

# Liquid Chromatography Electrochemical Determination of Nicotine in Third-Hand Smoke

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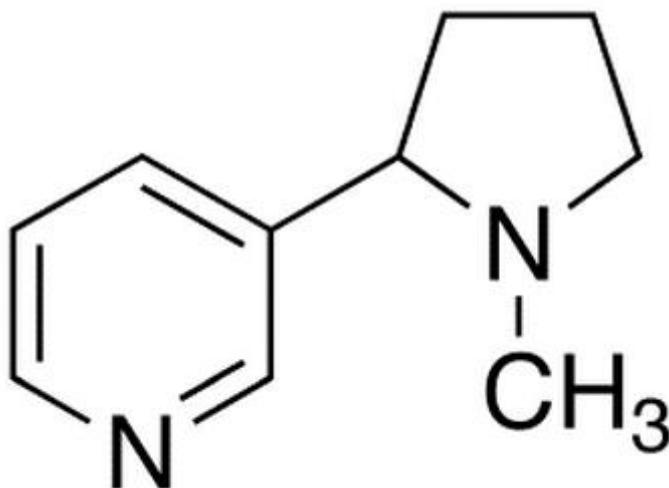
## Abstract

Third-hand smoke (THS) can be defined as the contamination of surfaces by second-hand smoke. This residue can form further pollutants which can be re-suspended in dust or be re-emitted into the gas phase. THS is a complex mixture and as a result studies have focused on nicotine as a marker of THS, it being the most abundant and indicative organic compound deposited. In this present study, the extraction of dust wipe samples and the subsequent chromatographic conditions required for the separation of nicotine by liquid chromatography with electrochemical detection were investigated and optimised. The optimum chromatographic conditions were identified as a 150 mm x 4.6 mm, 5  $\mu\text{m}$  C<sub>18</sub> column with a mobile phase consisting of 65 % methanol, 35 % pH 8 20 mM phosphate buffer. Hydrodynamic voltammetry was used to optimise the applied potential which was identified to be +1.8 V (vs. stainless steel). Under these conditions, a linear range for nicotine of 13 to 3240  $\mu\text{g/L}$  (0.26 ng – 65 ng on column) was obtained, with a detection limit of 3.0  $\mu\text{g/L}$  (0.06 ng on column) based on a signal-to-noise ratio of three. Dust wipe samples were extracted in methanol with the aid of sonication. Mean recoveries of 98.4 % (% CV = 7.8 %) were found for dust wipe samples spiked with 6.50  $\mu\text{g}$  of nicotine. Musk ketone, urea and stearic acid were found not to interfere. Communal entrance ways were found to be contaminated with THS nicotine levels of between 66.8 and 156  $\mu\text{g/m}^2$ .

**Keywords:** Third-hand smoke; dust wipe; nicotine; liquid chromatography electrochemical detection; voltammetry; amperometry.

## Introduction

Nicotine (i) is a pyridine alkaloid found in several species of fungi and plants. The most common source of nicotine is the plant *Nicotiana tabacum* which is commercially grown in many countries and processed into tobacco which is either chewed or smoke to release the nicotine present. Nicotine acts on the central nervous system causing an elevation of mood in the smoker and causing the individual to feel more relaxed. This effect is one of the features desirable to smokers resulting in their continued use of nicotine containing tobacco products. Nicotine is reported to equate to between 6.17 mg and 28.86 mg per cigarette and up to 50.89 mg/g in pipe tobacco [1] and it is the most abundant chemical found in tobacco smoke [2], making it a useful marker for the analysis of tobacco smoke in environmental [3] and biological samples [4,5].



(i)

Recent research [6-8] has demonstrated that not only are individuals at risk from smoking itself or second hand smoke produced, but they are also exposed to what is referred to as third hand smoke (THS). THS is formed from tobacco smoke or vapour and remains

deposited after the smoking has ceased. Studies have found that nicotine and other compounds can be found in house dust, cars and hotel rooms and on surfaces [6]. The deposited nicotine can react with pollutants, such as nitrous acid and ozone to form toxic and potentially mutagenic and cytotoxic compounds known as tobacco-specific nitrosamines (TSNAs) [7,8] which can be reemitted back into the environment by degassing or through movements of dust [6].

