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Preliminary Studies of Philippine Vodka as an HPLC Organic Modifier

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Abstract

In the United States, attempts have already been made to use distilled spirits as an organic modifier. However, the chromatograms produced were inferior in terms of number of theoretical plates (NTP) and noise compared with chromatograms produced using HPLC grade ethanol. This study aimed at finding a locally available vodka that produces chromatograms comparable with ACS HPLC grade ethanol in terms of NTP and noise. Three brands of locally produced vodka (Cossack, Antonov and Tosca) were tested and the brand that passed the ACS set limits for absorbance and residue on evaporation was selected. The brand that passed specifications was then used for isocratic and gradient HPLC separation. All experimental runs were carried out in triplicates. It was found out that Antonov Vodka passed the said set limits, which was then used in separating caffeine and benzoic acid under isocratic and gradient modes. Results revealed that the resulting chromatograms had NTP and baseline noise that were comparable to the ACS HPLC grade ethanol. In the case of caffeine, the NTP under isocratic conditions were 6006.67±36.35 and 5943.33±12.74 for ACS HPLC grade ethanol and Antonov Vodka, respectively. Using Antonov Vodka, good separation of caffeine and benzoic acid from other matrix components in three brands of energy drinks (Cobra, Lipovitan and Red Bull) was likewise observed. Hence, with the proper validation procedures in place like testing the robustness of the analytical method, Antonov Vodka may replace HPLC grade ethanol in the analysis of some samples.

***Keywords**:* ACS HPLC grade, ethanol, organic modifier, vodka

1. Introduction

Reverse Phase High Performance Liquid Chromatography (RP-HPLC) is a versatile analytical technique that can be used for the simultaneous determination of analytes in complex mixtures (Heydari *et al.*, 2013). The most commonly used organic modifiers in RP-HPLC are methanol followed by acetonitrile (Yabré *et al.*, 2018). These chemicals are considered as toxic and are an environmental concern. To address this problem, ethanol can be used as a substitute (Ribeiro *et al.*, 2004) for some analytes. However, ACS HPLC grade ethanol is more costly than the equivalent methanol.

HPLC grade chemicals are reagents pure enough (≥ 99%) to be used for HPLC analysis. For an organic modifier to be considered ACS HPLC grade, certain specifications set by the American Chemical Society (ACS) has to be met. For HPLC-ultraviolet (UV), the two most critical are the specifications for absorbance and residue on drying. A low absorbance would mean lower noise while a low residue on drying would mean lower chances of column clogging. Also, passing both specifications imply fewer contaminants that could possibly lower the number of theoretical plates (NTP) of the column.

In the United States, distilled spirits have already been tested for use in RP-HPLC (Welch *et al.*, 2015). However, these distilled spirits resulted in inferior chromatograms (e.g., low NTP and high baseline noise). This is partly because the distilled spirits failed to pass the ACS set limits for absorbance as seen by their UV-visible (VIS) spectra. Thus, finding a suitable distilled spirit would improve the quality of the analytical data. Considering the advancements in the equipment miniaturization (Lynch *et al.*, 2018) and the development of faster sample preparation techniques (Ghassempour *et al.*, 2008; Heydari and Mousavi, 2016; Heydari *et al.*, 2019; Rahimi *et al.*, 2019) using distilled spirits might help in doing HPLC, even in a non-laboratory setting like doctor's office.

Vodka is a tasteless and neutral distilled spirit that has undergone charcoal filtration (Hayman, 2003). This has also been used as a mobile phase for HPLC (Welch *et al.*, 2010) but with baseline noise still appearing to be inferior to ACS HPLC grade ethanol. The vodka used may be relatively pure but may not pass ACS HPLC grade specifications for limits on absorbance.

This paper aimed to find a locally made vodka that would produce chromatograms with NTPs and baseline noise comparable with those obtained using ACS HPLC grade ethanol. To achieve this objective, the values for absorbance and residue on evaporation of three cheap brands of locally made vodka (Antonov, Cossack and Tosca) were compared to an ACS HPLC grade ethanol. The best vodka was then used to obtain HPLC chromatograms for caffeine and benzoic acid. Performance characteristics (e.g., NTP and noise) were then compared to that of an ACS HPLC grade ethanol. These experiments helped in assessing whether or not the Philippine vodka can be used as a substitute.

