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Development and *in vitro*/*in vivo* evaluations of bioadhesive buccal tablets for nicotine replacement therapy

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Buccal bioadhesive tablet formulations of nicotine hydrogen tartrate (NHT) for nicotine replacement therapy (NRT) were developed using chitosan and carbomer at different ratios. Magnesium hydroxide was incorporated into the formulations as pH increasing agent. *In vitro* release and bioadhesion properties of the tablets were investigated. Release of NHT from the tablets was increased with the increasing amount of chitosan in formulations whilst the bioadhesion of the tablet was decreased. *In vivo* studies were carried out in healthy, non-smoker volunteers in comparison to a commercially available transdermal patch. Plasma nicotine and cotinine levels were determined using gas chromatography-mass spectrophotometry. No significant difference was found between the maximum plasma nicotine concentrations (C_{max}) obtained with the buccal tablet and the transdermal patch ($p > 0.05$). Time to reach the C_{max} was 2.9 ± 0.2 h and 11.5 ± 1.3 h, and AUC_{0-24} values were 59.3 ± 5.1 ng · h · mL^{-1} (0–12 h) and 204.1 ± 31.2 ng · h · mL^{-1} for buccal tablet and transdermal patch, respectively.

1. Introduction

Nicotine is being widely used to aid smoking cessation. Studies showed that temporary nicotine replacement reduced symptoms of smoking withdrawal and tripled the success rate of continuous smoking abstinence in the first year (Tønnesen et al. 1993; Blöndal 1989; Rose et al. 1990). Due to the first-pass metabolisation of nicotine following oral administration, the attempts are focused on alternative delivery of nicotine to the systemic circulation via such as oral mucosal, transdermal and nasal routes. Chewing gums and vapour inhalers are the dosage forms available on the market for delivery of nicotine across the oral mucosa which provide rapid and short lived peak plasma levels when compared to the transdermal patches (Fant et al. 1999; Molander et al. 1996). These delivery systems need to be administered at very short time intervals due to the short elimination half life of nicotine. In addition, for chewing gums, drinking and eating is not allowed.

Carbomers are extensively used in formulation of bioadhesive buccal tablets in combination with the non-ionic polymers such as hydroxypropylcellulose (HPC), hydroxypropylmethylcellulose (HPMC) and hydroxyethylcellulose (HEC) (Han et al. 1999; Perioli et al. 2004; Şenel et al. 1998; İkinci et al. 2000). In the last decade, the use of chitosan in drug delivery has been increased due to its bioadhesive, biodegradable and biocompatible properties (Giunchedi et al. 2002; Sandri et al. 2004). It also exerts bioactive properties such as wound healing, tissue regeneration and antimicrobial activity (Şenel et al. 2000; Aksungur et al. 2004; Kweon et al. 2003; Chou et al. 2003). Nicotine replacement therapy (NRT) products contain nicotine in different forms such as free base nicotine, nicotine salt, complex with an ion exchanger or nicotine inclusion complex (Blöndal 1989; Rose et al. 1990; Green et al. 1999; Berglund et al. 1997). In its free base form, it readily penetrates through membranes (Svensson 1987). However, free base nicotine is a labile compound that may easily evaporate and/or undergoe oxidative degradation whereas nicotine hydrogen tartrate (NHT) which is a crystalline powder is a stable salt form of nicotine (Mihranyan et al. 2004). The aim of this study was to develop a buccal bioadhesive tablet formulation which can be used for nicotine replacement therapy. Combination of chitosan and carbomer was used at different ratios to control the drug release as well as the bioadhesive properties of the tablet. Magnesium hydroxide which has been shown to increase the *in vitro* buccal permeation of NHT was also incorporated into the tablet formulations (İkinci et al. 2002).

2. Investigations and results

2.1. In vitro studies

2.1.1. Release of NHT from tablets

The release of NHT from buccal adhesive tablet was increased with increasing chitosan concentration in the formulations (Fig. 1a). At 20/80 ratio of chitosan/carbomer (CH-20), the released amount was $43 \pm 2\%$ in 4 h whereas at 80/20 ratio (CH-80) it was $90 \pm 4\%$. All for-