A number of different analytical methods have been utilised for the determination of nicotine in THS [9-21]. Nicotine has been determined in several different sample matrices such as toenails [22] and hair [23-26] utilising the liquid chromatography with electrochemical detection (LC-ED) method developed by Mahoney and Al-Delaimy [27], however to our knowledge, there have been no other reports on its determination by LC-ED. LC-ED offers a number of advantages, as it is both a sensitive and economic approach as has been demonstrated in number of previous reviews and monographs focused on its theory and application [28-30]. Table 1 describes earlier reported liquid chromatographic and electrophoretic approaches using electrochemical detection for the determination of nicotine. Previous reverse phase LC-ED approaches require mobile phase containing ion pairing agents and low concentrations of organic modifier; situations which can lead to stationary phase de-wetting and long conditioning times resulting in poor separation and overall analytical performance. These approaches have also utilised multiple electrode detection systems requiring complex **dedicated** equipment for their control and application. Our present report represents the first report of LC-ED using the much simpler and economic approach of single electrode amperometric detection [31]. **Our system requires only a standard potentiostat and a commercially available thin layer cell for its**

implementation; notably cheaper and easier to maintain than dedicated electrochemical detector systems. Our approach also allows for overall shorter run times and pre-conditioning times as we have avoided the use of ion-pairing reagents.

In the first part of this investigation we examined the electrochemical behaviour of nicotine by cyclic voltammetry, examining the effect of both scan rate and pH. The chromatographic conditions were then optimised and we then used hydrodynamic voltammetry to identify the optimum applied potential for its determination by LC-ED. The possibility of extracting nicotine from dust wipes was then investigated, and a number of real samples were examined using the optimised method.

Technique	Linear Range	Limit of Detection	Comments	Ref
Liquid chromatography with dual electrochemical detection at a porous graphite electrode.	4 to 640 ng/mg of human hair.	0.05 ng for 2 mg of human hair.	Determination of nicotine in children's hair using 2-phenylimidazole as an internal standard. The voltage settings for the conditioning cell and detectors 1 and 2 were +0.6, +0.6 and +0.9 V respectively.	[27]
Liquid chromatography with dual electrochemical detection at a porous graphite electrode.	0.2 ng to 5 µg		Dual electrode detection. Guard cell = +1.0 V, detector 1 = -0.5 V and detector 2 = +0.75 V. Reverse-phase C <sub>18</sub> stationary phase. Mobile phase primary buffer 92.5 % 2 mM NaH <sub>2</sub> PO <sub>4</sub> containing 0.25 mM Na octyl sulphate; secondary buffer 7.5 % methanol-acetonitrile (3:1) adjusted to pH 3.0 with H <sub>3</sub> PO <sub>4</sub> .	[32]
Liquid chromatography with dual electrochemical detection.	0.2 to 1.0 ng		Dual glassy carbon electrode, detection potential +0.75 V. Mobile phase of 2 mM NaH <sub>2</sub> PO <sub>4</sub> , 0.25 mM sodium octyl sulphate, 5 % organic modifier (acetonitrile : methanol, 3:1 by volume). Stationary phase C <sub>18</sub> . Samples of nicotine in dog plasma analysed after protein precipitation and diluted in mobile phase.	[33]
Micellar liquid chromatography with electrochemical detection	0.03 to 2 µg/mL	4 ng/mL	Nicotine in chewing gum, dermal patches, tobacco and serum samples. Mobile phase of SDS 0.15 M–6 % (v/v) pentanol–0.01M NaH <sub>2</sub> PO <sub>4</sub> (pH 6)–0.001 M KCl. Applied potential +0.8 V.	[34]
Capillary electrophoresis.	0.01 to 2.0 µg/mL	2 ng/mL	Detection at +0.95 V at a pencil carbon disc working electrode. Separation by capillary electrophoresis: fused-silica capillary, 25 µm internal diameter x 65 cm; working electrode: 0.3 mm diameter carbon disc electrode; running buffer: PBS, BB, and Tris-HCl solution with pH of 8.0, 60 mM. Separation voltage: 18 kV. Injection: 10 s/18 kV.	[35]
Capillary electrophoresis.	5.0×10 <sup>-7</sup> to 1.0×10 <sup>-4</sup> M	5×10 <sup>-8</sup> M	Detection potential of +1.20 V at a carbon fibre working electrode (33 µm diameter). 40 mM phosphate buffer (pH 2.0), a sample injection time of 10 s at 10 kV and a separation voltage of 16 kV.	[36]

Table 1. Liquid chromatography and electrophoresis electrochemical detection based methods for the determination of nicotine.

