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Determination of Eugenol Contents of Some Zinc Oxide-Eugenol Based Dental Cements¶

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The aim of this study was to analyze the eugenol content of some eugenol based commercially available dental cements. Eugenol analysis was made by gas chromatography equipped with flame ionization detector (GC-FID) and also results confirmed by gas chromatography-mass spectrometry (GC-MS). In all products, eugenol was found in different concentrations. It is noteworthy that there were variations in the concentration of eugenol even in products manufactured by the same company. Due to the fact that anaesthetic, sedative and analgesic effects are related to the eugenol concentration. It is critical to have eugenol content standardized.

Key Words: Zinc oxide-eugenol cement, Eugenol content, GC, GC-MS.

INTRODUCTION

Phenolic compounds have been widely used in dental treatments as sedatives for the dental pulp tissues, disinfectants for caries during cavity preparation and root canal medications. Despite of extensive clinical uses of these substances in dentistry, there are very few studies on their mode of action for possible pathobiological effect on human dental pulp tissues[1,2].

Eugenol (4-allyl-2-methoxy-phenol) is a natural pungent that is also an important constituent of the essential oils of many aromatic plant such as *Eugenia caryophyllus* (Spr.), *Dicipelium cariophyllatum, Pimenta dioica, Croton zehntneri* var. *eugenoliferum* and *Ocimum gratissimum*. Eugenol

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can be used as a food flavour, fragrant in cosmetic industry, in pesticides to attract the insects. It is commonly used in dentistry for the alleviation of toothache, for pulpitis and dental hyperalgesia. It has central nervous system effects in mammals, causes hypothermia, decreases in spontaneous motor activity, acts as an anticonvulsant and has general anesthetic effects[3]. Eugenol is a potent depressant of peripheral nervous activity. Eugenol was hypothesized to act, similar to Ca^{2+} channel antagonists, either through voltage-dependent or receptor-operated Ca^{2+} channels to induce its vasodilating effects on isolated rat aorta but not on the small resistance vessels[4]. Eugenol and β-caryophyllene oxide, which have relaxant effects on smooth muscle also block calcium channels of the heart[5]. Eugenol and isoeugenol are well known to possess antioxidant activities[6].

As a phenolate based cement, zinc oxide eugenol cement (ZOE) has been widely used in dentistry for indirect pulp capping, cementing agents for crowns and fixed partial dentures, temporary filling and as a root canal sealer. Eugenol is known to be an antioxidant and anti-inflammatory agent. However, eugenol at high concentrations has been reported to have some cytotoxic properties. In the presence of moisture the ZOE matrix is hydrolyzed to release eugenol, which could have pathological effects on the pulp or oral mucosa. Generally, ZOE is classified as a toxic material according to the cytotoxicity standard[7,8]. Some information supports the idea that eugenol has multiple effects, being able to act as a pro-mutagen, a weak carcinogen, as well as an antimutagen[9]. It has long been used in dentistry, first as cement and later as impression material[10]. The zinc oxide-eugenol cements have been used mainly as temporary restoratives during the treatment of teeth and prior to the placement of the final restorations due to its sedative effect on the pulp[2,10]. Zinc oxide-eugenol cements have always been regarded as the blandest in terms of their effect on the dental pulp. For this reason, these materials were commonly used as the control in biocompatibility pulp research studies on cements and restorative materials[2].

Zinc oxide eugenol cement (ZOE) has powder and liquid components, as the other conventional dental cements. The powder is essentially pure zinc oxide. Commercial materials may contain small amounts of fillers, such as silica. The liquid is purified eugenol or, in some commercial materials, oil of cloves (85 % eugenol). One per cent or less of alcohol or acetic acid may be present to accelerate setting together with small amounts of water, which is essential to the setting reaction. A chemial rection occurs between zinc oxide and eugenol, with the formation of zinc eugenolate (eugenat)[11].

The precise setting mechanism of ZOE is not fully understood, but the set mass contains residual zinc oxide particles bonded by a matrix of zinc

eugenolate and some free eugenol. Water is essential to the reaction which is accelerated also by zinc ions. The reaction is irreversible because the zinc eugenolate is easily hydrolyzed by moisture to eugenol and zinc hydroxide. Thus the cement disintegrates rapidly when exposed to oral conditions. The rate of reaction between the zinc oxide and eugenol depends on the nature, source, reactivity and moisture content of the zinc oxide and on the purity and moisture content of eugenol[11].

