Application Note: ANCCSSOLAEXVAN

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

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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 guaicol. 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 7.38[1]. 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/m. 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.

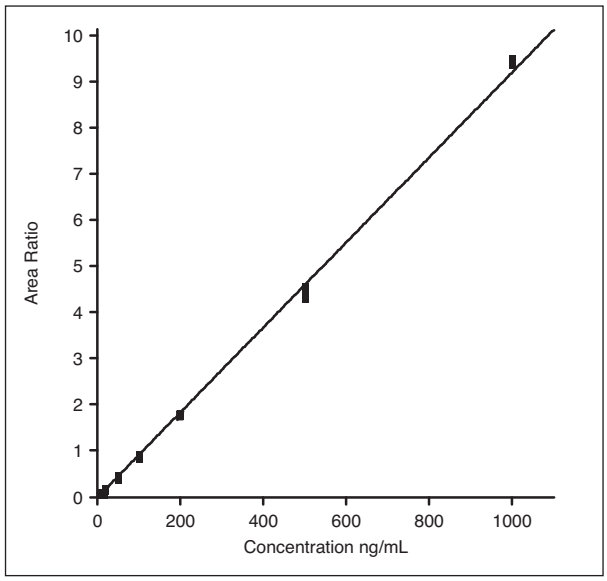

Figure 1: Calibration curve for the concentration range of area response of vanillin and IS.

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.

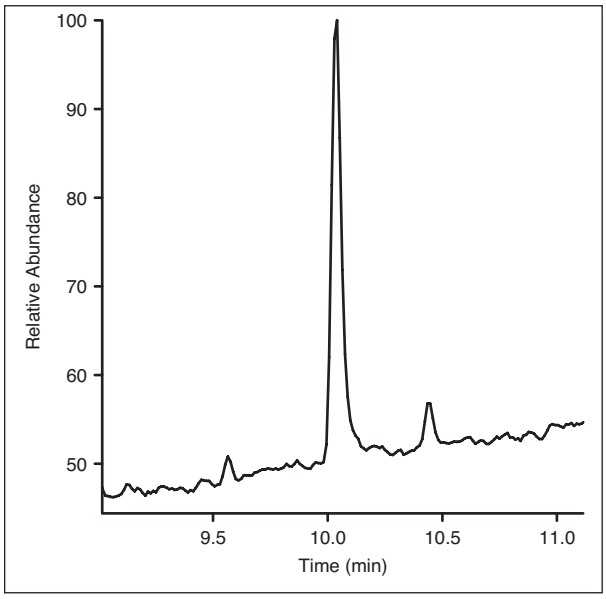

Figure 2: Chromatogram of 20 ng/mL of vanillin spiked in water after the extraction process

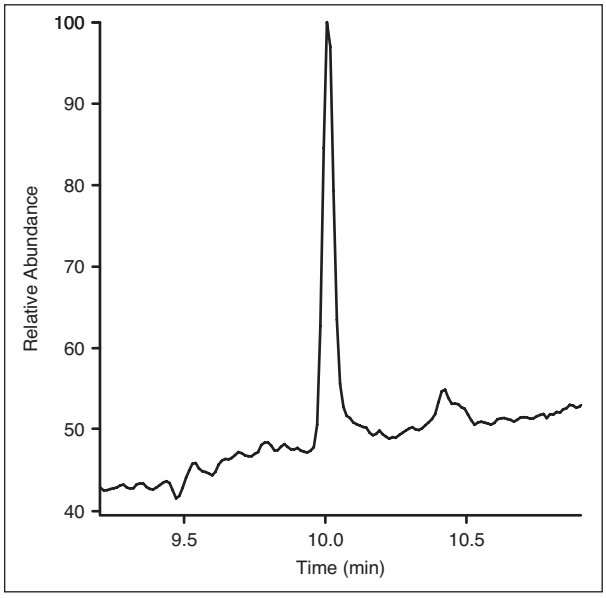

Figure 3: Chromatogram of vanillin in water after the extraction process

References

1. http://www.chem.unep.ch/irptc/sids/oecdsids/121335.pdf

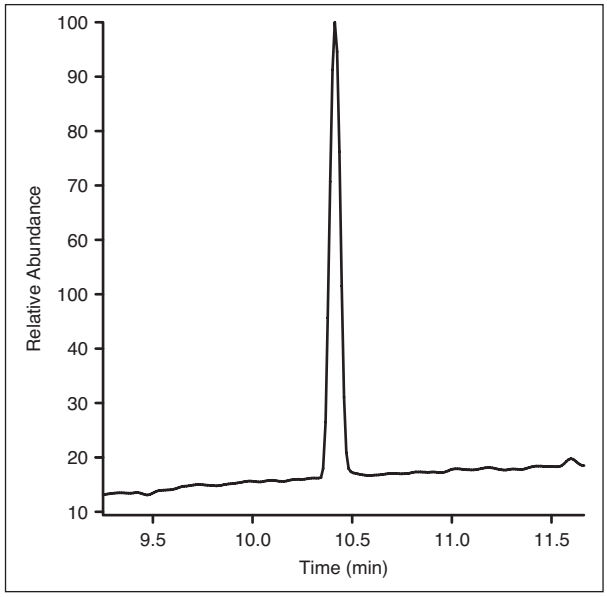

Figure 4: Chromatogram of 100 ng/mL benzyl benzoate (IS) in cola.

Extraction	Calculated Conc ng/mL	Recovery %	% Errorr
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).

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