## **2. Experimental**

### **2.1 Chemicals and Reagents**

All chemicals were obtained from Fisher (Loughborough, UK), unless otherwise stated. Deionised water was obtained from a Purite RO200 - Stillplus HP System, fitted with a Pur-1-te ion-exchanger (Purite Oxon., UK). A 20 mM phosphate pH 8.0 buffer was prepared by titration of a solution of 0.2 M trisodium phosphate, with 0.2 M phosphoric acid and subsequent dilution. Primary stock solutions of nicotine (Sigma-Aldrich, Dorset, UK), were prepared by dissolving the required mass in acetonitrile to give a concentration of 10 mM. Working standards, for initial voltammetric studies, were prepared by dilution of the primary stock in sufficient water, acetonitrile and phosphate buffer, to give an overall concentration of 2 mM nicotine in 10 % acetonitrile 0.1 M phosphate buffer. The surface-wipes were fabricated by cutting 10 cm<sup>2</sup> squares from a roll of tissue (one ply, Jangro White Centrefeed, Pattersons, Bristol, UK). Standards for LC-ED analysis were made by dilution of the primary stock solution in mobile phase.

### **2.2.2 High Performance Liquid Chromatography**

HPLC studies were undertaken using an Agilent 1100 HPLC system with a 250 mm x 4.6 mm Hypersil Gold C<sub>18</sub>, 5 µm column connected to a 7125 valve manual injector fitted with a 20 µL sample loop (Rheodyne, Cotati, USA). Sample extracts were determined using a mobile phase of 65 % methanol, (Fischer, Far UV, HPLC grade) 35 % 20 mM pH 8.0 phosphate buffer at a flow rate of 1.0 mL/min.

### **2.2.3 Electrochemical Detection**

The detector cell consisted of a two piece thin-layer cell, formed from an upper Kel-F block containing a GCE working electrode (3 mm diameter) and a bottom steel block serving as the pseudo-reference/counter electrode. Teflon gaskets were purchased from BAS, Congleton, Cheshire, UK. The inlet for the thin layer cell was connected directly to the outlet of the Agilent 1100 UV detector using a suitable PEEK connector and tubing. An Ivium CompactStat potentiostat (Ivium Technologies, The Netherlands) was used to control the potential at the thin layer cell at +1.8 V vs. the pseudoreference/counter stainless steel electrode (ss). Chromatograms were recorded

using an Ivium CompactStat potentiostat (Ivium Technologies, The Netherlands) interfaced to a PC for instrument control and data acquisition.

### **2.3 Cyclic Voltammetric Studies**

Cyclic voltammetry was undertaken using a  $\mu$ Autolab potentiostat interfaced to a PC for data acquisition and control using the GPES software version 4.9. Cyclic voltammograms were initially recorded in plain solutions of 10 % acetonitrile, 90 % phosphate buffer and then in the same solution containing 2.0 mM nicotine. A starting potential and an end potential of 0.0 V (vs. Ag/AgCl) was used, with a switching potential of +1.5 V (vs. Ag/AgCl). The effect of scan rate was studied over the range 20 mV/s to 200 mV/s.

### **2.4 Hydrodynamic Voltammetry**

Hydrodynamic voltammetry (HDV) was undertaken using an EG&G Princeton Applied Research (Princeton, NJ) model 362 scanning potentiostat to control the applied potential. Chromatograms were recorded using a Siemens Kompenosograph X-T C1012 chart recorder. The hydrodynamic voltammetric behaviour of nicotine was investigated by injecting fixed volumes of a standard solution of nicotine and varying the applied potential between +0.9 V and +2.0 V (vs. ss) in 100 mV steps. The hydrodynamic voltammogram was then constructed by plotting the recorded peak current against the applied potential. The optimum potential was determined from the position of the plateau on the hydrodynamic voltammogram.

