World Academy of Science, Engineering and Technology
International Journal of Chemical and Molecular Engineering
Vol:11, No:12, 2017

Characterization of Penicillin V Acid and Its Related Compounds by HPLC

Bahdja Guerfi, N. Hadhoum, I. Azouz, M. Bendoumia, S. Bouafia, F. Z. Hadjadj Aoul

Abstract**—**Background: 'Penicillin V' is a narrow, bactericidal antibiotic of the beta-lactam family of the naturally occurring penicillin group. It is limited to infections due to the germs defined as sensitive. The objective of this work was to identify and to characterize Penicillin V acid and its related compounds by High-performance liquid chromatography (HPLC). Methods: Firstly phenoxymethylpenicillin was identified by an infrared absorption. The organoleptic characteristics, pH, and determination of water content were also studied. The dosage of Penicillin V acid active substance and the determination of its related compounds were carried on waters HPLC, equipped with a UV detector at 254 nm and Discovery HS C18 column (250 mm X 4.6 mm X 5 µm) which is maintained at room temperature. The flow rate was about 1 ml per min. A mixture of water, acetonitrile and acetic acid (65:35:01) was used as mobile phase for phenoxyacetic acid 'impurity B' and a mixture of water, acetonitrile and acetic acid (650:150:5.75) for the assay and 4-hydroxypenicillin V 'impurity D'. Results: The identification of Penicillin V acid active substance and the evaluation of its chemical quality showed conformity with USP 35th edition. The Penicillin V acid content in the raw material is equal to 1692.22 UI/mg. The percentage content of phenoxyacetic acid and 4-hydroxypenicillin V was respectively: 0.035% and 0.323%. Conclusion: Through these results, we can conclude that the Penicillin V acid active substance tested is of good physicochemical quality.

Keywords**—**Penicillin V acid, characterization, related substances, HPLC.

I. Introduction

PHENOXYMETHYLPENICILLIN, bactericidal antibiotic of the betalactamin family is one of the common penicillins that has high activity against gram-positive organisms, gram-negative cocci, and non-beta-lactamase-producing anaerobic organisms, and low activity against gram-negative bacilli. It is sensitive to hydrolysis by beta-lactamases and inactivated by penicillinase [1], [4].

Penicillin V has the empirical formula $C_{16}H_{18}N_2O_5S$, its molecular weight is 350.39 g/mol. [2], [3]. Penicillin V differs from penicillin G by replacing the benzyl group with a phenoxymethyl group. This chemical modification confers stability in an acid medium and allows oral administration [5].

Penicillin V acid is indicated for adult and pediatric patients in the curative and preventive treatment of streptococcus A to beta-hemolytic tonsillitis, skin infections with susceptible organisms and in the treatment of pneumococcal infections in the splenctomized group major sickle cell disease and other asplenics function [4].

The present work deals with the identification and characterization of the active pharmaceutical ingredient Penicillin V acid and its related substances by HPLC.

Bahdja Guerfi is with the Laboratory of Medicinal Chemistry, Faculty of Medicine, University Saad Dahlab of Blida, Algeria and with the Principal Pharmacy, Anti-Cancer Center, Zabana, 09000, Blida, Algeria (e-mail: bahdja.guerfi@gmail.com).

N. Hadhoum is with the 4-Laboratory of Medicinal Chemistry, Faculty of Medicine, University Mouloud Maamri of Tizi ouzou, Algeria

I. Azouz, M. Bendoumia, and S. Bouafia are with the Laboratory of Medicinal Chemistry, Faculty of Medicine, University Saad Dahlab of Blida Algeria.

F.Z. Hadjadj Aoul is with the Laboratory of Medicinal Chemistry, Faculty of Medicine, University Ben youcef Ben Khedda – Algiers, Algeria.

II. Methods

Penicillin V acid was identified by an infrared absorption using Spectrum One FTIR spectrometer. The organoleptic characteristics, pH and determination of water content by the Karl Fischer method were also studied.

The determination of its purity, identification and the dosage of related substances of Penicillin were carried out using a Waters HPLC. [3]

A. Organoleptic Characteristics

Through a visual appreciation, we examined the appearance and color of our raw material.

B. Solubility

We tested the solubility in distilled water, acetone and ethanol 96 °.

