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RESEARCH ARTICLE

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Quantitative analysis of nicotinic acid, nicotinamide and 3-cyanopyridine in industrial effluent by high performance liquid chromatography

Abstract A rapid and reliable high performance liquid chromatographic(HPLC) method for the simultaneous determination of heterocyclic compounds, namely nicotinic acid, nicotinamide, and 3-cyanopyridine, in industrial effluent is described. A Φ4.6 mm × 150 mm, 5 μm C-18 reversed phase stationery phase, and a methanol-acetonitrile-water tertiary mobile phase (20:20:60 v/v) were used for separation. The detection wavelength of a diode array (DAD) was set at 216 nm with a bandwidth of 16 nm. Phenol was used as an internal standard. The regression equations revealed a linear relationship between the concentration of the analytes injected and the peak area detected by DAD. The limits of detection (S/N = 3) ranged from 0.70 to 1.18 mg L^{-1}, the recoveries ranged from 87% to 102% and the precision expressed as % RSD intra-day and inter-day varied from 0.9 to 3.9 and 1.2 to 5.6, respectively. This method is rapid, sensitive and suitable for the monitoring of nicotinic acid, nicotinamide, and 3-cyanopyridine in effluent of related pharmaceutical manufacturing plants.

Keywords heterocyclic compounds, high performance liquid chromatographic method, effluent

1 Introduction

Vitamin B3 (or PP) is part of the B complex group and is composed of nicotinic acid (or niacid) and nicotinamide (or niacinamide), structurally with a carboxylic or a carboxamide functional group respectively bounded on a pyridine ring. The conjugated π system of its heterocyclic aromatic structure allows detection by ultra-violet light. It is soluble in water and generally very stable. It is a well-known precursor in the synthesis of the coenzymes NAD^+ and NADP (nicotinamide adenine dinucleotide phosphate) involved in cell metabolism. A deficiency of them causes inflammation of mucus membranes and an illness known as pellagra.

A pharmaceutical manufacturing plant in Guangzhou, China synthesizes this vitamin B3 with 3-cyanopyridine as one of the intermediates. The residual amounts of nicotinic acid, nicotinamide and 3-cyanopyridine are the major waste products in the effluent that constitute a high measured biochemical oxygen demand arousing environmental concern. The main objectives of this study are to assess the initial level of them present in the effluent and to apply this method for the monitoring of the subsequent levels after biological treatment via bacterial degradation to be developed in future work. The level of all of the three compounds was found to exceed the maximum permissible level stipulated in integrated wastewater discharge standard GB8978-1996 [1] in China, bringing to public attention as environmental pollutants. A number of methods has been developed for the quantification of vitamin B3 and its metabolites in areas other than environmental, such as pharmaceutical formulations [2,3], biological fluids [4,5] and food [6,7]. However, the analysis of vitamin B3 and its synthetic intermediates in industrial effluent has not been reported elsewhere. To our knowledge, this report is the first of its kind that describes the simultaneous quantification of nicotinic acid, nicotinamide and 3-cyanopyridine in environmental monitoring.

In this work, an isocratic elution high performance liquid chromatographic (HPLC) method with a methanol-acetonitrile-water tertiary mobile phase and the most common stationary phase (RP-C18) was developed, and it is suitable for the analysis of the residual vitamin B3 and its synthetic intermediates in related environmental samples. This work was performed in the research laboratory of the Department of Science in the Hong Kong Institute of Education.

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2 Experiments

2.1 Apparatus and chemicals

The HPLC method was used on a HP1100 high performance liquid chromatographic system (Hewlett-Packard), equipped with an auto-injector, a vacuum degas module, a quaternary pump and a diode array detection. The data were collected using a HP ChemStation. A reverse-osmosis system (Orion) and resin columns (Aries) were used to purify and deionise water of 18 MΩ-cm resistivity.

All of the chemicals used in this study were of analytical-reagent grade, the deionised water was used to prepare a mobile phase and other solutions. Nicotinic acid, nicotinamide, 3-cyanopyridine and phenol were purchased from Fluka, the methanol was from Fisher Scientific and the acetonitrile was from Labscan. The effluent was collected from a pharmaceutical manufacturing plant in Guangzhou, China. Stock solutions of nicotinic acid, nicotinamide and 3-cyanopyridine were prepared in deionised water with a concentration of 1000 mg L^{-1}. The standard mixtures were diluted to 1, 5, 10, 20, 30 and 40 mg L^{-1} respectively using the described mobile phase, and a constant amount of the internal standard was dispensed into each of the standard mixtures.