2. Methodology

2.1 Chemicals and Materials

The ACS HPLC grade ethanol, acetic acid and benzoic acid were from Scharlab (Barcelona, Spain) while caffeine was from Sigma Aldrich (Missouri, United States). All chemicals were purchased from various local suppliers. Three Philippine Vodka brands, namely Antonov (Ginebra San Miguel, Philippines), Cossack (Tanduay Distillers Inc., Philippines) and Toska (Destilera Limtuaco & Co. Inc., Philippines) were purchased from different local supermarkets.

2.2 Comparison of the Limits on Absorbance and Residue on Evaporation of Locally Produced Vodka against Sigma Aldrich Specifications

2.2.1 Ultraviolet Absorption

The absorbance of an ACS HPLC grade ethanol and three brands of vodka were measured against water at different wavelengths (210, 220, 230, 240, 270 and 400 nm). The measured absorbances were then compared with specifications set by Sigma Aldrich for their ACS HPLC grade ethanol. All experiments were done in three trials. In the case of vodka, each trial represented a different production lot.

2.2.2 Non-Volatile Compounds

A total of 100 mL of Antonov Vodka was placed in a previously weighed beaker and evaporated to dryness using a hot plate. To ensure complete evaporation, the beaker was placed inside an oven at 130 °C for 2 h. The residue on evaporation of the vodka was then computed based on Equation 1. The results were compared to the specifications of HPLC-grade ethanol.

$$\%\left(\frac{W}{V}\right)=\frac{(W_1\text{-}W)}{V}\times 100 \tag{1}$$

where:

W_1 = weight of beaker with dry residue (g)
W = weight of empty beaker (g)
V = volume of HPLC-grade ethanol or Antonov Vodka (mL)

2.3 Comparison of Chromatograms obtained using Antonov Vodka and ACS HPLC Grade Ethanol as Organic Mobile Phase

Both ACS HPLC grade ethanol and Antonov Vodka passed through a 0.2 micron filter before they were used for the separation of caffeine and benzoic acid under isocratic and gradient modes. The system was equipped with two HPLC pumps (LC-20AD, Shimadzu, Japan) coupled to a UV-VIS detector (SPD-20AV, Shimadzu, Japan). Under gradient mode, a high pressure mixer with a volume of 0.5 mL was utilized. The column used was a C18 column (Prodigy, 150 mm x 4.6 mm) with a five micron packing. Caffeine and benzoic acid were used as test compounds.

2.3.1 Isocratic Mode

The test compounds, each having a concentration of 0.1 mg/mL, were run under isocratic conditions (column temperature: 40 °C; mobile phase: 0.17% acetic acid in 15/85 ethanol/water; flow rate: 1 mL/min; detection wavelength: 250 nm).

The NTP and noise of the chromatograms obtained using Antonov Vodka were then compared with that of the chromatograms obtained using an ACS HPLC grade ethanol (Scharlau, Spain).

2.3.2 Gradient Mode

Caffeine and benzoic acid were also run under gradient conditions. The gradient program was (a) 0.17% acetic acid in 10% ethanol from 0 to 12 min, (b) 0.17% acetic acid in 10-25% ethanol from 12-15 min (c) 0.17% acetic acid in 25% ethanol from 15-30 min. It is worth noting that the ratios of water and Antonov Vodka were adjusted accordingly to consider that the latter only has about 40% ethanol as confirmed by HPLC-RID. All runs were then done under similar conditions stated above except that the flow rate was 0.8 mL/min and the column temperature was maintained at 30 °C.

HPLC chromatograms of Lipovitan (Taishio Pharma Co., Japan), Red Bull (Red Bull GmbH, U.S.) and Cobra (Asia Brewery Inc., Philippines) energy drinks were also obtained using Antonov Vodka. The change in pressure was also monitored after running the mobile phase for 12 h.

2.4 Statistical Analysis

As mentioned, all experiments were done in triplicates. Test for statistical significance between two values was carried out using a T-*t*est at a 95% level of confidence.