Antibacterial, anesthetic, sedative and cytotoxic effects directly related to the eugenol concentration of the liquid components of ZOE. In addition, eugenol plays an important role on setting reaction[11-14]. Due to many properties depend on it, eugenol concentration is critical. The aim of this study was to analyze the eugenol content of seven different eugenol based dental cements that are commercially available.

EXPERIMENTAL

In this study, samples obtained from seven different commerical ZOE cement products were used.

Standard and sample preparation: For calibration curve 10, 20, 40, 60 and 100 µL were taken out of the standard solution (> 99 % eugenol pure for GC analysis, Merck) and dissolved in 10 mL of acetone (Merck). It was mixed by using vortex (Velp-Scientifica Cod. F 20220176) and 2 mL of the solution was filtered through 0.45 µm filter disc (milipore) was put in a vial and then injected to the gas chromatography (Shimadzu GC-17 A, Shimadzu Co., Japan). 50 µL of each ZOE cements and standards were taken out and test samples were prepared same as the standards solutions. Eugenol analysis method was performed according to Kildae *et al.*[15].

Analytical conditions

Gas-chromatography: A Shimadzu GC-17A GC, equipped with a flame ionization detector (FID), with electronic pressure control and split/splitless capillary inlet system was used. The apparatus was equipped with an auto injector system (Shimadzu Co. Japan) model AOC 20i with 10 µL syringe set at 1 µL delivery volume at fast injection speed. Analysis of eugenol was carried out on 30 m × 0.32 mm i.d. fused silica capillary column with 0.25 µm film of 95 % dimethylpolysiloxane 5 % diphenyl (Teknokroma, Spain). Carrier gas was nitrogen at constant flow 1.86 mL/min.

The initial oven temperature was set at 60°C for 1 min, programmed from 60 to 270°C at 10°C/min and held for 12 min. The injector and detector temperatures were 250 and 270°C, respectively.

After determination of eugenol, the samples injected to the GC-MS for confirmation of the peaks.

Gas chromatography-mass spectrometry: A GC-MS (ion trap) (Shimadzu GC-MS 5050, Shimadzu Co, Japan) was used. The GC-MS operating conditions were the same as above. The GC-MS interface temperature was 270°C and an electron impact ionization of 70 eV was used. The samples were injected to GC-MS in scan mod.

RESULTS AND DISCUSSION

GC chromatograms of the eugenol standarts and standart calibration curve were obtained and showed in Figs. 1 and 2.