C. Identification of Penicillin V acid by Infrared Absorption Using Spectrum One FTIR

In order to obtain the IR spectrum of Penicillin V, we mixed and grounded a very small amount of the substance (approximately 3 mg) with potassium bromide (approximately 300 mg) and then compressed the powder into a special mold under a pressure of 1.5 to 3 $MP.cm^{-2}$, to obtain a pellet of potassium bromide. [2]

TABLE I
Chromatographic Conditions

	Mobile phases	Column type	Injection volumes
Assay and 4-hydroxypenicillin V impurity	Water: acetonitrile: acetic acid [650:150:5.75].	Discovery HS: C18 (0.3 m x 4 mm x 5 µm), room temperature	10 µl
Phenoxyacetic acid impurity	Water : acetonitrile: acetic acid [65:35: 1]	Discovery HS: C18 (0.25 m x 4.6 mm x 5 µm), room temperature	20 µl
Flow rate: 1 ml/min			
Detection wavelength: 254 nm			

D.Standard and Sample Solutions for Dosage of Phenoxyacetic Acid [3]

- Standard solution: Dissolve 10 mg of 99.9% standard phenoxyacetic acid in 100 ml of the diluent.
- Preparation of diluent: Prepare a phosphate buffer at pH = 6.6. In a 200 ml vial, we mixed 50 ml of 0.2 M monobasic potassium phosphate solution and 16.4 ml of 0.2 M sodium hydroxide solution and made up with distilled water. At the end, we checked the buffer pH value
- Sample solution: Dissolve 1 g of the acidic penicillin V in 50 ml of the mobile phase.

E. Standard and Sample Solutions for Determination of Purity of Penicillin V Acid [3]

- Standard solution of penicillin V potassium RS: Dissolve 25 mg of penicillin V potassium in 10 ml of the mobile phase.
- Sample solution: Take 50 mg of acidic penicillin V in a 20 ml vial. Complete with the mobile phase.
- Standard solution of 4-hydroxypenicillin V: Dissolve 10 mg of 4-hydroxypenicillin V standard in 100 ml of the mobile phase.
- Preparation of the resolution solution: Dissolve 62.5 mg of penicillin G standard USP and 62.5 mg of penicillin V standard USP in 25 ml of the mobile phase.

