytical grade formic acid	F/1900/PB08

Sample Handling Equipment

Thermo Scientific HyperSep glass block manifold	60104-232
Chromacol 9mm screw 0.3 mL fixed insert amber Micro+ vials	03-FISV (A)
Chromacol 9mm screw 2 mL vial -amber	2-SVW(A)
Chromacol 9mm screw caps with silicone/PTFE septa	9-SC(B)-ST101

Sample Pre-treatment

Cola sonicated for 30 minutes prior to the application stage of SPE sample preparation.

Sample Preparation - SOLA

	Part Number
Compound:	vanillin and benzyl benzoate (internal standard)
Matrix:	cola
Cartridge type:	SOLA, 10 mg/1 mL 60109-001
Conditioning stage:	1 mL methanol, 1 mL water
Application stage:	200 µL of cola
Washing stage:	200 µL water
Elution stage:	200 µL methanol
Additional stages:	Add 200 µL of internal standard

Separation Conditions	Part Number	
Instrumentation:	Thermo Scientific Trace GC Ultra	
Septum:	BTO, 17 mm	31303211
Liner:	focus Splitless liner, 5 x 8 mm	45350033
Column ferrules:	100% Graphite ferrules for Trace injector 0.1-0.25 mmID	29053488
Colum ferrules:	graphite/vespel for transfer line 0.1-0.25mm ID	29033496
Column:	TraceGOLD TG-WaxMS A GC, 30 m x 0.25 mm x 0.25 µm	26087-1420
Carrier gas:	helium	
Split flow:	50 mL/min	
Column flow:	1.2 mL/min, Constant flow	
Oven temperature:	80 °C, 20 °C/min, 250 °C (6.50 min)	
Injector type:	split/splitless	
Injector mode:	splitless (1 min), constant septum purge	
Injector temperature:	250 °C	

MS Conditions

Instrumentation:	Thermo Scientific ISQ GC Single Quadrupole mass spectrometer
Transfer line temperature:	250 °C
Source temperature:	250 °C
Ionization conditions:	EI
Electron energy:	70 eV

Compound	Abbrev	SIM (start time/min)	Quan ion (Qual Ion)	Dwell time/s
Vanillin	-	4.0	152 (151)	0.1
Benzyl benzoate (Internal standard)	ISTD	4.0	212 (105, 91)	0.1

Table 1: SIM Scan parameters

Injection Conditions

Instrumentation:	Thermo Scientific TriPlus Autosampler
Injection Volume:	1 µL
Pre and Post Injection	
Dwell Time:	1 second
Wash solvent:	1:1 methanol/water

Data Processing

Software:	Thermo Scientific XCalibur™
-----------	-----------------------------

Solutions

Preparation of Standards

Internal standard

An internal standard of 200 ng/mL of benzyl benzoate was prepared in methanol.

Calibration Standards

A stock standard solution of 1000 µg/mL of vanillin was prepared in methanol to give STD A.

This was then used to prepare standard solutions in methanol of 1000, 500, 200, 100, 50, 20 and 10 ng/mL. These were Standards B-H

For construction of the calibration curve the standards B-H were mixed 50:50 with the Internal Standard (200 ng/mL)

Spiked sample: A 20 ng/mL vanillin standard was spiked in water and taken through the extraction process. The internal standard (IS) added after eluent was collected giving a ratio of 1:1 of eluent and IS.

Results

The method linearity on a TraceGOLD TG-WaxMS A GC 30 m x 0.25 mm x 0.25 µm column was confirmed with a calibration constructed for vanillin using benzyl benzoate as an internal standard (IS). The concentration range studied was between 10 and 1000 ng/mL. The correlation coefficient (R²) between the area ratio of vanillin and IS was 0.9987, demonstrating a good method linearity (Figure 1).

The extraction of 20 ng/mL of vanillin spiked in water and cola was performed six times using SOLA cartridges. This required the application of only 200 µL of the cola followed by elution in 200µL of methanol into the small volume fixed insert vials. Whereas using the equivalent conventional SPE cartridges, solvent volumes for washing and elution would require more than 1 mL of solvent volume.