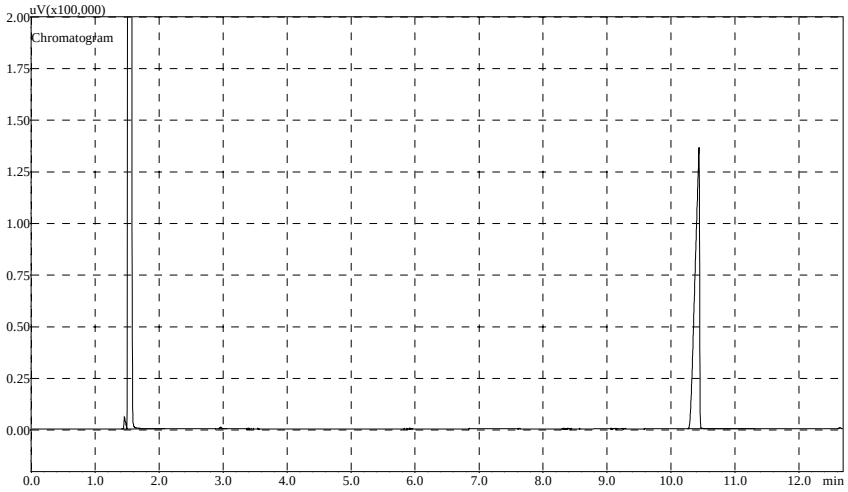

Fig. 1. GC chromatogram of eugenol standard

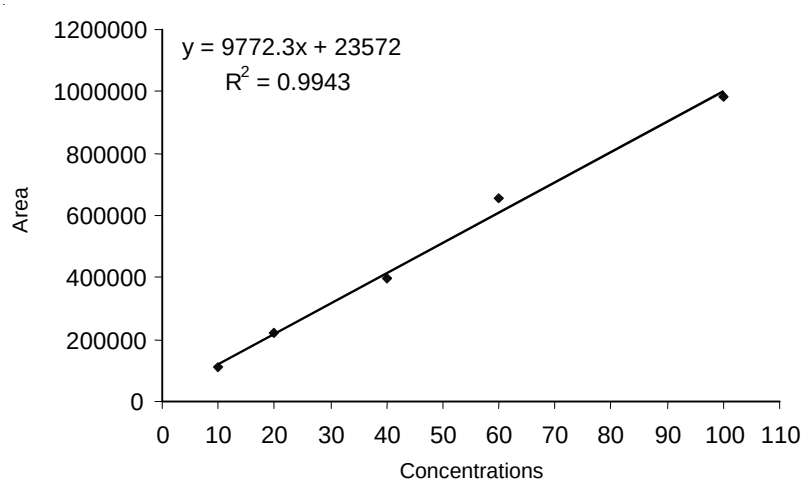

Fig. 2. Calibration curve of eugenol standard

Four peaks were determined on the sample B (Fig. 3) chromatogram and confirmed by GC-MS (Fig. 4). The four peaks were eugenol, β- caryophyllene, α-caryophyllene and eugenol acetate, respectively.

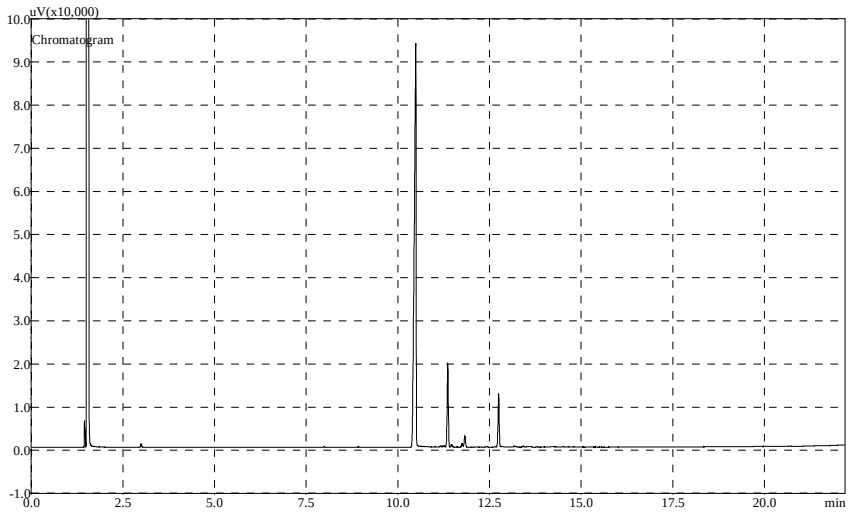

Fig. 3. GC chromatogram of sample B

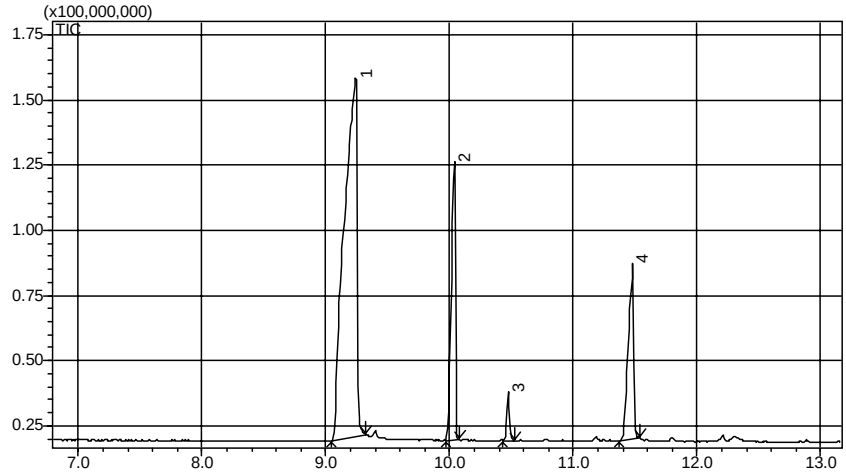

Fig. 4. GC-MS chromatogram of sample B. (1) Eugenol, (2) β-caryophyllene, (3) α-caryophyllene and (4) Eugenol acetate

In the other GC chromotograms of the ZOE materials showed only eugenol peak as in the sample A (Fig. 5).