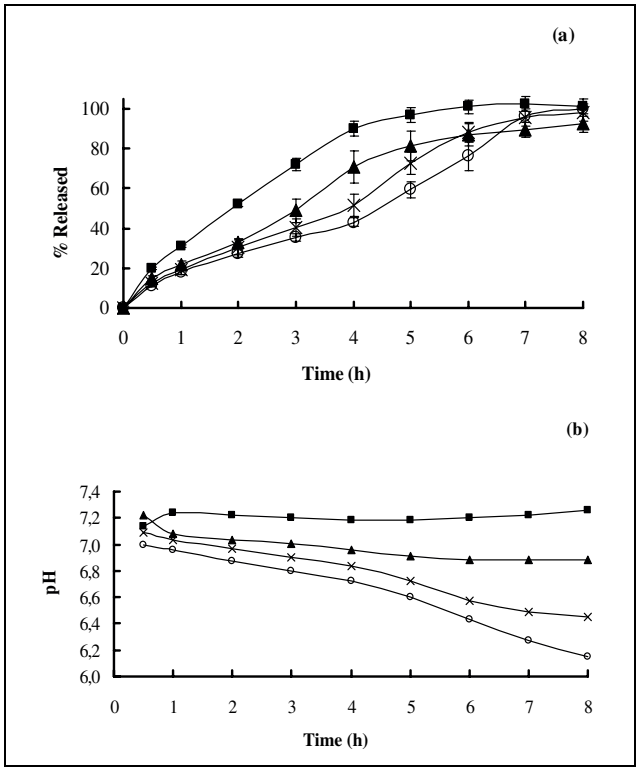

Fig. 1: Release of NHT from tablets (a), and pH of the dissolution medium over the release period (b); CH-80 (■); CH-60 (▲); CH-40 (x); CH-20 (o) (mean ± SD, n = 6)

Table 1: Release exponents (n) and determination coefficients (r^2) for buccal tablets

Formulation	n	r^2
CH-80	0.713 ± 0.022	0.991 ± 0.004
CH-60	0.725 ± 0.025	0.966 ± 0.011
CH-40	0.794 ± 0.029	0.970 ± 0.008
CH-20	0.795 ± 0.018	0.951 ± 0.006

mulations remained intact during the 8 h release period. Release data was analysed using Eq. (1):

$$M_t/M_\infty = kt^n \quad (1)$$

where M_t/M_∞ is the drug fraction released at time t, k is the release rate, n is the diffusional coefficient related to the release mechanism. The n values were found to be between 0.713–0.795 indicating an anomalous, or non-Fickian, release mechanism (Table 1).

A significant decrease in pH of the dissolution medium was observed with the increasing CP concentration in the tablet formulation whereas no change in the pH was observed with formulation CH-80 in which the carbomer concentration was the lowest (Fig. 1b).

2.1.2. Bioadhesion studies

The bioadhesion forces (detachment force and work of adhesion) of the buccal tablets were found to be affected by the change in polymer ratios (Fig. 2). The formulation containing chitosan/carbomer at the ratio of 20/80 (CH-20) showed the highest bioadhesion. The lowest adhesion was observed with the formulation containing chitosan/carbomer at the ratio of 80/20 (CH-80) ($p < 0.05$). No significant difference in bioadhesion forces was observed between formulations CH-80 and CH-60 ($p > 0.05$).

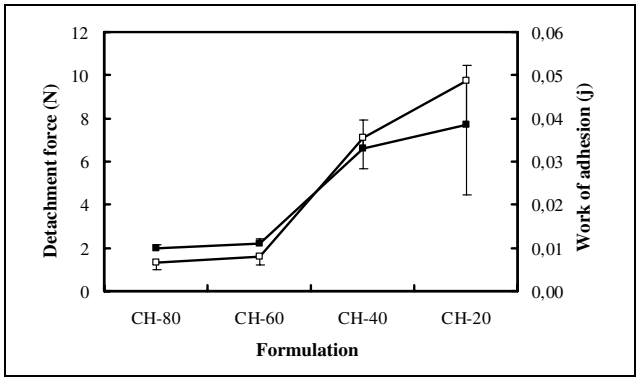

Fig. 2: Bioadhesion test results for tablets in contact with bovine buccal mucosa: (□) work of adhesion; (■) detachment force (mean ± SD, n = 3)

2.2. In vivo studies

The tablets applied on buccal mucosa remained attached for 4 h without any discomfort (Fig. 3). No irritation or pain on the mucosa was reported.