Fig. 1 Appearance of Penicillin V acid

Fig. 2 Solubility tests in distilled water, ethanol 96° and acetone

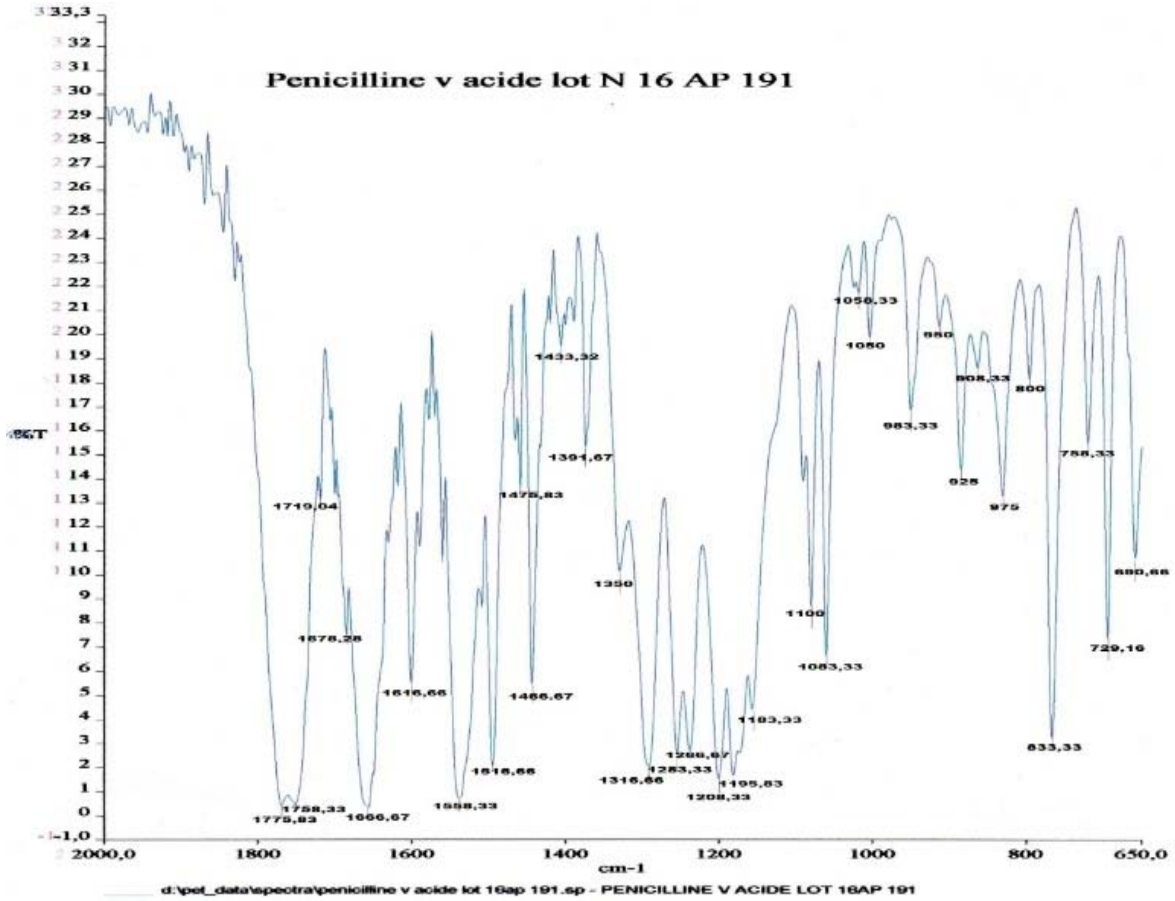


Fig. 3 Penicillin V acid Infrared Spectra

III. Results and Discussion

A. Organoleptic Characteristics

Penicilline V acid is a white, crystalline powder. This aspect is in accordance with the US Pharmacopoeia USP 35 edition.

B. Solubility

- Penicillin V acid is very slightly soluble in water, easily soluble in 96 ° ethanol and acetone.
- The pH of solution containing 30 mg/ml of Penicillin V acid was pH= 2.73. Norms of USP 35: 2.5- 4.0.
- The water content determined using a Karl-Fischer apparatus was about 0.11%. USP 35 (≤ 2%).

C. Dosage of Phenoxyacetic Acid

The standard deviation (RSD) for a series of standard injection is 0.4% so is not more than 2%, according to the USP 35.

