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Validation of a high performance liquid chromatography method for the stabilization of epigallocatechin gallate

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ABSTRACT

Epigallocatechin gallate (EGCG) is a green tea catechin with potential health benefits, such as anti-oxidant, anti-carcinogenic and anti-inflammatory effects. In general, EGCG is highly susceptible to degradation, therefore presenting stability problems. The present paper was focused on the study of EGCG stability in HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) medium regarding the pH dependency, storage temperature and in the presence of ascorbic acid a reducing agent. The evaluation of EGCG in HEPES buffer has demonstrated that this molecule is not able of maintaining its physicochemical properties and potential beneficial effects, since it is partially or completely degraded, depending on the EGCG concentration. The storage temperature of EGCG most suitable to maintain its structure was shown to be the lower values (4 or −20 °C). The pH 3.5 was able to provide greater stability than pH 7.4. However, the presence of a reducing agent (i.e., ascorbic acid) was shown to provide greater protection against degradation of EGCG. A validation method based on RP-HPLC with UV–vis detection was carried out for two media: water and a biocompatible physiological medium composed of Transcutol®P, ethanol and ascorbic acid. The quantification of EGCG for purposes, using pure EGCG, requires a validated HPLC method which could be possible to apply in pharmacokinetic and pharmacodynamics studies.

1. Introduction

In the last years, extensive studies have been published about the biological and pharmacological activities of tea catechins, which are the main components of the green tea leaves (*Camellia sinensis* L.). The main component is the epigallocatechin gallate (EGCG), which is present in about 70% (Nagle et al., 2006). There are several studies reporting its effects on human health, including anti-carcinogenic (Du et al., 2013; Rathore et al., 2012; Suganuma et al., 1999; Zhong et al., 2012a), antioxidant (Henning et al., 2005; Hu and Kitts, 2001; Zhong et al., 2012c; Zhong and Shahidi, 2012), anti-inflammatory (Cavet et al., 2011; Tedeschi et al., 2002; Zhong et al., 2012b) and anti-microbial (Du et al., 2012; Gordon and Wareham, 2010; Güida et al., 2007), based on the anti-oxidative property and inhibitory effect of tea catechins on some enzymes (Wang et al., 2006). Despite its proven health benefits, the use of EGCG for pharmaceutical purposes is not clear. The main problems that affect this catechin are related to its stability. To understand the underlying mechanisms and to study the beneficial health effects related to EGCG, it is essential to stabilize the molecule. The majority of catechins, particularly EGCG, possess certain physicochemical limitations. They are highly unstable in solution and degrade through oxidative and epimerization processes (Mochizuki et al., 2002). The catechins are hydrogen

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donors and the anti-oxidant capacity is dependent on the number and position of hydroxyl (—OH) groups and their conjugation. Thus, EGCG has greater beneficial health effects than other similar catechins due to the presence of the galloyl moiety, which is partly responsible for the chelating and radical scavenging properties (Heim et al., 2002). The stability of tea catechins, including EGCG, is pH and temperature dependent. The main problem is when EGCG is in aqueous solution, demonstrating limited stability when pH is below 4, and is highly unstable at pH above 6 (Chen et al., 2001; Komatsu et al., 1993; Lun Su et al., 2003; Zimeri and Tong, 1999). Thus, the *in vivo* and *in vitro* evaluation of this catechin becomes a problem regarding its dissolution on physiological buffers such as phosphate buffered saline (PBS) and HEPES (which is composed by 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid). In addition, storage temperature also significantly affects its stability (Chen et al., 2001; Kumamoto et al., 2001; Wang et al., 2008). The main reactions that occur with the degradation of EGCG (Fig. 1A) is the epimerization, which can be caused by the temperature and leads to release of other catechins products, namely (−)-gallocatechin gallate (GCG) (Fig. 1B).

Some studies report that epimerization processes of catechins, including EGCG, do not significantly alter the anti-oxidant activity, absorption and metabolism of the original catechins (Xu et al., 2004). In addition, it is proven that (−)-GCG is more effective in reducing plasma cholesterol and triglyceride concentrations than EGCG (Ikeda et al., 2003; Lee et al., 2008). However, the epimers of the catechins revealed that stereochemistry influences radical scavenging activity of galloylated catechins (Muzolf-Panek et al., 2012). Unfortunately, there is a lack of information related to the epimerization reactions; however, some authors suggested that epimerization of catechins occurred followed by thermal degradation (Wang et al., 2006, 2008). Some studies already evaluated EGCG in a liquid model system, where the reaction temperature was up to 100 °C with varied pH values ranging from 4 to 7. The involved oxygen concentration was taken into account for the thermal degradation of EGCG, i.e., the conversion of EGCG to its epimer (−)-GCG and vice versa (Wang et al., 2008).