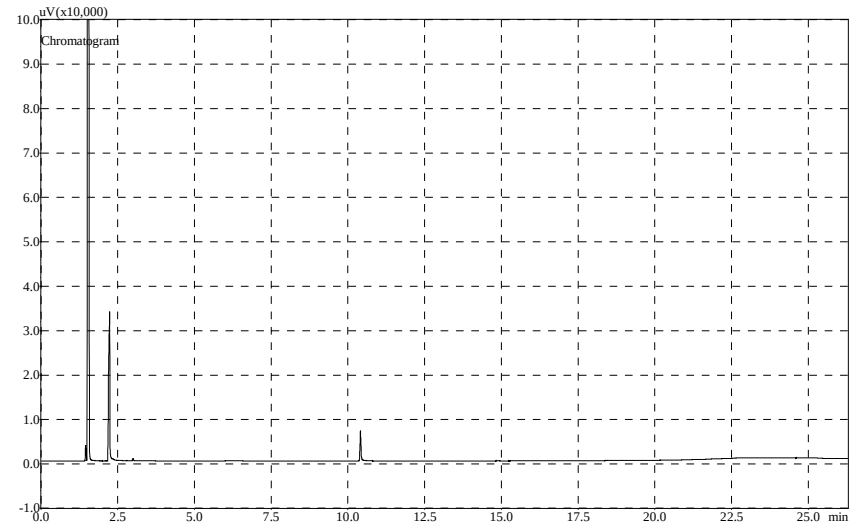

Fig. 5. GC chromatogram of sample A

The eugenol amount of seven samples was showed in Table-1. Not only between the different brands but also in the same brand products, the eugenol levels varied. The difference of eugenol level was the highest in the samples from sample F while the others had much or less differences.

TABLE-1
EUGENOL CONTENTS OF ZOE CEMENTS

Preparations	Area	Concentration of Eugenol (%)
A	(1) 29652*	1.20
	(2) 28987	1.11
B	(1) 334731	63.68
	(2) 341570	65.08
C	(1) 436541	84.50
	(2) 418946	80.91
D	(1) 392067	75.41
	(2) 401813	77.41
E	(1) 379798	72.90
	(2) 421616	81.46
F	(1) 483403	94.10
	(2) 332468	63.21
G	(1) 400010	77.04
	(2) 454864	88.26

*Mean values of two injections of each preparation.

In the present study, the eugenol amount of seven ZOE cement commercially available in Turkey was investigated. Due to less attention in such studies, it is difficult to discuss and compare our results. Since the eugenol content of the analyzed preparations was not given on the boxes of the products or the prospectus them, the concentration was calculated from a calibration curve constructed with standards and peak areas of the samples prepared from standard solutions were used.

The reproducability of this study was > 99 %, indicating that this method is proper for eugenol analysis (Fig. 1).

According to the eugenol standards set by Turkish Standards Institution (Turkish standard TS 7805/January 1990) the amount of zinc oxide eugenol was lower than the amount stated on the package, which was a serious fault. Similarly, the US and European standards stipulate the amount of eugenol be stated on the package and the content should not be less than the amount stated on the package. In all samples analyzed, eugenol content was different (Table-1). It is an interesting fact that there are differences in content among products manufactured by the same company. Furthermore, one of the products claimed to contain eugenol on the package while the analyses confirmed that it contained oil of clove because it contains eugenol, β-caryophyllene, α-caryophyllene and Eugenol acetate (*Oleum caryophylli*) (Figs. 3 and 4).

While eugenol contents of some of the products analyzed (cements A, B, C, D and E) were similar, we found a difference in concentrations as much as 30 % among certain products (preparations F and G) manufactured by the same company.

Since anesthetic, sedative and analgesic effects of zinc oxide eugenol cement is concentration-dependent, we conclude that standardization of active ingredient contents of materials used in dentistry and market control at certain intervals are mandatory.

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