### **2.5 Dust Wipe Sampling**

Dust wipe samples were collected in a similar manner to that described previously [37]. The fronting of doors and windows facing onto entranceways were chosen for investigation. Dust wipe samples were obtained by wiping from the upper left corner of the sample area; in "S" shape manner, wiping from side-to-side whilst moving down the sample area. The exposed wipe was then folded in half, exposed side to exposed side and another "S" shape was made in the opposite direction wiping up and down instead of side-to-side. The folded wipe was placed in a glass vial, which was also used as the extraction vessel and sealed. A new pair of gloves was also used for each sample. A procedural

blank was obtained by taking a tissue onsite but without sampling the surface. The area sampled was measured so that a comparison of concentration values ( $\mu\text{g}/\text{m}^2$ ) between different surfaces could be made.

## 2.6 Sample Extraction and Analysis

Five mL of methanol was added to the glass vessel containing the dust wipe sample. This was then sealed and the dust wipe extracted by sonication for 15 minutes at room temperature. A 200  $\mu\text{L}$  aliquot of this was taken and 100  $\mu\text{L}$  of 50 mM pH 8 phosphate buffer added and investigated by liquid chromatography electrochemical detection.

## 3. Results and Discussion

### 3.1 Cyclic Voltammetry

Initial cyclic voltammetric studies were performed with a 2 mM solution of nicotine, dissolved in 0.1 M phosphate buffer pH 10, in the presence of 10 % acetonitrile (10 mL). Figure 1 shows the cyclic voltammogram obtained at a GCE using a scan rate ( $\nu$ ) of 50 mV/s. Two oxidation peaks were recorded, which we have designated as O1 and O2. There a number of different theories which have been postulated to explain the voltammetric behaviour of nicotine [32,38-42]. The majority of reports show nicotine to be oxidised in a single oxidation wave. However, in our present study, at pH values above pH 8 two oxidation peaks are recorded, similar to the cyclic voltammetric behaviour reported by Cinková *et al* [39]. The effect of scan rate was studied at pH 2, 4, 6, 8 and 10, over the scan rate 10 to 200 mV/s. For both oxidation processes peaks current ( $i_p$ ) values were found to be proportional to the square of scan rate ( $\nu^2$ ), demonstrating diffusion controlled processes. The oxidation peak observable at +1.6 V was concluded to results from the oxidation of common alkene [43] impurities present in the acetonitrile (*i.e.* acrylonitrile, acrolein, *etc.*), it being present in both the sample and the blank supporting electrolyte. Figure 2 shows the effect of pH on the cyclic voltammetric  $i_p$  for both oxidation peaks O1 and O2. The oxidation process O2 was found to independent of pH over the range studied. However, the more negative peak O1 shows a maximum at pH 8 and as a result further investigations were made at this pH.

### 3.2 Hydrodynamic Voltammetry

Figure 3 shows the HDV obtained over the potential range +1.0 to +1.9 V (vs. ss). The peak current response for nicotine was found to increase with increasing potential from +1.0 V to +1.7 V (vs. ss). At potentials more positive than this, between +1.7 V and +1.9 V (vs. ss), the response was found to plateau and become constant. Interestingly, under hydrodynamic conditions a single oxidation process is obtained, differing from the two separate electrochemical processes observable by cyclic voltammetry in quiescent solution (Figure 1). The oxidation mechanism would appear to be different under hydrodynamic conditions compare to that as obtained under quiescent conditions. Consequently, further LC ED studies were undertaken using an applied potential of +1.8 V (vs. ss).

### 3.4 Calibration, Limit of Detection, and Precision

A linear range of 13 µg/L to 3240 µg/L ( $R^2 = 0.999$ ) was obtained with an associated detection limit of 3.0 µg/L, based on a signal to noise ratio of three. Dust wipes were spiked with 6.50 µg nicotine (n = 5) and extracted using the procedure described. A mean recovery of 98.4 % with a coefficient of variation of 7.8 % was calculated.

