PURIFICATION OF VANILLIN: A COMPARATIVE STUDY BETWEEN PREP HPLC AND CPC

APPLICATION NOTE AN1035

APPLICATION BENEFITS

- 500 mg of pure vanillin obtained from 6.3 g of crude extract for further isotopic analysis to confirm its naturality.
- Comparison of prep HPLC and CPC columns on vanillin purification.

ADDRESSED ISSUES

- One Prep LC system (PLC 2050) for both prep HPLC and CPC.
- CPC (CPC 250 PRO) is the best option for complex extract treatment in comparison to prep HPLC.
- Drastically reduce the solvent consumption for lower cost and environmental impact.

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INTRODUCTION

Vanillin (4-hydroxy-3-methoxybenzaldehyde), (Figure 1) is the major component of natural vanilla, which is one of the most widely used and important flavouring materials worldwide.

Vanillin can be extracted from the bean, or pod of the tropical Vanilla orchid (principally *Vanilla planifolia* Andrews)[1], produced recombinantly, or synthesized. The origin of pure vanillin used in food formulation can be distinguish by its isotopic signature[2]. This method may require a preliminary purification step.

In this study, an ethanolic crude extract of vanilla is used to show the capacity of the PLC 2050 system to work with different types of chromatographic columns. Vanillin separations were performed on an HPLC preparative column and CPC 250 PRO column. Vanillin purity, solvent consumption, and quantity injected are compared between both techniques.

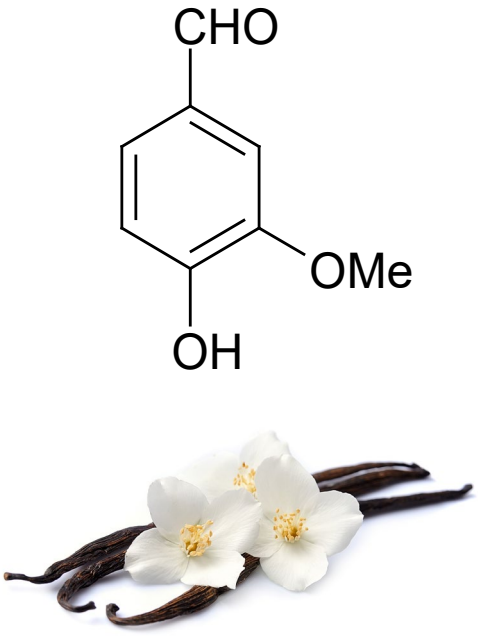

Figure 1
Vanillin

MATERIALS AND METHOD

Systems

A Gilson CPC 250 PRO column (Figure 2), or Prep HPLC column, Hibar 250 X 25, C18, 5µm from Merck (Figure 3) were connected to a Gilson PLC 2050 system, configured with a 50 mL/min quaternary gradient pump, UV/Vis detector, fraction collector and Gilson Glider software.

HPLC analysis was performed on LaChrom Elite HPLC system (VWR) equipped with Photodiode Array Detector, (PDA), (200-800 nm).

Figure 2
PLC Purification System (right) shown with Centrifugal Partition Chromatography (CPC) Column (left)

Figure 3
PLC 2050 shown with a Prep LC column

Sample

Crude extract was first analyzed by HPLC (Table 1). Vanillin is identify at rt = 9.9 min and 63.7% of peak area at 280 nm, (Figure 4). The content of vanillin in the crude extract is estimated at 9% (w/w) after external standard calibration. Most of the molecules are not detected by UV in this extract.

A 28 g/L solution of Vanillin extract was prepared for CPC injection while a lower concentration of 0.08 g/L was used for prep HPLC to avoid any over pressure at the injection.

Table 1
Analytical HPLC conditions

HPLC column:	Purosphere RP18, 250 X 4.6 mm, 5 µm
Mobile phase A:	Water at pH 2.8 (HCl)
Mobile phase B:	Methanol
Time program:	isocratic mode : 30%B-70%A
Flow rate:	1 mL/min
Injection volume:	2 µL
Temperature:	30°C

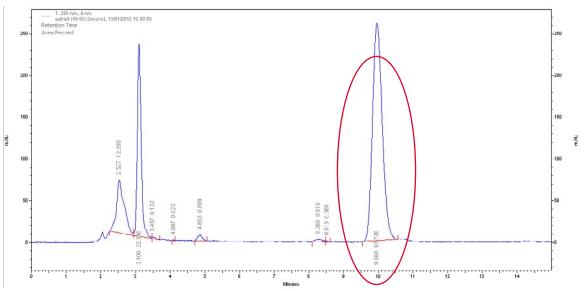

Figure 4
HPLC analysis at 280 nm of crude mixture.

Methods

For both preparative HPLC and CPC runs, the methods used are detailed in tables 2 and 3 respectively. Preparative HPLC was performed by injecting 90 mg of crude oil with an isocratic mode at 20 mL/min. CPC run was done in ascending mode at 30 mL/min by injecting 6.3 g of crude oil.