2.2 Preparation of samples

The samples of effluent were stored in refrigerator at 4°C, and they were thawed at 23°C for about 30 min before use. A constant amount of the internal standard was dispensed into each sample. They were filtered through a 0.45 μm membrane filter and then diluted 200 times by the mobile phase prior to the HPLC run.

2.3 HPLC Conditions

A separation column of Φ4.6 mm × 150 mm, 5 μm C-18 reversed phase and a guard column of the same material (Alltech) were used. The column temperature was operated at 23°C in a controlled laboratory environment. The mobile phase used was methanol-acetonitrile-water (20:20:60 v/v) at a flow rate of 1.5 mL min^{-1} isocratic. The detection wavelength was set at 216 nm, and the injection volume of samples was 20 μL by an auto-injector.

3 Results and discussion

3.1 Resolution of nicotinic acid, nicotinamide and 3-cyanopyridine

The content of methanol in the mobile phase exerted a greater influence on the capacity factor of 3-cyanopyridine and phenol than that of nicotinic acid and nicotinamide. When increasing the content of methanol, the 3-cyanopyridine and phenol eluted much more quickly. Acetonitrile of the mobile phase, on the other hand, improved the overall peak shape, especially minimizing the effect of peak broadening. To select an optimum composition of the mobile phase, the content of methanol starting from scratch was increased by 5% at a time (0%, 5%, 10%, 15%, 20%, 25%) at the expense of the same content of acetonitrile (40%, 35%, 30%, 25%, 20%, 15%) while holding the content of water to a constant at 60%. The peaks of nicotinic acid and nicotinamide were found to be too close together for quantification when the content of methanol and acetonitrile reached 25% and 15% respectively. Using methanol-acetonitrile-water (20:20:60 v/v) as the mobile phase, the analysis period was shortened to about 6 min. while the peaks of nicotinic acid and nicotinamide were still evidently baseline resolved that was sufficient for a quantitative analysis of the three analytes. The peak of the internal standard was well separated from the three analytes. As a result, the methanol-acetonitrile-water (20:20:60 v/v) was employed as the mobile phase in the entire work. The chromatograms of a standard mixture of nicotinic acid, nicotinamide, 3-cyanopyridine and an internal standard, and a sample of effluent at the selected chromatographic conditions are depicted in Fig. 1. The elution order of the analytes was consistent with their polarity. Nicotinic acid with the average retention time, 1.2 min was eluted first, followed by nicotinamide (1.7 min), 3-cyanopyridine (3.1 min), and then phenol (5.7 min).

3.2 Linearity and limit of detection

Mixtures of nicotinic acid, nicotinamide and 3-cyanopyridine with different concentrations were prepared for calibration curves. The regression equation between the peak area (mAu*s) and the concentration of the analytes injected (mg L^{-1}) can be expressed as $y = mx + c$, where y is the ratio of the peak area of the analyte to the peak area of the internal standard, x is the concentration of the analytes, c is the intercept and m is the slope (sensitivity). It was obvious that similar, though not identical, slopes were obtained for the analytes, indicating that they had matching sensitivities. The linear range and limit of detection for the three analytes were investigated, and the findings are shown in Table 1. The correlation coefficients of all regression lines were over 0.999. The limit of detection was calculated from the extrapolation of the regression line of the data, i.e. the intercept plus three times the standard deviations of the noise, assuming a variation of normal distribution [8,9]. Using the above definition, the limits of detection for the method ranged from 0.70 to 1.18 mg L^{-1}.

3.3 Precision and recovery

The precision of the chromatographic determination in relation to the proposed method, expressed as a relative standard deviation (% RSD), was found by analyzing the effluent samples five times on five different days (intra-day