### 3.5 Studies of Possible Interferences

A wide range of compounds could be potentially present in dust wipe samples resulting from cosmetics, soaps and human skin contact which could interfere with the determination of nicotine. In this present study we investigated; musk ketone, a nitroaromatic compound present detergents, perfumes and cosmetics, stearic acid, also present in cosmetics, soap, *etc.*, the biological metabolite of nicotine, cotinine and urea, present in urine and to a lesser extent in sweat. None of these compounds were found to give any chromatographic response under the conditions employed and consequently did not interfere.

## 4. Analytical Application

The glass fronting of doors and windows facing onto entranceways were chosen for investigation. Figure 4 shows representative chromatograms obtained for extracted dust wipe samples. A well

resolved peak for nicotine was obtained at a retention time of 185 s. The levels found at the different sample locations are summarised in table 2. The injection-to-injection time was only 210 s including a 100 s equilibration time.

Sample	Nicotine, $\mu\text{g}/\text{m}^2$
1	110
2	150
3	ND
4	66.8
5	156

Table 2. Nicotine levels obtained for dust-wipe samples. ND = not detected.

## 5. Conclusions

A method using LC-ED has been successfully developed for the determination of trace levels of nicotine in THS dust wipe samples. The chromatographic separation is achieved using a  $\text{C}_{18}$  reversed phase column in conjunction with a methanol-phosphate buffer based mobile phase. Amperometric detection using an applied potential of +1.8 V (vs. ss) was employed and well-resolved peak free from interferences was obtained with a retention time of only 185 s. This is an improvement on our previously reported liquid chromatographic method utilising UV detection [37] as it is nearly twice as fast, is more selective and exhibits a better theoretical limit of detection. In this investigation levels of nicotine of between 66.8 to 156  $\mu\text{g}/\text{m}^2$  were found, comparable to those we previously reported [37]. The developed method is more economic and simpler compared to LC/MS and related approaches, but has been shown to be able to determine levels comparable to those reported by other techniques [10,11, 20].

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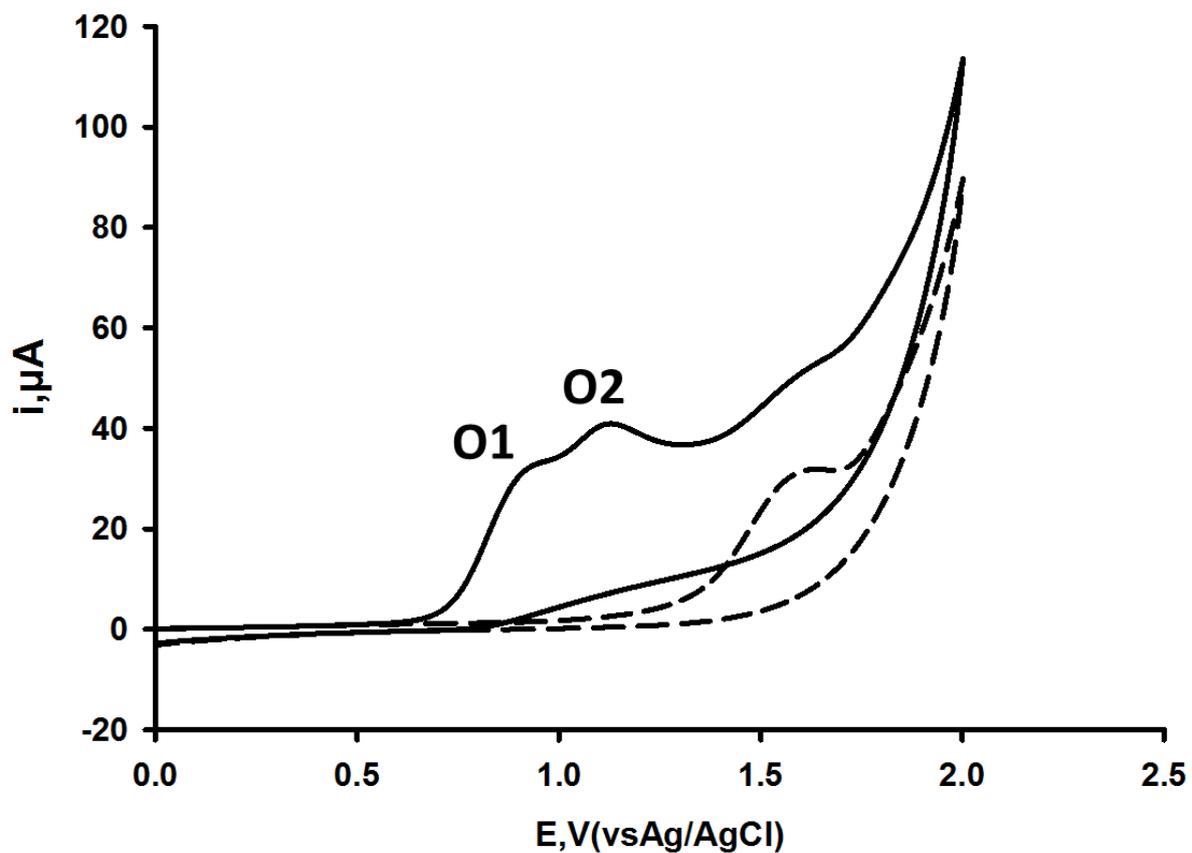