3. Results and Discussion

3.1 Absorbance and Residue on Evaporation

All three local vodka brands had absorbances that were way below the specified limit (Table 1). However, it was observed that there was a "non-linear" decrease in absorbance when ACS HPLC grade ethanol was diluted to 40%. Instead of the expected 2.5X, the decrease in absorbance at 210 and 230 nm were around 6.8X and 8.9X, respectively (Table 2). Therefore, the very low absorbance of vodka at those wavelengths might also be a dilution effect. Regardless, the absorbance values at those wavelengths would still pass ACS HPLC grade specifications even when corrected for dilution effects. In the case of Antonov Vodka, the corrected values were 0.34 at 210 nm and 0.09 at 230 nm – still below the specified limits which were 0.40 and 0.15, respectively, for pure ethanol.

Only Antonov Vodka appeared to have a residue on evaporation low enough to be at least comparable with the ACS HPLC grade ethanol (Table 1). It was observed that the amount of residue on evaporation for Cossack and Toska was consistent within production lots. Therefore, the residues were probably substances added in the manufacturing process. Additives like glycerol (Buglass and Caven-Quantrill, 2012) have been known to be added to cheap distilled spirits (Interest, 2016) to mask the "sharp edges" brought by trace amounts of impurities. This might not be necessary for Antonov Vodka because it has already been double distilled. To the authors' knowledge, it is the only double-distilled vodka produced in the Philippines and yet it is surprisingly cheap (4.4 U.S. dollar per liter).

Table 1. Absorbance and residue test results of ACS HPLC grade ethanol and Antonov Vodka

Parameter	ACS HPLC Grade Specification	ACS HPLC Grade Ethanol	Antonov Vodka	Cossack	Toska
Wavelength (nm)			Absorbance		
210	≤ 0.40	0.44±0.01	0.05±0.02	0.03±0.01	0.04±0.01
220	≤ 0.25	0.24±0.00	0.02±0.01	0.02±0.00	0.03±0.00
230	≤ 0.15	0.11±0.00	0.01±0.01	0.02±0.00	0.02±0.01
240	≤ 0.05	0.05±0.00	0.00±0.00	0.02±0.00	0.01±0.01
270	≤ 0.01	0.01±0.00	0.00±0.00	0.01±0.00	0.00±0.00
400	≤ 0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Residue on Evaporation	≤ 0.001	0.001±0.001[a]	0.001±0.001[a]	0.262±0.025	0.280±0.005

All values within a row with the same letters are statistically different at $\alpha = 0.05$.

3.2 Isocratic and Gradient Modes

Under both isocratic and gradient modes, the chromatographic parameters such as NTP for caffeine, NTP for benzoic acid and noise were comparable with the ACS HPLC grade ethanol (Table 3). In the case of benzoic acid, there was a small but significant difference in the NTP.

Table 2. Absorbance of 100% versus 40% ACS HPLC grade ethanol

Wavelength (nm)	Absorbance 100% Ethanol	Absorbance 40% Ethanol	X Decrease in Absorbance when Diluted to 40%
210	0.46±0.01	0.07±0.01	6.68X
220	0.22±0.0	0.06±0.00	3.87X
230	0.11±0.0	0.01±0.00	8.94X

Table 3. Isocratic and gradient elution chromatographic parameters for caffeine and benzoic acid

Chromatographic Parameters	ACS HPLC Grade Ethanol	Antonov Vodka
	Isocratic Mode	
Noise	0.02±0.01	0.02± 0.00
NTP (caffeine)	6006.67±36.35	5943.33±12.74
NTP (benzoic acid)	10168.33±19.76^{a}	9985.00±19.31^{a}
	Gradient Mode	
Noise at 10% ethanol	0.02±0.01	0.01±0.01
Noise at 25% ethanol	0.06 ± 0.06	0.01±0.01
NTP (caffeine)	8150.00^{b}	8249.33±9.8^{b}
NTP (benzoic acid)	30593.00^{c}	27838.33±179.34^{c}

All values within a row with the same letters are statistically different at $\alpha = 0.05$.

Looking closely at Figure 1, it can be observed that caffeine and benzoic acid peaks shift in the same direction when the mobile phase was changed from ethanol to vodka. They only differ in extent. Under isocratic conditions (Figure 1a), there was a “very slight shift” of the caffeine peak to the left while the benzoic peak had a shift in the same direction but to a greater extent. Under gradient conditions (Figure 1b), there was a slight shift of the caffeine peak to the right while the benzoic peak also had a shift in the same direction but to a greater extent. This is commonly observed if the elution strength of the mobile phases differs. Peaks that elute longer have been observed to be more sensitive (Fountain, 2017). Elution strength may vary because the actual ethanol composition of the mobile phases might not be accurate. In this study, the mobile phase were individually prepared first before being vacuum filtered. This might slightly change the ethanol composition due to evaporation.