Table 2
Prep HPLC conditions

Prep HPLC conditions:	Lichrospher 100 RP18 250 X 50 mm, 5 µm
Elution flow rate:	20 mL/min
Mobile phase A:	Water at pH 2.8 (HCl)
Mobile phase B:	Methanol
Method:	Isocratic 70% Eluent A- 30% Eluent B
Sample	1 mL of mixture (crude/MeOH 1/9; v/v)

Table 3
CPC 250 PRO conditions

CPC column volume:	250 mL
Elution flow rate:	30 mL/min
Rotation Speed:	2000 rpm
Solvent System:	Hept/AcOEt/MeOH/W
Method:	Ascending
Sample	6.3 g in 20 mL of lower phase

RESULTS AND DISCUSSION

Preparative chromatograms are shown in Figure 5 and 6. The blue area on both chromatograms represent the vanillin fraction collected. Those fractions were then analyzed by HPLC at 280 nm (Figure 7 and 8).

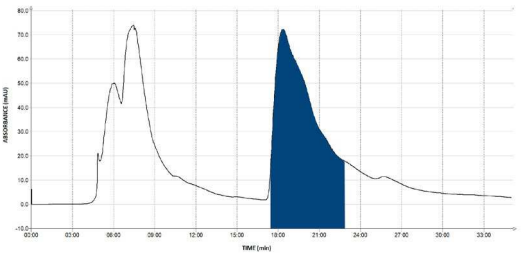

Figure 5

UV chromatogram at 280 nm of prep HPLC run

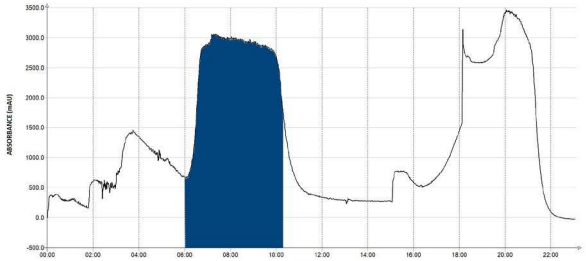

Figure 6

UV chromatogram at 280 nm of CPC 250 PRO run

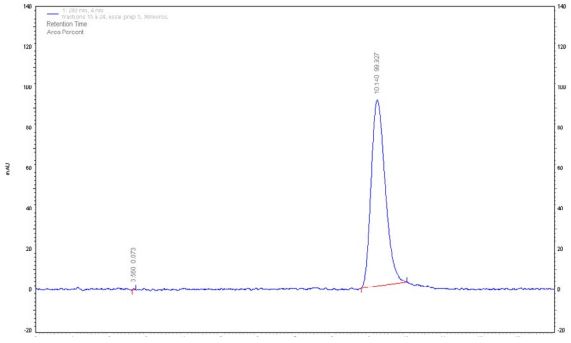

Figure 7

HPLC profile at 280 nm of pooled vanillin fractions from prep HPLC column run

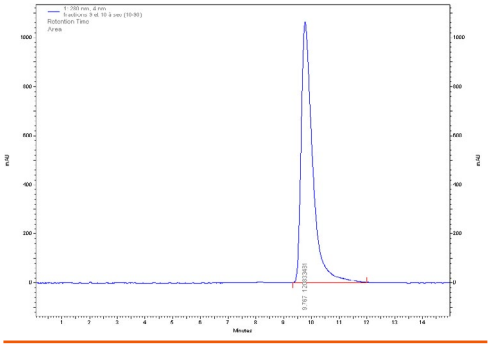

Figure 8

HPLC profile at 280 nm of pooled vanillin fractions of CPC 250 PRO column run

Both technologies yield vanillin with a purity greater than 99% (Table 4). In addition, using CPC yields a recovery greater than 91.8% (Table 4). This shows the capability of CPC to avoid sample loss due to potential irreversible absorption. 500 mL of solvent was necessary to elute vanillin in prep HPLC (Figure 5) as opposed to 360 mL which was used in CPC (Table 4) resulting in 5.3 L of solvent per gram injected in prep HPLC, versus 0.1 L for CPC.

The mass and yield of vanillin recovered after prep HPLC was not determined in this case due to the low quantity injected.

Table 4

Results comparison of two runs performed for vanillin purification

	PREP HPLC	CPC 250 PRO
Crude solution injected	0.08 g/L vanillin	28 g/L vanillin
Masse of crude extract injected	90 mg	6.3 g
Mass of vanillin injected	8 mg	560 mg
Mass of vanillin recovered	n.d.	514 mg
Yield of vanillin purification	n.d.	91.8%
HPLC purity of vanillin (at 280 nm)	99.9%	99.1% (300 mg) ; 100% (200 mg)
Separation time and solvent consumption	25 min ; 500 mL	12 min ; 360 mL

CONCLUSION

The PLC 2050 is able to be efficiently and easily used in combination with both CPC and HPLC columns for purification work. This example illustrates the advantages of CPC compared to HPLC preparative columns (Table 4). CPC allows a larger quantity of sample to be injected than HPLC and achieves similar separation efficiency, with lower solvent consumption and no chromatographic solid phase e.g. silica. The resulting gains in productivity and profitability observed with these laboratory systems can then be amplified with CPC industrial solutions like the Gilson VERITY® CPC Process.

REFERENCES

1. Vanillin; Walton N.J. and Al; Phytochemistry, 63, (2003), 505-515
2. Zietlow *et. al.* Journal of Agricultural and Food Chemistry 50(22):6271-5

Note

This application note has been produced and edited using information that was available when the data was acquired for each article. This application note is subject to revision without prior notice.