Figure 1. Typical cyclic voltammogram, obtained at a scan rate of 50 mV/s, for dashed line, in the absence of and solid line in the presence of 2 mM nicotine in 10 % acetonitrile, buffered with 0.1 M phosphate at pH 10. Starting potential 0.0 V; switching potential +2.0 V.

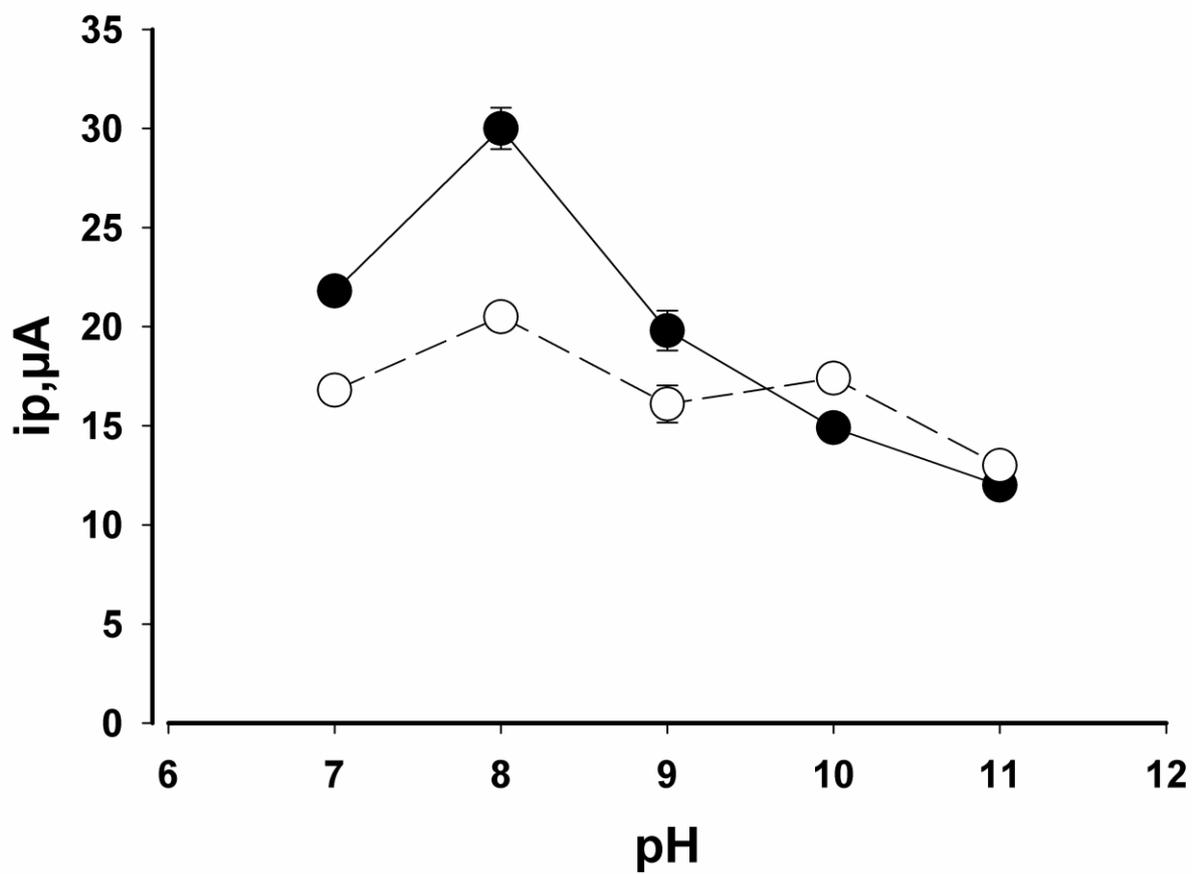