After the extraction, the IS was added to the collected eluent. Figure 2 shows the chromatogram of 20 ng/mL of vanillin (m/z 152) spiked in water to which internal standard was added giving an extraction concentration of vanillin to be 10 ng/mL. Figure 3 shows the chromatogram of vanillin extracted from cola followed by the addition of IS and Figure 4 shows the chromatogram of the IS in the cola extract.

The extraction percent recoveries of vanillin spiked in water were measured between 96-103% giving an average recovery of 99% over six extractions with an RSD of 2.7% (Table 2). The average accuracy of the six spiked extractions of vanillin was -0.7%, which demonstrates SOLA cartridges can give greater accuracy and precision data. The vanillin extract from cola gave concentrations between 23.2 – 26.7 ng/mL with an average RSD of 4.6% over six replicate extractions (Table 3). The high precision and accuracy shown by the extraction of vanillin from cola does not require the eluted solvent to be reconstituted therefore eliminating drying time which can take more than 1 hour.

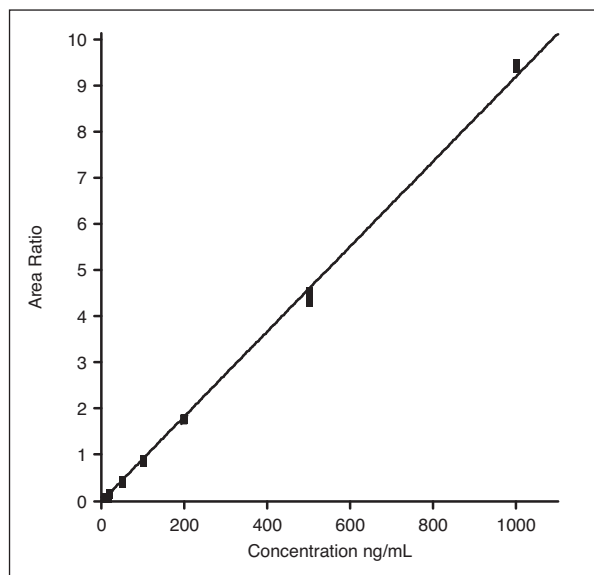


Figure 1: Calibration curve for the concentration range of area response of vanillin and IS.

Conclusion

SOLA cartridges and TraceGOLD TG-WaxMS A GC columns can extract and quantify vanillin from cola with a quick and simple method. The advantage of SOLA cartridges ensures a reduction in elution solvent volume and subsequently reduced drying times in comparison to the loose-packed SPE sorbent. In addition, greater recoveries, accuracy and precision can also be achieved using SOLA cartridges, demonstrating the capabilities of the product.

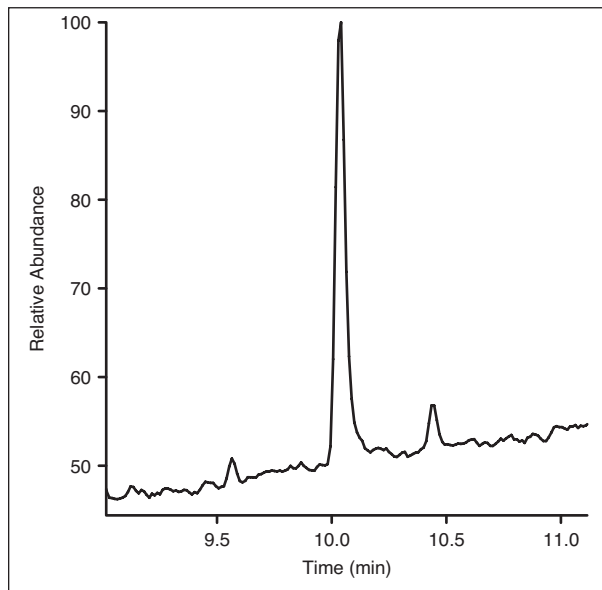


Figure 2: Chromatogram of 20 ng/mL of vanillin spiked in water after the extraction process

