**Supporting Information**

# Rapid and sensitive determination of etomidate based on colorimetric assays with chromium(III) complex†

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## Experimental

**Materials and Instruments.** All reagents and solvents were of reagent or HPLC grade and used as received. Chromium(III) nitrate nonahydrate and chromium(III) acetate were purchased from Aladdin Scientific. Etomidate, metomedate, isopropoxate, and nicotine were kindly provided by the Hong Kong Government Laboratory. Analytical LC/MS was performed on an Agilent 1260 series HPLC system (Agilent Technologies, Stockport, UK) equipped with a diode-array detection (DAD) detector (monitored at 220 nm), a mass detector (MSD), and an Agilent Poroshell 120 Column (C18, 2.7μm, 3.0×150 mm) at the gradient on Table S1. All ultraviolet-visible absorption spectra were measured in the range of 250-800 nm using an Agilent Cary 60 UV-visible spectrophotometer using a 10 mm pathlength cuvette.

**Live Subject Statement.** All experiments were performed in compliance with the relevant laws and institutional guidelines of Hong Kong Polytechnic University, in which human serum albumin (HSA) were purchased and the sample were supplied by Sigma Aldrich.

**Table S1**. The timetable of the gradient for analytical LC/MS.

| **Time (min)** | **A % ($H_2O$ + 0.1% FA)** | **B % ($CH_3CN$ + 0.1% FA)** | **Flow (mL/min)** |
|---|---|---|---|
| 0 | 80 | 20 | 0.5 |
| 10 | 20 | 80 | 0.5 |
| 10.5 | 0 | 100 | 0.5 |
| 12.5 | 0 | 100 | 0.5 |

**Preparation of EtoChrom.** A 10% NaCl (v/v) solution was first prepared by dissolving NaCl in deionized water. $Cr(NO_3)_3 \cdot 9H_2O$ was then dissolved in this solution to form a mixture with a 10 mM concentration, followed by tuning the pH to 5.0.

**Optimization for Etomidate Detection.** $Cr(NO_3)_3 \cdot 9H_2O$ or chromium(III) acetate were dissolved in deionized water, and etomidate was dissolved in methanol to form the stock solutions of chromium (III) ions (both 10 mM) and etomidate (100 mg/mL). For $[Cr(H_2O)_6]^{3+}$ ion-depending assay, etomidate was added to two aqueous chromium (III) solutions and diluted to 1 mg/mL concentration. For pH-dependent assay, the pH value of aqueous $Cr(NO_3)_3 \cdot 9H_2O$ solution (10 mM) was tuned by either diluted HCl or NaOH solution to 1.0, 3.0, 5.0, 7.0, 9.0, or 11.0. For the buffer-dependent assay, $Cr(NO_3)_3 \cdot 9H_2O$ was dissolved in either PBS or sodium acetate buffer solution (1, 10, or 100 mM) to give a 10 mM solution. The absorption spectra of these solutions were recorded after mixing for 1 min at room temperature. For

the time-dependent assay, the stock solution of etomidate was diluted to 1 mg/mL by adding to **EtoChrom** (10 mM) and their absorption spectra were recorded after mixing over a period of 3 min at room temperature. Triplicates were performed to obtain the data.

**Studies of Etomidate Detection with EtoChrom**

***Stability assay of EtoChrom.*** **EtoChrom** (10 mM) was placed inside a glass bottle and stored at room temperature for 90 d. The absorption spectra were recorded at the corresponding time points. On days 28, 56, and 90, the stock solution of etomidate was diluted to 1 mg/mL by adding it to **EtoChrom** (10 mM), and their absorption spectra were recorded. Triplicates were performed to obtain the data.

***Sensitivity assay of EtoChrom.*** The stock solution of etomidate was diluted to different concentrations of etomidate (from 0 to 40 mg/mL) by adding to **EtoChrom** (10 mM). The mixture was shaken for 1 min before measuring the absorption spectra. Triplicates were performed to obtain the data. The limit of detection (LOD) was calculated by using the $3\sigma/\kappa$ rule based on the above experiments. Where $\sigma$ is the standard deviation of blank measurement ten times and $\kappa$ is the slope of the maximum absorbance of the $^4A_{2g} \rightarrow {}^4T_{1g}(F)$ transition band plotted against the etomidate concentration.