Plasma nicotine and cotinine levels were found to increase rapidly following application of the buccal tablet when compared to the transdermal patch (Fig. 4). The maximum plasma concentration (C_{max}) values were between 9.4–18.7 ng · mL^{-1} with the buccal tablet whereas with the transdermal patch they were between 7.0–26.4 ng · mL^{-1} (Table 2). No significant difference was found between the maximum plasma nicotine concentration (C_{max}) values for buccal tablet and transdermal patch ($p > 0.05$). Time to reach the C_{max} was 2.9 ± 0.2 h and 11.5 ± 1.3 h for buccal tablet and transdermal patch, respectively. The AUC_{0-24} value following application of transdermal patch containing 35 mg nicotine was 204.1 ± 31.2 ng · h · mL^{-1}; while AUC_{0-12} value was 59.3 ± 5.1 ng · h · mL^{-1} (0–12 h) for buccal tablets. The intersubject variability in nicotine plasma levels was found to be higher with the transdermal system when compared to that of buccal tablet.

3. Discussion

3.1. In vitro release of NHT

NHT release from the tablets was increased with increasing chitosan amount in the formulation (Fig. 1a). The percentage of release for formulations CH-20, CH-40, CH-60 and CH-80 at the end of 4 h was 43, 52, 71 and 90, respectively. The chitosan used in formulations is the water soluble form. It is thus envisaged that an increased amount of chitosan in the formulation would result a fast hydration of the tablet and formation of porous channels in the matrix due to its water solubility, resulting in a faster release of NHT. The release of NHT from tablets showed anomalous, non-Fickian behaviour which indicates that the release was controlled by combination of polymer swelling, erosion and diffusion (Table 1) (Choi et al. 2000). On the other hand, pH of the release medium was found to decrease with the increasing carbomer amount in the formulation and it was highest for formulation CH-80 (above 7.2). Hydration of carbomer in alkaline medium would be more rapid which could also result in increased release.

In our previous study, we have shown that the permeation of nicotine through bovine buccal mucosa was increased in presence of magnesium hydroxide which was used as pH increasing additive (İkinci et al. 2002). These results

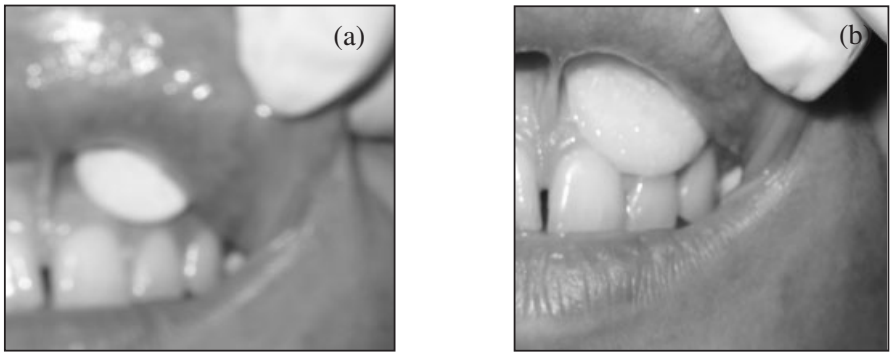

Fig. 3:
Appearance of the buccal tablet, rightafter (a) and 4 h after (b) application

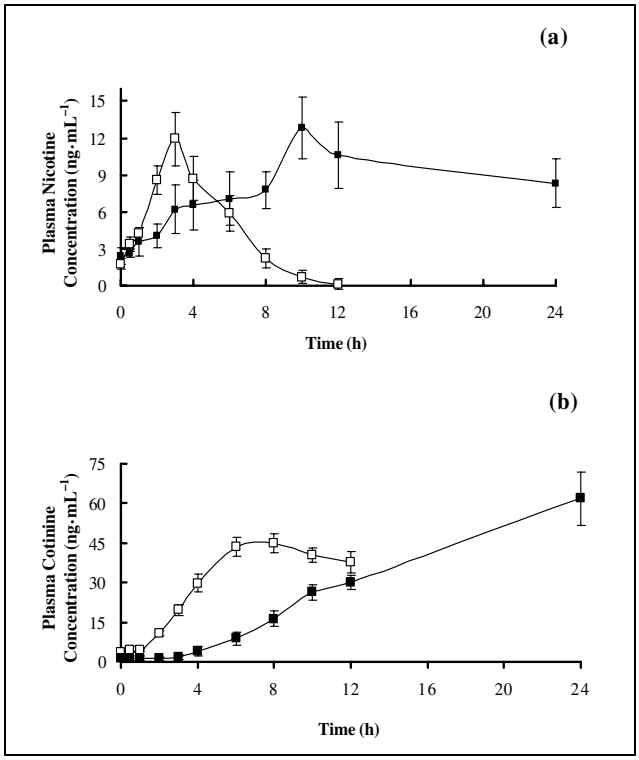

Fig. 4: Plasma levels of nicotine (a) and cotinine (b) in healthy volunteers following administration of buccal tablet (□) and transdermal patch (■) (mean ± SD, n = 8)

