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Analytical Methods

Simultaneous determination of diethylene glycol, diethylene glycol monoethyl ether, coumarin and caffeine in food items by gas chromatography

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ABSTRACT

A simple gas chromatography–flame ionisation method was initially developed for the simultaneous determination of the prohibited flavours, namely diethylene glycol (DEG), diethylene glycol monoethyl ether (DEGME) and coumarin in food samples. The analytes were extracted using methanol, and was sonicated for 10 min. The extracts were filtered and directly injected into the GC unit that was fitted with a capillary AT^{TM}-AQUAWAX column. Caffeine, which was found in some of the soft drink samples were also successfully separated. Excellent separation of these flavours and caffeine was achieved in about 23 min. Limit of detection, linear range, and reproducibility of the retention time were evaluated. Average recoveries in the ranges of 93.44–97.54% (RSD, 2.68%) for DEGME, 92.99–101.45% (RSD, 2.99%) for DEG, 90.64–100.00% (RSD, 1.99%) for coumarin and 94.62–97.50% (RSD, 2.54%) for caffeine were obtained. None of the food items analysed was found to contain coumarin, DEG or DEGME. However, of the 35 soft drinks and fruit juices that were analysed, eleven samples were found to contain caffeine, but only one exceeded the legal limit.

1. Introduction

The Food Act 1983 and the Food Regulations 1985 of Malaysia regulates the various issues of food in the country. This includes standards, hygiene, import and export, advertisement and accreditation of laboratories (Foreign Agricultural Service, *GAIN* Report, 2003). The Eight Schedule (Regulation 22) of the Act listed fifteen flavouring substances that are prohibited to be used in food, of which coumarin, diethylene glycol (DEG) and diethylene glycol monoethyl ether (DEGME) (Fig. 1) represent three of the list. Since there is scarce information on the prevalence of these flavourings in local food, a study was commissioned by the relevant regulatory agency (Ministry of Health Malaysia) to shed some light on the matter. An important objective of the task is to develop a practical analytical method, using common instruments (e.g., spectrophotometry, gas chromatography (GC), high performance liquid chromatography (HPLC)) that enable the simultaneous separation of the three components, which finally can be adopted by the satellite laboratories of the Ministry throughout the country.

Coumarin or 1,2-benzopyrone and its derivatives occur abundantly in nature, both in the free state and as glycosides (e.g., in legumes, citrus fruits, orchids, and grasses). Coumarin that is commonly isolated from the tonka beans has a sweet odour and is widely used in soaps and cosmetics (Lake, 1999). It can be found in several foods, in particular in cereals (e.g., wheat, maize, barley, and oat) and several derived products, such as corn flakes, flour, infant foods, malt, and beer (Dall'Asta et al., 2004). Coumarin glycosides have been shown to have blood-thinning, anti-fungicidal, and anti-tumour activities. The maximum limit for coumarin in foodstuffs and beverages according to the European Union standards is 2 mg kg^{-1} in flavouring and other food ingredients. Exception and special restriction are permitted, e.g., 10 mg kg^{-1} in certain types of caramel confectionery and alcoholic beverages and 50 mg kg^{-1} in chewing gums (European Council Directive, 1988).

DEG and DEGME are highly flammable, colourless, moderately volatile liquids with very good solubility properties in water and organic solvents. They are used in paints, stains, inks and surface coatings, silk-screen printing, photographic and photo lithographic processes (Johanson, 2000). DEG is also used as a preservative for food packaging adhesives with polyethylene glycol. The chemical migration from plastic packaging materials or regenerated cellulose film into food, leading to DEG poisoning resulting from the ingestion of the migrated glycol has been reported (Knight & Creighton, 2004). In 2007, several safety alerts on the presence of DEG in dental products have been issued by several institutions after the Food and Drug Administration of the USA found DEG in

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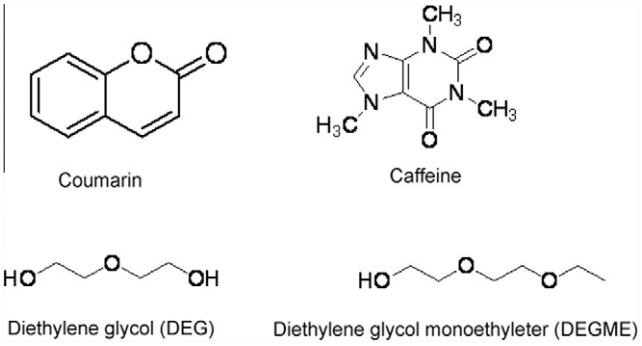

Fig. 1. Chemical structures of the flavouring agents and caffeine.

certain imported toothpastes. The limit allowed for DEG and DEGME in food items has not been stipulated. However the maximum concentration of DEG allowed in finished cosmetic products is 0.1% (European Council Directive, 2009).