The main problems are related to the oxidation of EGCG, which leads to degradation of products (Fig. 1C and D). There are two major oxidation routes, namely: (i) the condensation with coexisting epicatechin and its gallate, yielding theaflavins, pigments with a benzotropolone moiety (Tanaka et al., 2010), and (ii) the production of some epigallocatechin dimers, such as theasinensins and oolongtheanins (Matsuo et al., 2008). The first oxidation route which leads to the formation of theaflavins, another group of polyphenols, is promoted by the enzymatic oxidation and leads to brown solutions, easily visible at naked eye (Lun Su et al., 2003). This formation could form dimers with benzotropolone structures linked through the B-ring, namely dehydrotheaflavin and theanaphthoquinone, and is associated with the co-oxidation of the hydroxyl groups of the gallate group of EGCG, namely in the ortho and tri positions (Sang et al., 2004). In the other route, EGCG degradation leads to the formation of theasinensins and oolongtheanins by the dismutation of the molecule (Tanaka et al., 2010).

Since EGCG is a potent anti-oxidant, it tends to be oxidized within the biological environment, which leads to a lower bioavailability and short half-life limiting its therapeutic efficiency. Therefore, the stability of tea catechins must be evaluated, so that reliable results can be obtained from any biopharmacokinetic or biopharmacodynamic studies. The addition of an anti-oxidant to EGCG has been reported to improve their pharmaceutical effect as also to prevent degradation of the molecule (Hatano et al., 2008). Hatano et al. (2008) evaluated the capacity of several food additives to maintain the EGCG in solution, and the ascorbic acid was the most potent in preserving the initial concentration. In addition, some authors use ascorbic acid as reducing agent to prevent EGCG oxidation in the analysis medium (Dube et al., 2010a, b). For this purpose, the effect of the presence of an anti-oxidant molecule, namely ascorbic acid, in the degradation of EGCG has been analyzed.

Being EGCG one of the most prevalent and biomedical interesting catechin, its stability in various media was examined in this study. In addition, a sensitive analytical method for the determination of EGCG in a mimetic biological medium has been

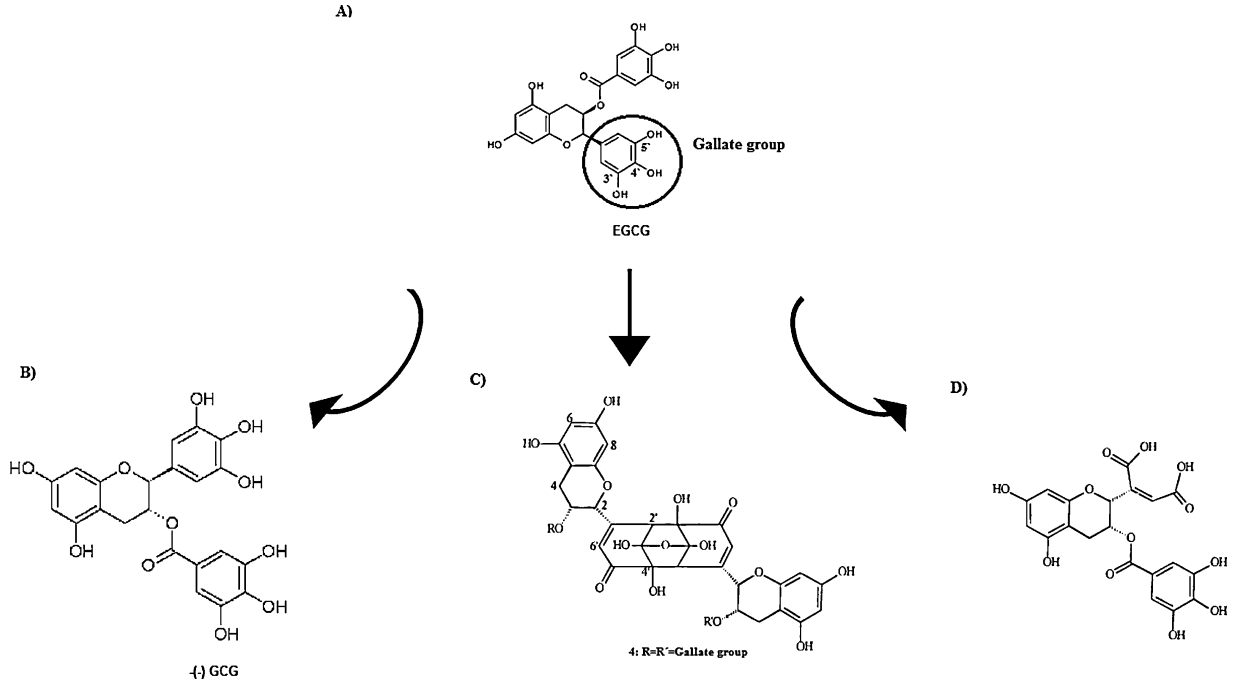

Fig. 1. Degradation products of EGCG. Structure of EGCG (A), the product resulting from its epimerization (−)-GCG (B), and the products derived from its oxidation (C and D).