DETERMINATION OF ASPARTAME, BENZOIC ACID AND CAFFEINE IN SOFT DRINKS USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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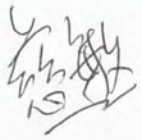

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ABSTRACT

A simple and rapid analysis of soft drinks by high performance liquid chromatography with diode array detector allows the simultaneous determination of aspartame, benzoic acid and caffeine. The separation was performed on an Agilent Zorbax Eclipse XDB-C18 (5 µm, 4.6 x 150 mm) column at 50°C within 5 min at 1.0 mL/min flow rate by an isocratic elution with acetonitrile (0.05 % TFA) and ionized water (0.1 % TFA) (20:80, v/v). The determination was set at 210 nm, 230 nm and 280 nm for aspartame, benzoic acid and caffeine respectively. The optimization on mobile phase polarity and flow rate show a significant effect on retention time and peak height signal, while temperature shows significantly small changes and can be ignored. The retention time for caffeine, aspartame, and benzoic acid were at 2.155 min, 2.810 min and 4.139 min respectively. Good linearities (r^2=.0999) between concentration of all analytes and relevant peak area and peak height responses were achieved over the range 100 – 500 ppm. All samples used for analysis requires minimal sample treatment. Aspartame only found in Pepsi Original at 88.11 mg in each bottle of 355 mL. Amount of benzoic acid in Pepsi Twist and Pepsi Max were 8.18 mg and 23.754 mg respectively in each bottle of 355 mL, not found in Pepsi Original. Amount of caffeine contain in each bottle of 355 mL soft drink were 31.19 mg in Pepsi Original, 33.41 in Pepsi Max and 38.03 mg in Pepsi Twist. The observed level of additives in soft drink samples are significantly lower than the permitted level stated in Malaysia Food Regulation.

ABSTRAK

PENENTUAN ASPARTAME, BENZOIK ZCID DAN KAFEIN DALAM MINUMAN RINGAN MENGGUNAKAN KROMATOGRAFI CECAIR PRESTASI TINGGI

Satu analisis mudah dan cepat dapat dijalankan dengan Kromatografi Cecair Prestasi Tinggi Fasa-Berbalik dengan pengesan diod teratur membenarkan pengesanan serentak untuk aspartame, asid benzoik dan kafein. Pemisahan telah dijalankan dengan menggunakan turus analitikal Agilent Zorbax Eclipse XDB-C18 (4.6 x 150 mm, 5 µm saiz zarah) pada suhu 50°C dalam tempoh 5 min pada kadar aliran 1.0 mL/min dengan menggunakan fasa gerak secara isokratik yang mengandungi asetonitril (0.05 % TFA) dan air dinyahion (0.1 % TFA) (30:70, v/v). Pengesanan dilakukan masing-masing pada 210 nm, 230 nm dan 280 nm untuk aspartame, asid benzoik dan kafein. Pengoptimuman kekutuban pelarut fasa bergerak dan kadar aliran pelarut menunjukkan kesan yang ketara ke atas tempoh masa penahanan dan ketinggian puncak isyarat, manakala pengoptimuman pada suhu menunjukkan perubahan yang sangat kecil dan boleh diabaikan. Tempoh masa penahanan untuk kafein, aspartame, dan asid benzoik adalah 2.155 min, 2.810 min dan 4.139 min masing-masing. Kelinearan yang baik (r^2 = 0999) ditunjukkan antara kepekatan semua analit dalam julat 100 – 500 ppm dengan luas puncak dan ketinggian puncak. Kesemua sampel yang digunakan untuk analisis hanya memerlukan rawatan sampel yang minimum. Aspartame hanya didapati dalam Pepsi Original sebanyak 88.11 mg dalam setiap botol minuman 355 mL. Kandungan asid benzoik dalam Pepsi Twist dan Pepsi Max dalam setiap botol 355 mL adalah masing-masing 8.18 mg dan 23.754 mg, tetapi tidak ditemui dalam Pepsi Original. Bersamaan kandungan kafein dalam setiap botol minuman ringan 355 mL yang dianalisiskan adalah 31.19 mg dalam Pepsi Original, 33.41 dalam Pepsi Max dan 38.03 mg dalam Pepsi Twist. Didapati paras analit dalam contoh-contoh minuman ringan yang dipilih adalah nyata sekali lebih rendah daripada paras yang dibenarkan oleh Peraturan Makanan Malaysia.