Table 2: Pharmacokinetic parameters in healthy volunteers following administration of a buccal tablet and transdermal patch (mean ± SD, n = 8) (CV%)

	Buccal tablet	Transdermal patch
C_{max} (ng · mL^{-1})	12.7 ± 0.8 (25%)	13.1 ± 3.3 (55%)
t_{max} (h)	2.9 ± 0.2 (29%)	11.5 ± 1.3 (44%)
$AUC_{0\text{-}12}$ (ng · h · mL^{-1})	59.3 ± 5.1 (34%)	–
$AUC_{0\text{-}24}$ (ng · h · mL^{-1})	–	204.1 ± 31.21 (61%)

were in good correlation with the studies carried out with cell culture or excised porcine mucosa (Nielsen and Rassing 2002; Nair et al. 1997; Chen et al. 1999). Therefore in this study magnesium hydroxide was incorporated into the tablet formulations to provide an alkaline medium to enhance the nicotine absorption. Nicotine has two pKa values at 3.04 and 7.84. Various ratios of nicotine species (diprotonated, monoprotonated and un-ionized) are present in the solution depending on the pH. The permeability of nicotine across buccal mucosa is reported to follow the pH-partitioning theory characteristics for passive diffusion (Nair et al. 1997). The differences obtained in pH values of the release medium with different formulations can be attributed to the differences in the amount of the anionic (CP) or cationic (chitosan) polymers and the released amount of NHT (Fig. 1). With the formulation containing the highest chitosan amount (CH-80), no significant change in the pH of the release medium was observed during the release period whereas increasing the amount of carbomer in the formulation resulted in a decrease in the pH of the release medium. This result can be attributed to the ionization of the carboxyl group in carbomer. Similar results were obtained for non-ionic polymer (HPMC) and carbomer containing systems (İkinci et al. 2004; Khanna et al. 1997).

3.2. Bioadhesive properties

Bioadhesion was increased with the increasing amount of CP in the formulations (Fig. 2). Polymer type, the ratio of the polymers in the formulation and the pH of the medium was found to affect the bioadhesive properties of the tablets. The bioadhesion obtained with formulations CH-20 and CH-40 was higher than that of CH-60 and CH-80. This can be explained by the high concentration of carbomer in these formulations. The oligosaccharide chains which are present in the mucus would bind more strongly with the carboxyl groups of carbomer when compared to that with amine groups of chitosan (Duchêne and Ponchel 1992; Agarwal and Mishra 1999). With an increasing amount of chitosan in the formulation, upon exposure to the moist surface, the pH of the medium becomes alkaline and carbomer swells rapidly in the alkaline medium and the number of carboxyl groups of the polymer which interact with the mucin would decrease resulting in a decrease in bioadhesion. On the other hand, the bioadhesive property of carbomer is reported to decrease at pH values above 6 due to loss of hydrogen bonding (Satoh et al. 1989). The lower pH values of the medium can be another reason of the higher bioadhesion obtained with formulations CH-40 and CH-20 when compared to the formulations CH-80 and CH-60.

3.3. In vivo studies

The absorption of nicotine was found to be faster from the oral mucosa when compared to the absorption from skin (Table 2) even though the ratio of the amount of nicotine in the tablet and the transdermal patch was 1 : 5.5. The C_{max} values obtained with both delivery systems were found to be similar. This is a very important result as faster absorption of nicotine is a desirable situation in replacement therapy for acute relief of craving.