Coumarin and related compounds are commonly analysed using reversed-phase HPLC (Ahn, Lee, Kim, & Sung, 2008; de Jager, Perfetti, & Diachenko, 2007; He et al., 2005; Naik & Nagalakshmi, 1997; Sproll, Ruge, Andlauer, Godelmann, & Lachenmeier, 2008), although other techniques such as gas chromatography–mass spectrometry (GC–MS) (Yang et al., 2009), capillary electrophoresis (CE) (Bogan, Deasy, O'Kennedy, Smyth & Fuhr, 1995; Ochocka, Rajzer, Kowalski, & Lamparczyk, 1995) and spectrophotofluorometry (Tan, Ritschel, & Sanders, 2006) have also been reported. By virtue of their volatility, GC technique is the method of choice for the determination of DEG and DEGME (Maurer, Peters, Paul, & Kraemer, 2001; Savchuk, Brodskii, & Formanovskii, 1999; Williams, Shah, Maggiore, & Erickson, 2000).

This study initially deals with the analytical method development for the simultaneous determination of the prohibited flavourings, DEG, DEGME and coumarin in food. As mentioned earlier, an important consideration in the exercise is to use straight-forward sample preparation techniques in conjunction with common instruments. Thus, a simple solid–liquid and liquid–liquid extraction sample preparation procedures for solid and liquid samples, respectively, together with GC with flame ionisation detector (FID) was chosen. The suitability of the AQUAWAX capillary GC column, which was claimed to be suitable for the analysis of aqueous extracts (Alltech Heliflex® AT-AquaWAX-DA Capillary Columns), was also evaluated.

2. Experimental

2.1. Chemicals and standards

DEGME (99.5%), DEG (99%), coumarin (99%) and caffeine (99%) standards were purchased from Sigma Aldrich (Steinheim, Germany). Methanol (HPLC grade) was obtained from Fisher Scientific (Loughborough, UK). Nanopure water (18 MΩ cm^{-1}) was generated from a NANOpure Diamond™ unit from Barnstead, USA.

2.2. Preparation and storage of standards

Stock standard solutions (5000 mg mL^{-1}) of each of the analytes (DEGME, DEG, coumarin and caffeine) were prepared in methanol. The flask was sonicated in an ultrasonic bath (10 min) until a homogenous and clear solution was formed. The stock solution was stored in a freezer (4 °**C**) for a maximum of 1 month. Before use, standard working solutions were prepared by diluting appropriate amounts of the stock solution in methanol.

2.3. Food samples

One hundred-fifty five food samples were analysed, comprising soft drinks and juice (35), infant formula and infant food (10), cereal (27), flour (26) and snacks (57). Samples were purchased from local supermarkets around the states of Perlis, Kedah and Penang, Malaysia.

2.4. Apparatus

The following apparatus were used during the course of sample preparation: kitchen grinder (Pensonic Model PB-325, Penang, Malaysia), ultrasonic bath, Power Sonic 405 (Hwashin Technology, Seoul, Korea), and PTFE membrane filters (Whatman 0.5 μm × 45 mm, Shanghai, China).

2.5. Extraction procedure

Homogeneous solid sample (1 g) was placed into a 13-mL screw-capped vial and placed in an ultrasonic bath (room temperature) for 10 min. The extracts were filtered through disposable syringe filters and 1 μL sample was injected into the GC unit. The same procedure for liquid samples was adopted. The extractions were carried out in triplicates.

2.6. GC analysis

GC analyses were performed on a 7890A GC System (Agilent Technologies, USA) equipped with a split/splitless (1:10) capillary injector and a FID. Analytical separation was carried out either on an AT™-AQUAWAX capillary column (30 m × 0.25 mm i.d.) (Alltech, USA) or a SBP-35 capillary column (30 m × 0.32 mm i.d.) (Supelco, USA). The film thickness for both columns was 0.25 μm. Oxygen free nitrogen was used as carrier gas (1 mL min^{-1}). The air, hydrogen and auxiliary gases (N_2) flow to the detector were kept at 400, 30 and 25 mL min^{-1}, respectively. The temperature of the injector and detector was at 260 °C. The oven temperature was set at 100 °C, increased to 225 °C at 7.5 °C min^{-1}, then to 250 °C at 20 °C min^{-1} and held for 5 min, and finally to 260 °C at 15 °C min^{-1} and was held for another 6 min. The peak areas were used to calculate the levels of the analytes.

3. Results and discussion

3.1. Sample preparation

A rapid and simple sample preparation procedure is of great importance in analytical method development. The main aim of the sample preparation procedure is to isolate the analytes in as pure form as possible before the analytical determination. During the process, particulates as well as interfering matrix components are removed, resulting in an enhancement of the method sensitivity. In our study, the extraction was carried out using a relatively environmentally-friendly solvent, methanol, and the extraction was enhanced by sonication. For soft drink samples, the extracts could be directly injected into the GC system after the membrane filtration. Products with high water content (e.g., milk products, juice and concentrated cordial) were diluted with methanol to obtain a water–methanol (1:10) mixture. The disposable syringe filter was effective in removing particulates before the GC injection.

The extraction method used provides extracts with negligible matrix interferences and does not form emulsions even for fat-containing food items as confirmed by Sproll et al. (2008). The overall extraction was enhanced by subjecting to ultrasonic sonication for