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SYMBOL AND ABBREVIATION LIST

ε	Absorptivity
%	Percentage
°C	Celsius
atm	Atmosphere
mΩ	Miliampere
mAU	Mili arbitury unit
min	Minutes
mg	Milligram
mg/kg	Milligram per kilogram
mL	Millilitre
mL/min	Millilitre per minutes
L	Litre
g/L	Gram per litre
cm	Centimetre
mm	Millimetre
nm	Nanometre
µL	Microlitre
µm	Micrometre
kcal/g	Kilo calorie per gram
ppm	Parts per million
ADI	Average daily intake
AOAC	Association of Official Analytical Chemist
AS	Aspartame
BA	Benzoic acid
CA	Caffeine
CH_3CN or ACN	Aceotnitrile
FDA	Food and Drug Administration
TFA	Trifluoroacetic acid
HPLC	High performance liquid chromatography

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Aspartame (AS), benzoic acids (BA) and caffeine (CA) are considered as additives with different function when they are added into food and beverages. They are classified as artificial sweeteners, preservatives and flavour respectively. They are widely used throughout the world in beverages (Chen & Wang, 2001).

Additive define as "any substance not normally consumed as a food in itself and not normally used as a characteristic ingredient of a food whether or not it has nutritive value, the intentional addition of which to food for a technological purpose in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food result, or may be reasonably expected to result, in it or its by-products becoming directly or indirectly a component of such foods" in the book named *Food Preservatives* by Russell and Gould (2003). It is also used to preserve flavour or improve the taste and appearance of food, but we have to keep in mind that an additive is intended as an aid, for some purpose or another, not as an ingredient (Ruiter & Voragen, 2002).

Saag (1988) stated that there are more than hundreds of additives that had been listed in the list of additives, those additives which are permitted by government can be taken safely. Not all additives can be used at the same times, they are only applied to certain food at certain amount and condition as permitted by government and are compliance to the Food Regulation 1985 of Malaysia.

AS is considered the most popular ingredients in carbonated soft drinks, and, historically, most BA and CA present in soft drinks (Bidlingmeyer & Schmitz, 1991). They were widely used throughout the world to improve the taste and maintain the quality of food (Chen & Wang, 2001).

AS is a safe and common artificial sweetener used as dietary sugar which can help those who had diabetes and hypoglycaemia and control calorie intake to decrease body weight (Chen *et al.*, 1997). It can only be accepted by Food and Drug Administration (FDA) at an average daily intake (ADI) of 50 mg/kg body weight (Witt, 1997). AS cannot be taken by phenylketonuria sufferer who is sensitive to phenylalanine, because it can undergo hydrolysis process and convert aspartame to non-sweet compounds, phenylalanine, one of the metabolite of aspartame (Saag, 1988).

BA is a widely used preservative, as exhibit inhibitory activity against a wide variety of fungi, yeasts, molds and bacteria, including foodborne pathogents. The ADI of benzoic acid is less than 5 mg/kg body mass (Chen & Wang, 2001). The pH range for optimum microbial initiation by BA is 2.4 to 4.0. It was reported to be found in

natural fruits such as fresh apple, apricot, green tea, fresh plum and etc. (Burdock, 2005).

CA is also known as 1,3,7-trimethylxanthine and is the most widely consumed psychoactive substance in the world from coffee, tea, chocolate product and carbonated drinks(Carmago & Toledo, 1999). It is used as a flavour when added to the soft drinks. The excessive intake of CA will cause many undesirable side effects. CA is also accompanied by theophylline and theobromine (Chen & Wang, 2001).

High performance liquid chromatography (HPLC) is the most frequently used method in determine the amount of aspartame, benzoic acid and caffeine in food and beverages (Chen & Wang, 2001). It is a very useful instrument in quantitative determination as it is effective, highly selective and widely applicable to many types of samples and only small amount of sample is required (Bovanová & Brandšteterová, 2000). Quanlitative determination can be adapted by respective sample's standard (Skoog *et al.*, 2004).

HPLC is a technique that has arisen from the application of liquid chromatography of the theories and instrumentation that were originally developed for gas chromatography. Good understanding in principle and instrumentation of HPLC is important to attempt a good methodology (Lindsay, 1991).

1.2 Objectives

The aims of this study were,

a. To optimize experimental conditions for separation of aspartame, benzoic acid and caffeine using HPLC.
b. To determine the aspartame, benzoic acid and caffeine in the soft drinks samples.
c. To compare the concentration of aspartame, benzoic acid and caffeine in selected soft drinks samples.

1.3 Scope of study

The study focused on the optimization to obtain an optimum condition in determination of the amount of AS, BA and CA contains in several commercial soft drinks. Qualitative analysis of the AS, BA and CA is done using standard solution, and at the same time the conditions of HPLC separation were optimized. Determinations of peak in a separation of the mixed standard solutions were done by compare the retention time with the peak obtain from individual standard solution. When the determination of optimum conditions of HPLC was done, the peak obtained is important because it will affect the quantitation of samples. In another word, the study also focuses on optimizing the HPLC separation. Therefore, factors like types of mobile phase used, flow rate and temperature were studied as well.