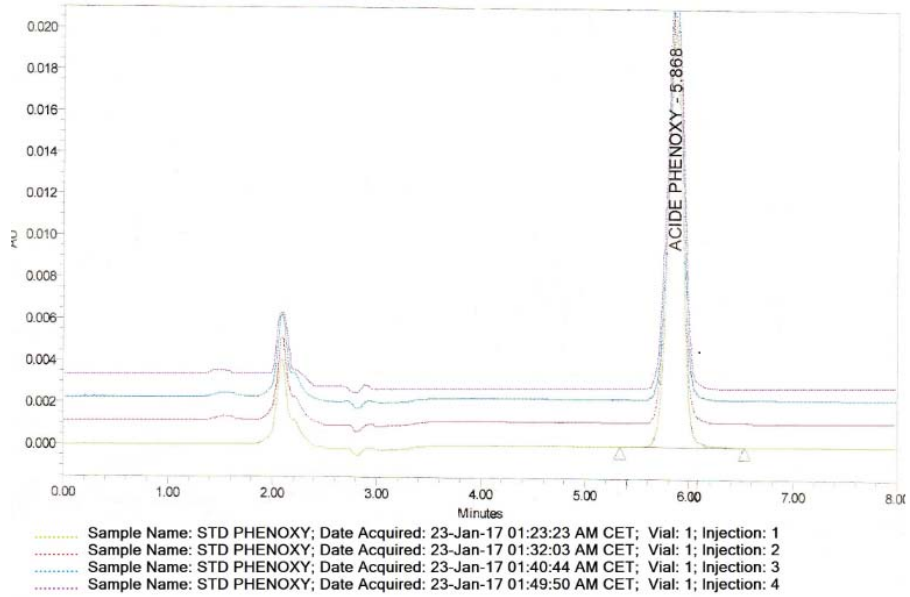


Fig. 4 Chromatograms of standard solution of phenoxyacetic acid 0.1 mg/ml

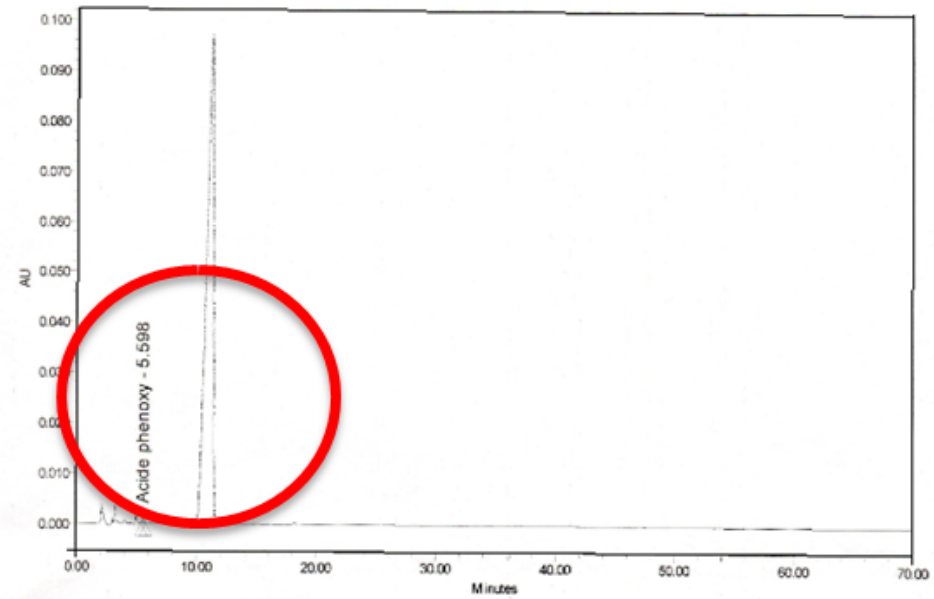


Fig. 5 Chromatograms of test solution (20 mg/ml)

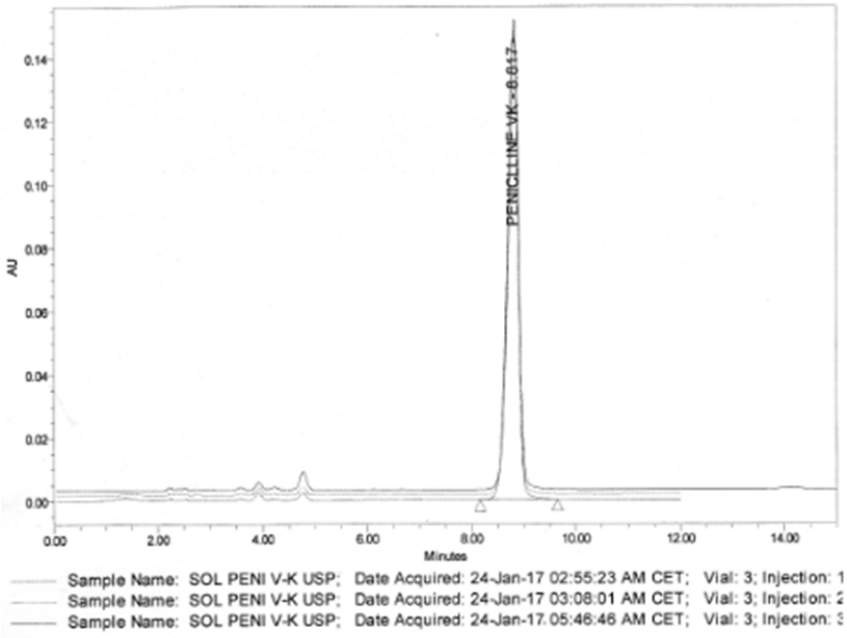
The percentage content of phenoxyacetic acid was calculated using:

$$\mathbf{T\%}=\left(\frac{AUCEch}{AUCStd}\right)x\left(\frac{CStd}{CEch}\right)x\ 100 \quad (1)$$

P (%) of phenoxyacetic acid = 0.035%. Norms of USP 35 < 0.5%).

D.Dosage of Penicillin V Acid and 4-Hydroxypenicillin V "Impurity D"

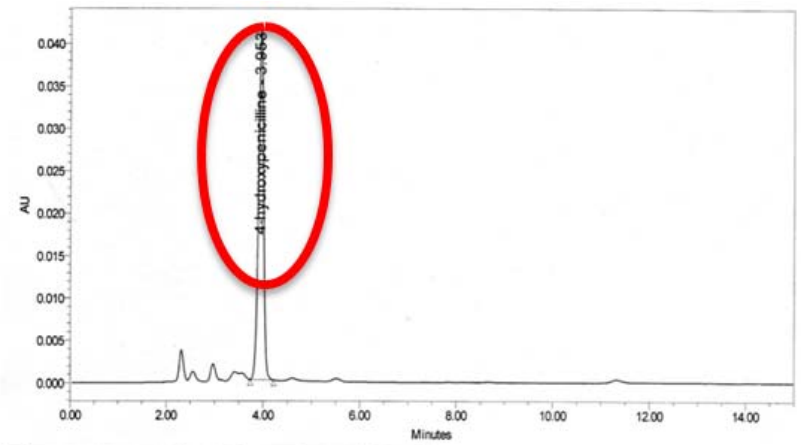
The standard deviation (RSD) for a series of V-K USP standard injection is 0.5% so is not more than 1%, according to the USP 35.