The difference in NTP could be attributed to differences in ethanol composition and not due to contaminants found in Antonov Vodka. This is especially true since the peak shape of benzoic acid appears to be almost the same when using vodka and HPLC grade ethanol. Regardless, shifting from ACS HPLC grade ethanol to Antonov Vodka would not significantly affect the quality of the present results.

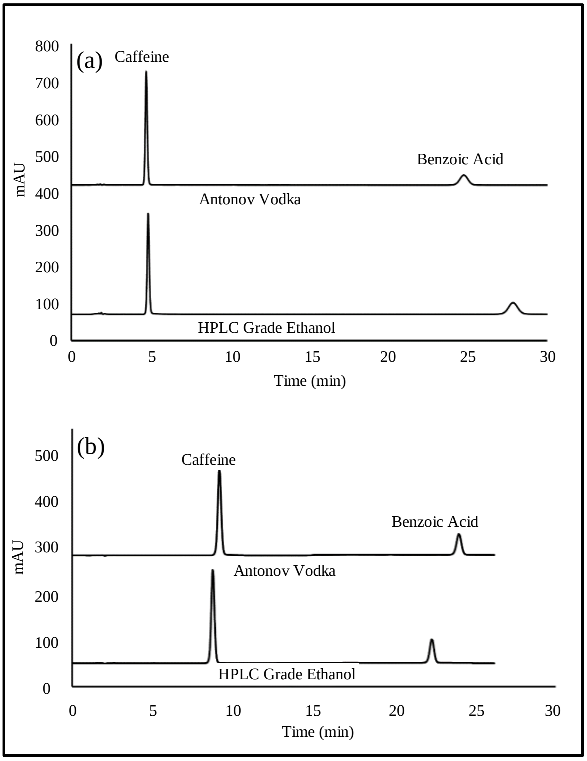

Figure 1. Chromatograms of caffeine and benzoic acid under isocratic (a) and gradient (b) modes using Antonov Vodka and ACS HPLC grade ethanol as organic modifier

Antonov Vodka was then utilized in analyzing various caffeine and benzoic acid in different energy drinks. It appears that the caffeine and benzoic acid peaks are well separated from the other interfering substances (Figure 2). Most of the caffeine and benzoic acid had a relative standard deviation (RSD) under 2% (Table 4).

Table 4. RSD of the results of caffeine and benzoic acid under isocratic and gradient modes

Sample	% RSD of Caffeine (Isocratic)	% RSD of Benzoic Acid (Isocratic)	% RSD of Caffeine (Gradient)	% RSD of Benzoic Acid (Gradient)
Cobra	0.90	0.73	0.77	1.21
Red Bull	0.76	1.21	0.50	1.75
Lipovitan	0.49	0.40	3.59	2.69

Under gradient conditions, the baseline noise for Antonov Vodka was not statistically different from that of the ACS HPLC grade ethanol (Figure 3). This is probably due to the low absorbance of Antonov Vodka at 250 nm. In the previous study of Welch *et al.* (2010), the mobile phase prepared from vodka had a higher baseline noise at 220 nm than the mobile phase prepared with HPLC grade ethanol. This stemmed from significantly higher absorbance of that particular vodka at 220 nm. Antonov Vodka had a low absorbance at 220 nm. Unfortunately, the authors were not able to compare the chromatograms at that wavelength.

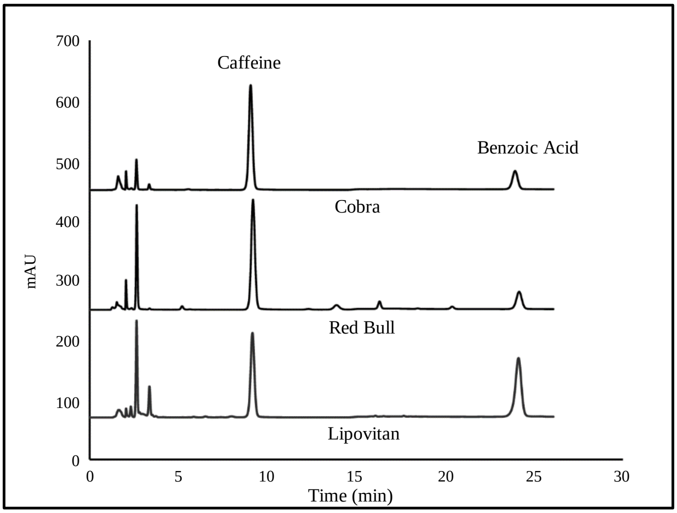

Figure 2. Chromatograms of various energy drinks using Antonov Vodka as organic modifier