CHAPTER 2

LITERATURE REVIEW

2.1 High-Performance Liquid Chromatography (HPLC)

2.1.1 History

Chromatography was invented by a Russian botanist named Tsweet somewhere around the turn of the last century; his work involved separating plant pigments by eluting a mixture on a column of calcium carbonate. His technique was to allow a plant extract to percolate through a bed of powdered calcium carbonate. He reported his findings at the Biological Section of the Warsaw Naturalist's Society in 1903 (Scott, 1994).

Tsweet carried out experiment on chlorophyll extracts in petroleum spirit with over 100 adsorbents. Although most of these adsorbents are now no longer important, it is interesting to note in the list the inclusion of materials such as silica, alumina, charcoal, calcium carbonate, magnesia and sucrose which are still in use. He also confirms the identities of the fractions obtained by spectrophotometry at various wavelengths thus anticipating the commonest mode of detection in liquid

chromatography (Lough & Wainer, 1996). The coloured bands produced on the separation process, combining the Greek word *chromos* meaning colour with *grafe* meaning writing (Scott, 1994).

In 1930 in Germany, Edger Lederer drew upon the work of Tswett and Palmer in using chromatography in an investigation into the pigments in egg yolk. Because of the relative speed of the technique it was possible to avoid the degradation of the carotene molecules. Thereafter there was steady success including the development of forms of chromatography other than column liquid chromatography and of instrumental methods of analysis, for examples, infrared spectroscopy and mass spectroscopy which would much later be incorporated into instrumental chromatography (Lough & Wainer, 1996).

In 1938 Eastern European workers carried out planar chromatography in which the powder was spread on a glass plate. Thin-layer chromatography had its origins in this work but at this time the plate had to be horizontal otherwise the layer of powder would be displaced (Lough & Wainer, 1996).

The major breakthrough that would eventually lead to many of the developments in modern chromatography came in 1941 with the work of Martin and Synge. They carried out partition chromatography of amino acids using silica wetted with water and treated with an indicator. The more important was that they produced the first mathematical treatment of chromatographic theory for which they won the Nobel Prize in 1952 using plate theory and predicted may of the developments in chromatography that were later become possible (Lough & Wainer, 1996).

With developments in technology it was possible to apply chromatographic theory to the developments of column liquid chromatography and fulfil the predictions made many years earlier by Martin and Synge. The important improvement upon classical open-column liquid chromatography which came with HPLC was the use of very small particles for the solid adsorbent stationary phase. Because of this the bed of packing material had much lower permeability so that it become necessary to use a pump to generate sufficient pressure to produce a fast enough flow rate. This gave rise to the improved technique being called High-Speed Liquid Chromatography and High-Pressure Liquid Chromatography. Soon these separate terms were replaced by the new term High- Performance Liquid Chromatography (HPLC), the new instrumental technique having better 'performance' in term resolving power, detection and quantitation as well as speed (Lough & Wainer, 1996).

2.1.2 Principles

The principle of chromatographic separation is very straightforward. A mixture is allowed to come into contact with two phases, one referred to as the stationary phase and the other as the mobile phase. The stationary phase is contained in a column or sheet through which the mobile phase moves in a controlled manner relative to the stationary phase, carrying with any material that may prefer to mix with it. In preparative chromatography a device may be attached to the end of a column to collect the separated components of a mixture. The nature of the stationary and mobile phase in a particular chromatographic experiment determines the efficacy of component separation in a particular mixture (Kaiser, 1993).

In the study, the chromatography mode used was a reverse phase HPLC (RP-HPLC). Reverse phase indicates that the stationary phase is less polar than the solvent, which mean that the RP-HPLC is performed on a non-polar stationary phase, C_{18} with a polar mobile phase, water. Adsorption of a solute to a reverse phase is driven by hydrophobic interaction between the solute and the non-polar hydrocarbon stationary surface (Shah & Maryanoff, 2001).

The non-polar components of a sample interact more with the relatively non-polar hydrocarbon column packing and thus elute later than polar components. The elution order of solutes in RP-HPLC is in the order of decreasing polarity or increasing hydrophobicity. RP-HPLC is predominating in the analysis of small organic molecules (Shah & Maryanoff, 2001).

2.1.3 Instrumentation

The components of a typical apparatus for HPLC include a high pressure pump and a supply of mobile phase, a column packed with a high efficiency stationary phase, an injection unit for introducing the samples on to the column, an in-line detector of displaying the detector signal (Lindsay, 1991).

Any part of the system that is contact with the mobile phase must be made of materials that are not attacked by any of the solvents that are to be used. The wetted parts are usually made of stainless steel of PTFE although other materials, such as sapphire, ruby or ceramics are sometimes used. Everything on the high pressure side, for example, from the pump outlet to the end of the column, must be strong enough to

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