Figure 2. Plot of (a)  $i_p$  vs. pH for the two nicotine oxidation peaks. Voltammetric conditions as Figure 1. O1 solid line, O2 dashed line. Error bars represent  $\pm \sigma$ .

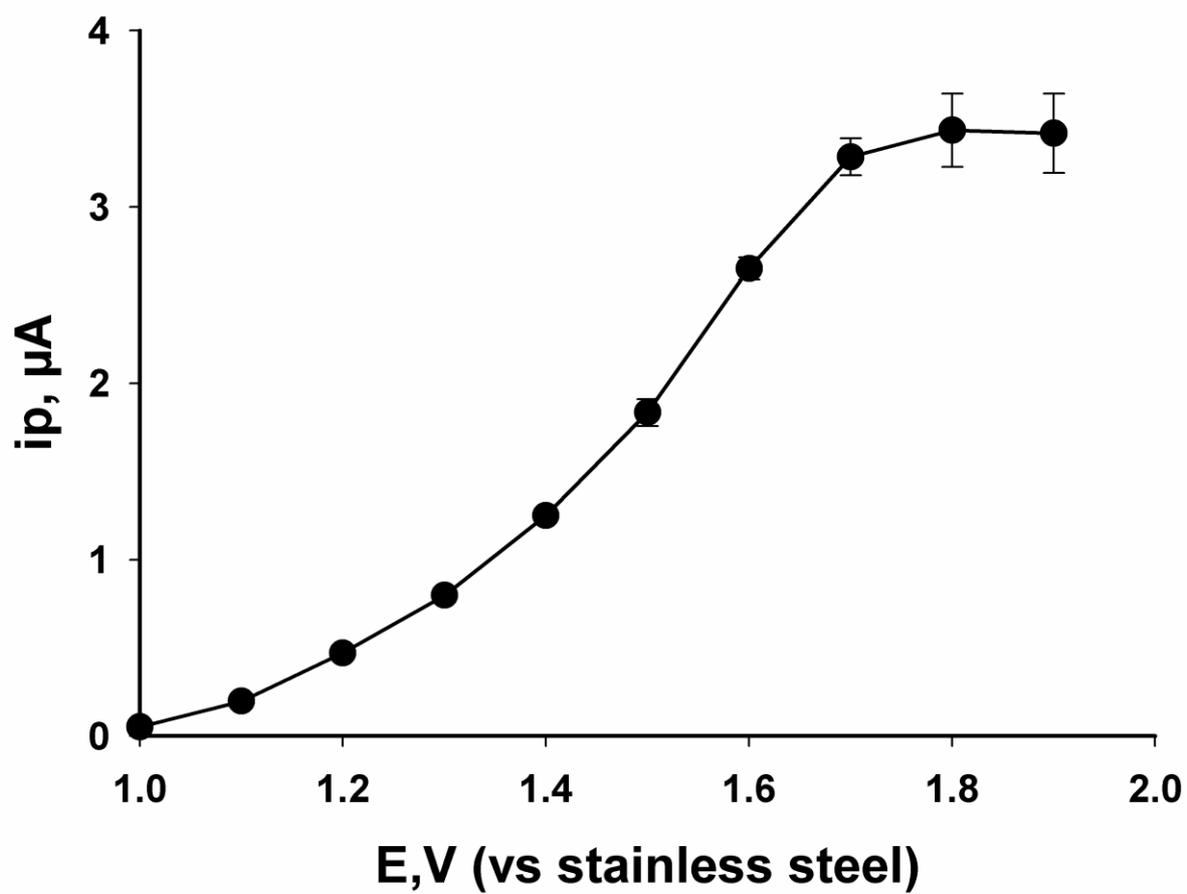


Figure 3. Hydrodynamic voltammogram for 2.6 μg injections of nicotine. Error bars represent  $\pm \sigma$ .