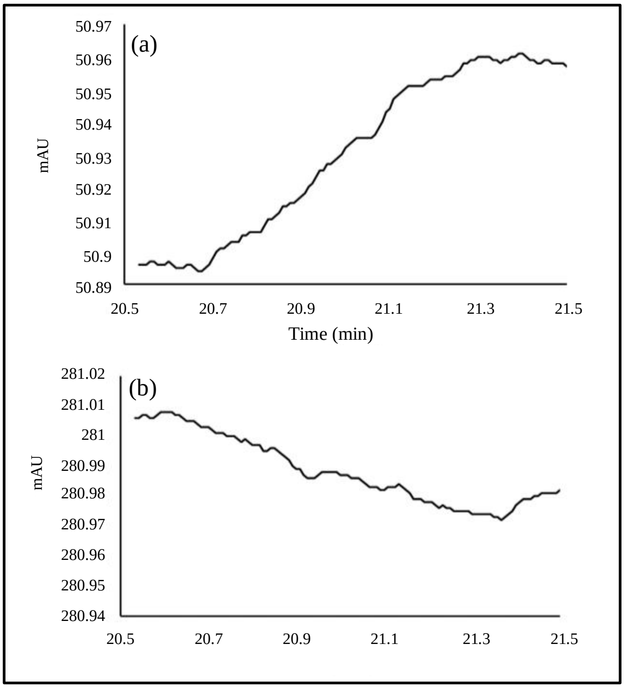

Figure 3. Chromatographic noise under gradient mode at 25% ethanol using ACS HPLC grade ethanol (a) and Antonov Vodka (b). Note that the x-axis was kept at only 1 min zoom in the noise.

3.3 Changes in Column Pressure after 12 h

When running the gradient protocol using Antonov Vodka for 12 h, there was only a slight increase in the system pressure (97.32 to 98.54 MPa) at 25% ethanol. This suggests that Antonov Vodka does not contain additives that could clog the column.

Based on the above results, Antonov Vodka had comparable performance characteristics (NTP and noise) with that of an ACS HPLC grade ethanol. Therefore, Antonov Vodka may be used as a cheap organic modifier without sacrificing the quality of the analytical results.

There are imported brands of vodka (e.g., Smirnoff) that claim to be charcoal filtered and triple distilled. These expensive brands of vodka might be able to pass ACS HPLC grade specification for residue on evaporation. However, these distilled spirits cost at least four times higher than the Antonov Vodka. Also, there is no assurance that these expensive vodkas are additive-free. Thus, finding another “cheap” vodka comparable with an ACS HPLC grade ethanol may be a challenge.

4. Conclusion and Recommendation

Among the three brands of vodka, only Antonov had comparable results with the ACS HPLC grade ethanol for residue on evaporation. When used as an organic phase modifier under both isocratic and gradient modes, all chromatographic parameters (NTP and noise) appeared to be comparable with the ACS HPLC grade ethanol. There was a small difference in the NTP of benzoic acid. However, it could be due to the small difference in the actual percent of ethanol of the two mobile phases. There was only a small increase in system pressure suggesting that column clogging is minimal. With the proper validation procedures in place like testing the robustness of the analytical method, Antonov Vodka may be able to replace HPLC grade ethanol for the analysis of some samples.

It is worthy to note that most, if not all, of the official RP-HPLC protocols use methanol or acetonitrile. In the case of service laboratories, shifting to ethanol like Antonov Vodka would involve extensive steps to comply with regulatory requirements. However, this can easily be adapted to teaching laboratories where procurement and waste management have always been a problem. Antonov Vodka is a cheap organic modifier that can be obtained from the grocery store and directly thrown under the sink.

To extend the use of ethanol (Antonov Vodka), it is necessary to validate its substitution for methanol in a number of United States Pharmacopoeia (USP) HPLC protocols in the future.

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