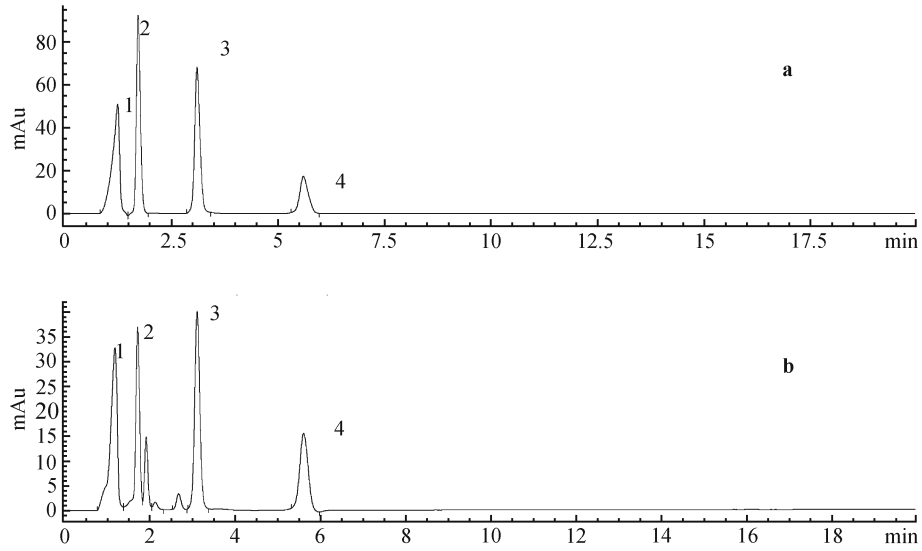

Fig. 1 HPLC chromatograms of standards
(a) Effluent samples; (b) analytes: 1, nicotinic acid; 2, nicotinamide; 3, 3-cyanopyridine; 4, phenol

Table 1 Calibration curves, calibration ranges and detection limits for the three analytes

Compound	Regression equation	Calibration range (mg·L^{-1})	Correlation coefficient/r	*Detection limit (mg·L^{-1})
Nicotinic acid	$y = 0.1251x + 0.02751$	1.0–40	0.9997	1.10
Nicotinamide	$y = 0.1226x + 0.03758$	1.0–40	0.9999	0.70
3-cyanopyridine	$y = 0.1274x + 0.02641$	1.0–40	0.9997	1.18

*S/N = 3

and inter-day). As summarized in Table 2, the RSD of the chromatographic determination varied from 0.9 to 3.9 and 1.2 to 5.6 for intra-day and inter-day respectively. The recovery method, including all experimental procedures from the addition of an internal standard, sample preparation to the chromatographic analysis, was measured using the effluent samples. Known amounts of each standard were spiked into the samples which were analyzed in triplicate using the developed HPLC method. The amount found of the recoveries were calculated on the basis of the difference between the total amount determined in the spiked samples and the amount determined in the non-spiked samples and expressed as a mean percentage ratio between the amounts found and the amount spiked. As shown in Table 3, the recoveries varied from 87% to 102%.

3.4 Analysis of effluent samples

The developed method in this study was applied to the effluent samples, namely LZ-A, LZ-B, LZ-C, LZ-D and LZ-E, collected on five different days from a pharmaceutical manufacturing plant. The concentrations determined ranged from 1.79 to 2.59, 1.75 to 2.45 and 0.90 to 2.21 g L^{-1} for nicotinic acid, nicotinamide and 3-cyanopyridine respectively. The concentrations are summarized in Table 4 below.

4 Conclusions

A rapid and reliable HPLC method for the simultaneous determination of nicotinic acid, nicotinamide, and

Table 2 Precisions for the chromatographic determination

	Intra-day *RSD (%, $n = 5$) a			Inter-day RSD (%, $n = 5$)		
Sample	Nicotinic acid	Nicotinamide	3-cyanopyridine	Nicotinic acid	Nicotinamide	3-cyanopyridine
LZ-A	3.3	2.1	0.9	2.3	2.5	1.2
LZ-B	1.7	2.9	1.1	3.5	2.1	3.5
LZ-C	2.4	3.9	3.0	2.6	3.1	5.6
LZ-D	3.1	2.6	2.3	4.7	2.2	3.9
LZ-E	3.3	3.5	2.7	4.8	4.0	2.9

*RSD = relative standard deviation.

Table 3 Recoveries of the method

	*Mean recovery (%, $n = 3$)		
Sample	Nicotinic acid	Nicotinamide	3-cyanopyridine
LZ-A	95	90	96
LZ-B	98	92	90
LZ-C	93	87	95
LZ-D	102	91	94
LZ-E	99	93	92

*Expressed as [(amount found) / (amount spiked)] × 100.

Table 4 Determination results of the three analytes in the effluent samples

Sample	Nicotinic acid	Nicotinamide	3-cyanopyridine
LZ-A	2.33	2.45	0.90
LZ-B	1.79	2.10	0.98
LZ-C	2.59	2.04	2.21
LZ-D	2.56	1.79	1.98
LZ-E	2.41	1.75	1.58

Unit in $g \cdot L^{-1}$

3-cyanopyridine in effluent was developed. This method requires nothing but a common stationary phase and a simple tertiary mobile phase, which, more importantly, gives good resolution and peak shapes. Besides, it requires a shorter analytical time (6 min) and covers a wide linear range, and is thus useful for the monitoring of these heterocyclic compounds in related industrial effluent.

5 Future work

One of the methods for solving this environmental contamination is by means of biotechnology. Various stains of bacteria were isolated and purified from the effluent samples in the laboratory. The capability of them to degrade the three heterocyclic compounds will be investigated with a view to finding the most suitable candidates to clean up the compounds in consideration of degradation efficiency, as well as their synergistic or antagonistic interactions in the coming future.

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