***Selectivity assay of EtoChrom.*** Stock solutions of $NaHCO_3$ (2000 mmol/L), KCl (3000 mmol/mL), $CaCl_2$ (250 mmol/L), $MgCl_2$ (25 mmol/L), $Na_2PO_4$ (3000 mmol), HSA (200 mg/mL), diacetyl, 2,3-pentanedione, acetoin (all 50 mg/mL), and nicotine (1800 mg/mL) in deionized water were prepared. These solutions were then added to **EtoChrom** (10 mM) and diluted 100-fold to the corresponding concentration. Glycerol, propylene glycol and their ratio of 2:8, 3:7, and 4:6 (v/v) were directly added to **EtoChrom** (10 mM) without dilution. For the detection of etomidate in the presence of nicotine, the stock solution of etomidate was added and diluted to 1 mg/mL with the mixture containing **EtoChrom** (10 mM) and nicotine (18 mg/mL). For the analogue assay, a stock solution of etomidate, metomedaite, and isopropoxate (100 mg/mL) was diluted to 1 mg/mL by incubation with **EtoChrom** (10 mM). The absorption spectra of all solutions were recorded after 1 min of shaking. Triplicates were performed to obtain the data.

**Calculations.** All complexes were modelled using Avogadro.[1] Geometry optimizations were carried out using density functional theory (DFT) on ORCA 4.2.0[2,3] employing PBE0/def2-SVP level of theory. Single point energy calculations were performed on the same level of theory. The nature of the optimized structures was checked using frequency calculations. The molecule geometries were visualized using UCSF Chimera (version 1.12).[4] Solvent effects of water were taken into account by the Conductor-like Polarizable Continuum Model (CPCM) model.[5,6]

**Real Vape Juice Sample Analysis**. E-cigarette pods and vape juice insides were kindly provided by the Hong Kong Government Laboratory. For the LC/MS analysis, the samples (1 μL) were diluted 1 x 106 times with 20% acetonitrile in water and the integrated peak area of the total ion count (TIC) chromatogram was recorded and the etomidate concentration was quantified with the calibration curve of etomidate prepared using the same LC/MS with gradient shown in Table S1. For the spectroscopic assay, 20 μL of real samples were mixed with 1.98 mL of EtoChrom (10 mM) with 1 min mixing time. The absorption spectra were obtained afterwards and the concentration was calculated using the calibration curve from Fig. 3d. Triplicates were performed to obtain the data.