Peak Summary with Statistics
Name: PENICLLINE VK

	Sample Name	Vial	Inj	Name	Retention Time (min)	Area	% Area	Height
1	SOL PENI V-K USP	3	1	PENICLLINE VK	8.817	2116932	100.00	147180
2	SOL PENI V-K USP	3	3	PENICLLINE VK	8.837	2140924	100.00	148842
3	SOL PENI V-K USP	3	2	PENICLLINE VK	8.818	2123656	100.00	147943
Mean						2127170.8		
% RSD						0.6		

Fig. 6 Penicillin V-K USP Standard solution chromatogram



	Peak Name	RT	Area	% Area	Height
1	4-hydroxypenicilline	3.953	312969	100.00	41567

Fig. 7 Hydroxypenicillin V Standard solution chromatogram

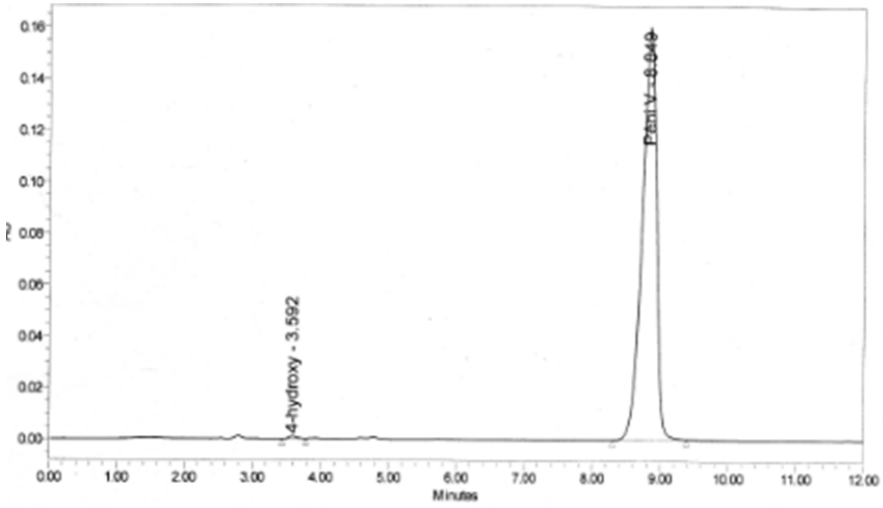


Fig. 8 Test solution Chromatogram

The percentage content of Penicillin V acid was calculated using:

$$T\% = \left(\frac{AUCEch}{AUCStd}\right) x \left(\frac{CStd}{CEch}\right) x\ purity \quad (2)$$

P (%) of Penicillin V acid = 1692.22 UI.

Norms: (1525 – 1780) UI/mg. USP 35

The percentage content of 4-hydroxypenicillin V "Impurity D" was calculated using:

$$100rp/rs \quad (3)$$

rp: 4-hydroxypenicillin V Air; rs: sum of 4-hydroxypenicillin V and penicillin V acid airs. P (%) of 4-hydroxypenicillin V= 0.323% (Norms of USP: ‹ 5%).

IV. Conclusion

The identity of penicillin V acid active substance was confirmed by infrared absorption spectroscopy.

The purity of Penicillin V acid and the impurity content analyses were carried out by HPLC. The results obtained meet the standards required by the American Pharmacopoeia 35th edition [3].

Our drug substance is therefore consistent with the standards required by the US pharmacopoeia, reflecting its good physicochemical quality.

References

[1] André Bryskier. Antibiotique Agent Antibactériens et Antifongiques. Ellipses 1999.
[2] European Pharmacopoeia 8th edition.
[3] United States Pharmacopoeia Convention: United States Pharmacopoeia 35.
[4] Bertram G Katzung. Pharmacologie fondamentale et clinique. Traduit par la la direction de Georges Lagier Paris le 23 Avril 2000.
[5] D Lebrun, M Bonnet, A Limelette, C de Champs. Pénicillines et inhibiteurs de bétalactamases. Elsevier Masson SAS 2017.