Several studies have been reported on the development of buccal bioadhesive tablet formulation for nicotine delivery (İkinci et al. 2004; Park and Munday 2002, 2004), yet no pharmacokinetic evaluations were reported except for a sublingual tablet containing 2 mg nicotine (Molander and Lunell 2001). The $AUC_{0-\infty}$ value was reported to be 17.0 ± 5.0 ng · h · mL^{-1} after a single tablet administration. When two tablets were applied at the same time, the $AUC_{0-\infty}$ values were 27.6 ± 7.6 ng · h · mL^{-1} and 36.5 ± 13.1 ng · h · mL^{-1} after administration of three tablets. When compared to the sublingual tablet, a higher AUC value (59.3 ng · h · mL^{-1}) was obtained in our study with one tablet which contained 6.4 mg nicotine. The higher AUC values obtained with the buccal tablet indicates that rapid dilution with saliva and swallowing of nicotine before absorption from the oral cavity was avoided. As the volunteers were all non-smokers faintness and dizziness were observed both with the buccal tablet and transdermal patch application.

Oral mucosal delivery of nicotine with bioadhesive tablets exerts advantages over the chewing gum. The release of drug from tablets is not dependent on the subject and the bioadhesive system does not stimulate saliva secretion, therefore the retention time of the drug in the oral cavity is expected to be longer with the buccal tablet than that with the chewing gum. Moreover, with the buccal tablet formulation, side effects such as bad taste, irritation of the tongue, mouth and throat caused by chewing gum are eliminated.

As a conclusion, when compared to the transdermal patch, similar plasma nicotine levels were obtained with the developed buccal adhesive tablet but in a significantly shorter time. The developed formulation is very promising to relieve the acute craving and it could be used in combination with the transdermal patch for NRT.

4. Experimental

4.1. Materials

Nicotine hydrogen tartrate (NHT), cotinine (Sigma, St. Louis, MO USA), chitosan (Protosan CL212, degree of deacethylation 73%, molecular weight 272000) (Pronova Biomedical, Norway), carbomer (CP) (Carbopol® 974P NF, BF Goodrich, Cleveland, USA), magnesium stearate, potassium carbonate, diphenylamine (internal standart), acetic acid, methanol, diethyl ether and magnesium hydroxide (E. Merck, Darmstadt, Germany) were used as received.

Table 3: Composition of the buccal tablet formulations

Ingredients	Tablet formulations			
	CH-80	CH-60	CH-40	CH-20
NHT	20	20	20	20
Polymer ratio (Chitosan/Carbomer)	80 : 20	60 : 40	40 : 60	20 : 80
Magnesium hydroxide	30	30	30	30
Magnesium stearate	1.5	1.5	1.5	1.5

4.2. Preparation of buccal adhesive tablets

Chitosan and carbomer were used at different ratios in the tablet formulations. Magnesium hydroxide was used as the pH increasing additive and magnesium stearate as lubricant. Each tablet contained 20 mg of NHT (equivalent to 6.4 mg nicotine base). Composition of the tablet formulations is given in Table 3. Tablets were prepared by direct compression using a single punch-tablet machine Korsch EK/O. A flat non-beveled punch of 12 mm diameter was used. The weight of a tablet was 151.5 mg and the thickness between 1.0 and 1.2 mm.

4.3. Release studies

Release of NHT from tablets was studied using modified Franz diffusion cells (İkinci et al. 2004). The dissolution medium was 22 mL phosphate buffer saline (PBS) pH 7.4 at 37 °C. Uniform mixing of the medium was provided by magnetic stirring at 300 rpm. To provide unidirectional release, each bioadhesive tablet was embedded in paraffin wax filled in a glass die with a 12 mm central hole, and it was placed on the top of the receptor site. Samples of 2 mL were taken from the medium at certain time intervals and replaced with the same amount of PBS. The samples were filtered and assayed for NHT spectrophotometrically at 259 nm. pH of the dissolution medium at each sampling time interval was measured during the release studies.

4.4. Bioadhesion studies

Bioadhesive properties of the tablets were examined *ex vivo* using bovine buccal mucosa which was obtained freshly from local slaughterhouse (İkinci et al. 2004). The peak force of detachment (N) and the work of adhesion (joule) was measured on a tensile strength apparatus using 1 kN load cell (Zwick Z010, Germany). Cyanoacrylate adhesive was used to fix the tablet to the upper metallic support of the apparatus. Bovine buccal tissue ($\sim 5 \times 5$ cm^2) was fixed on polystyrene and placed on the base of the tensile test apparatus with the mucosal side facing upward. After hydrating the mucosa with 20 µL PBS pH 7.4, the tablet was brought into contact with a force of 0.5 N and kept in this condition for 5 min. The tensile test was performed at a constant speed of 0.5 mm · min^{-1}.