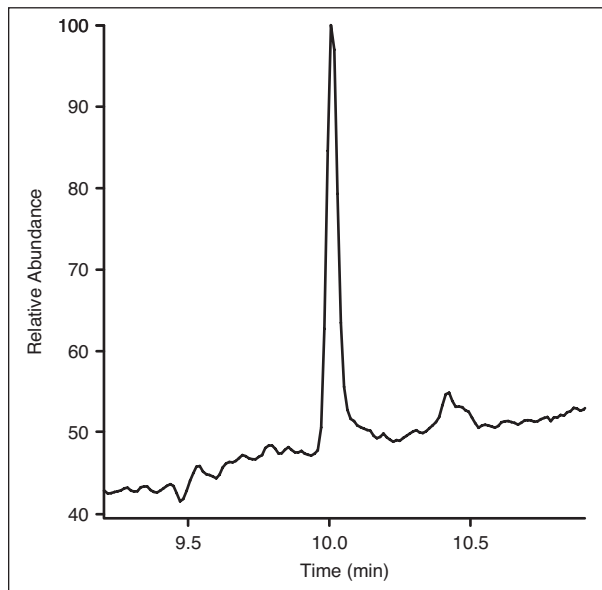


Figure 3: Chromatogram of vanillin in water after the extraction process

www.thermoscientific.com/chromatography

© 2011 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details.

References

1. <http://www.chem.unep.ch/irptc/sids/oecd/sids/121335.pdf>

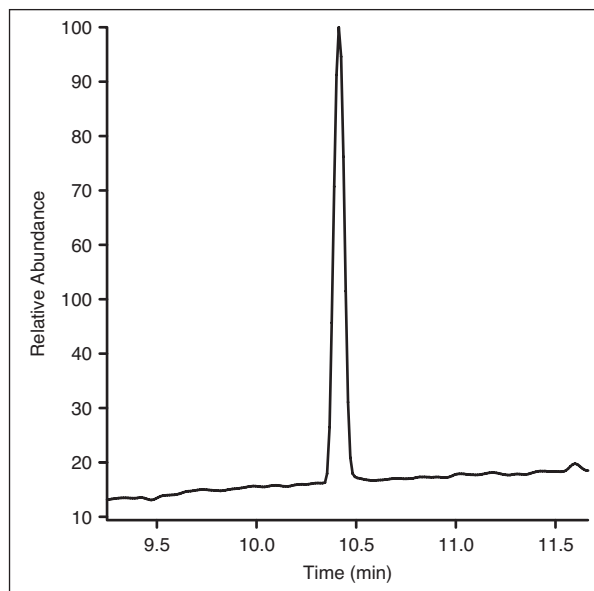


Figure 4: Chromatogram of 100 ng/mL benzyl benzoate (IS) in cola.

Extraction	Calculated Conc ng/mL	Recovery %	% Error
1	19.6	97.9	-2.1
2	19.8	99.0	-1.0
3	19.4	96.8	-3.2
4	19.4	96.8	-3.2
5	20.6	103.2	3.2
6	20.3	101.7	1.7
Average	19.9	99.3	-0.7
Std Dev	0.53	2.67	-
%RSD	2.7	2.7	-

Table 2: Method precision (%RSD) and recovery for the 20 ng/mL of vanillin (data calculated from six replicate extractions).

Extraction	Calculated Conc ng/mL
1	25.6
2	26.7
3	26.0
4	26.0
5	25.7
6	23.3
Average	25.6
Std Dev	1.18
%RSD	4.6

Table 3: Method precision (%RSD) and recovery for vanillin extracted from cola soft drink (data calculated from six replicate extractions).

In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

**North America
USA and Canada**
+1 800 332 3331

**Europe
France**
+33 (0)1 60 92 48 34

Germany
+49 (0) 2423 9431 -20
or -21

United Kingdom
+44 1928 534110

**Asia
Japan**
+81 3 5826 1615

China
+86-21-68654588
or +86-10-84193588
800-810-5118

India
+91-22-6742 9494

**Thermo Fisher
Scientific Australia
Pty Ltd**
1300 735 292 (free call
domestic)

**Thermo Fisher
Scientific New
Zealand Ltd**
0800 933 966 (free call
domestic)

All Other Enquiries
+44 (0) 1928 534 050

**Technical
Support**

North America
800 332 3331

**Outside North
America**
+44 (0) 1928 534 440

ANCCSOLAEXVAN 1211