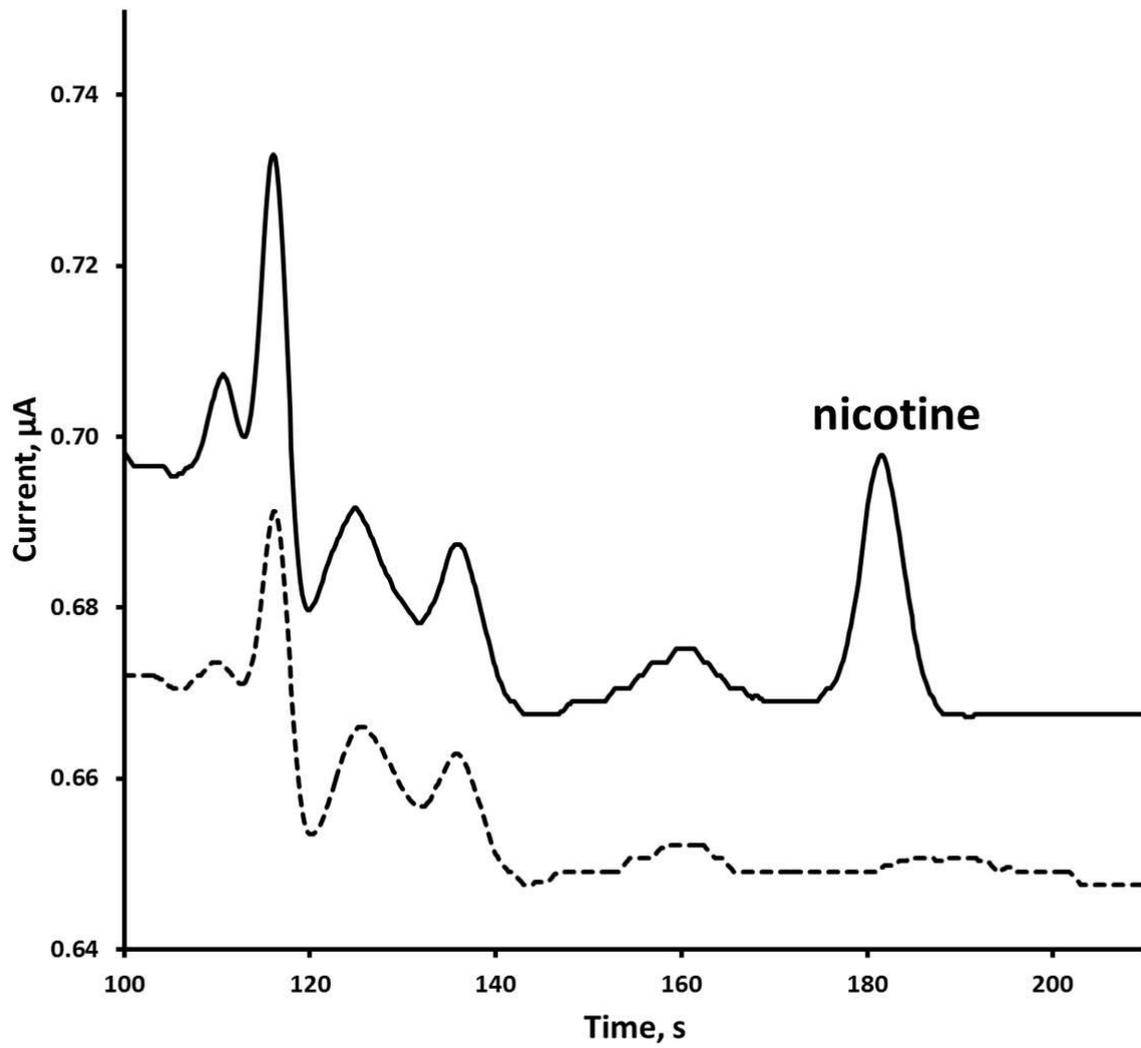


Figure 4. Representative chromatograms obtained for THS dust-wipe samples. Dashed line procedural blank, solid line THS dust wipe sample.

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