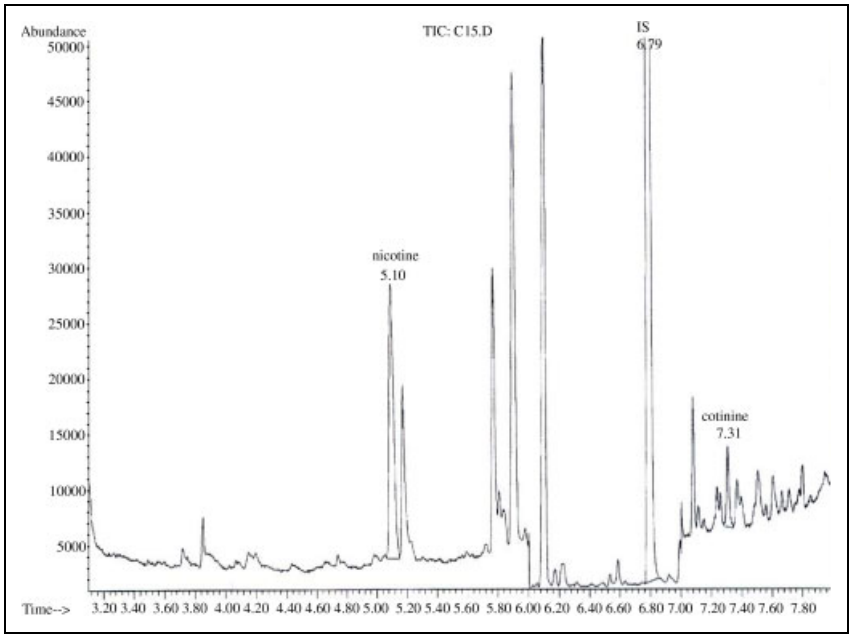

Fig. 5: Chromatograms of nicotine and cotinine extracted from plasma sample taken at 2 h after application of buccal tablet

4.5. In vivo studies

4.5.1. Study design

This study was approved by the Human Ethics Committee of Hacettepe University and the National Ethics Committee. A written informed consent was obtained from each subject. Eight healthy, non-smoker volunteers (3 male and 5 female, aged 27 ± 3) participated in this study. All subjects had a normal medical history and were in good health, confirmed by physical examination and appropriate laboratory tests and electrocardiogram.

Based on the results of the *in vitro* studies, formulation CH-80 was evaluated *in vivo*. In each volunteer, a buccal tablet was applied on the buccal sulcus above the canine tooth and removed after 4 h. After a washout period of one week, the transdermal patch (Nicotinell®) containing 35 mg nicotine was applied on a dry, hair free area on the upper arm for 24 h. All studies were conducted in a smoke-free area.

Venous blood samples (5 mL) were collected in citrated test tubes immediately before and 0.5, 1, 2, 3, 4, 6, 8, 10, 12 h after application. Additional samples at 24 h were withdrawn after transdermal patch application. Following centrifugation, the plasma fraction was transferred into glass tubes and stored at −80 °C until analysis. C_{max}, t_{max} and AUC values for nicotine were determined and the bioavailability of the tablet was evaluated in comparison to the transdermal patch.

4.5.2. Plasma assay

Nicotine and cotinine concentrations in plasma were determined using of the method given by Shin et al. (2002) with some modifications. 1 mL plasma sample was added to 1 mL of aqueous potassium carbonate solution at $200\ mg \cdot mL^{-1}$ concentration and 50 µL of diphenylamine ($1\ \mu g \cdot mL^{-1}$ in methanol) was added as internal standard. Extraction was carried out with 6 mL of diethyl ether by mechanical shaking for 10 min. The organic layer was transferred to another test tube containing 20 µL acetic acid and evaporated to dryness under nitrogen stream. The residue was dissolved in 100 µL of methanol, and 2 µL of the resulting solution was injected automatically into the GC-MS and analyzed in the SIM mode.

The peak of nicotine, cotinine and internal standard were distinctly separated from each other (Fig. 5). Retention time for nicotine, internal standard and cotinine was 5.1, 6.7 and 7.2, respectively. Coefficient of variations (CV%) was found to be less than 10% for $1-50\ ng \cdot mL^{-1}$ of nicotine and cotinine. The limit of detection for nicotine and cotinine was found to be $0.1\ ng \cdot mL^{-1}$ with a variation coefficient of 16% and 32%, respectively.

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