**References**


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## Supporting Figures

**Table S2**. Comparison of recent instrumental and non-instrumental detection methods of etomidate (ET).

| Methodology | Apparatus | Detection Range & LOD | Sample Matrix | Advantages & Disadvantage | Reference |
|---|---|---|---|---|---|
| *Colorimetry* | *N/A* | *0 - 2 mg/mL (after dilution); 0.030 mg/mL* | *Vape Juice (E-Cigarette)* | ✓ *Instrument- & pre-treatment-free*<br>✓ *Obvious colour changes visualised by the naked-eye*<br>✓ *Rapid (< 1 min) & inexpensive (< HKD $2)*<br>✓ *Quantitate & qualitative results with actual vape juice sample*<br>× *Relatively narrow upper & lower detection limit* | *This Work* |
| Chromatography | LC-MS/MS | 0.4 – 120 ng/mL; 0.01 ng/mL | Urine | ✓ Can detect ET & its metabolites<br>✓ Ultralow LOD with high accuracy even after 24 d of sampling<br>× Require apparatus & can only apply to urine sample | **5** |
| Chromatography | LC-MS/MS | 0.25 - 50 pg/mg; 0.05 pg/mg | Hair | ✓ Can detect ET & its metabolites<br>✓ Ultralow LOD & detectable even ET was taken for a long time<br>× Require pre-treatment, apparatus & unavailable for on-site test | **6** |
| Chromatography | LC-MS/MS | 0.5 - 50 ng/mL; 0.2 ng/mL | Urine, Liver, Kidney | ✓ Can detect ET & its metabolites<br>✓ Low LOD & applicable for routine forensic evaluation of ET<br>× Require pre-treatment, apparatus & inapplicable for on-site test | **7** |
| Raman Spectroscopy | Raman Spectrometer | 1 - 50 ppm; 17 ppb | Vape Juice | ✓ Can detect ET & its metabolites<br>✓ Practical for on-site analysis<br>× Tedious & expensive synthesis to prepare the gold array substrate for the Raman spectrometer | **8** |
| Indicator displacement Assay | Fluorescence Spectrometer | 0 - 10 μM; 86 nM | Beverages & Vape Juice (Spiked) | ✓ Eye-catching emission colour change<br>✓ Ratiometric for quantitative analysis<br>× Portable instruments are either expensive or the signal-to-background ratio is limited<br>× Require pre-treatment for vape juice | **9** |
| Fluorescence Quenching Assay | Fluorescence Spectrometer | 0 - 500 ng/mL; 10 ng/mL | Vape Juice (Real&Spiked); Urine | ✓ Fast (< 10min) & accurate result<br>✓ Quantitative for real sample analysis<br>× Require apparatus & unable to perform on-site test | **10** |
| Lateral Flow Immunoassay | Self-constructed Testing Kit | 0.08 - 1.31 μg/kg; 0.3 μg/kg (visual) | Water, Urine and Serum | ✓ Can detect ET & MT at the same time<br>✓ User-friendly test strips for on-site test to give accurate result < 10 min<br>× High cost to prepare monoclonal antibodies and assemble the kit | **11** |

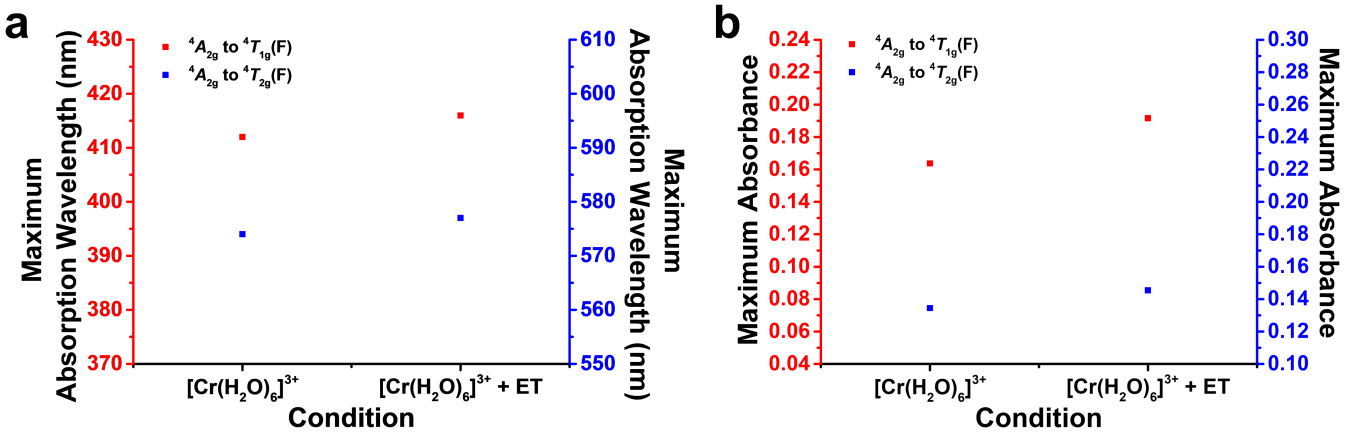


**Fig. S1.** (a) Maximum absorption wavelength and (b) maximum absorbance of $^4A_{2g} \rightarrow {}^4T_{1g}(F)$ and $^4A_{2g} \rightarrow {}^4T_{2g}(F)$ transition bands of $[Cr(H_2O)_6]^{3+}$ in DI water (10 mM) with or without the addition of ET (1 mg/mL).

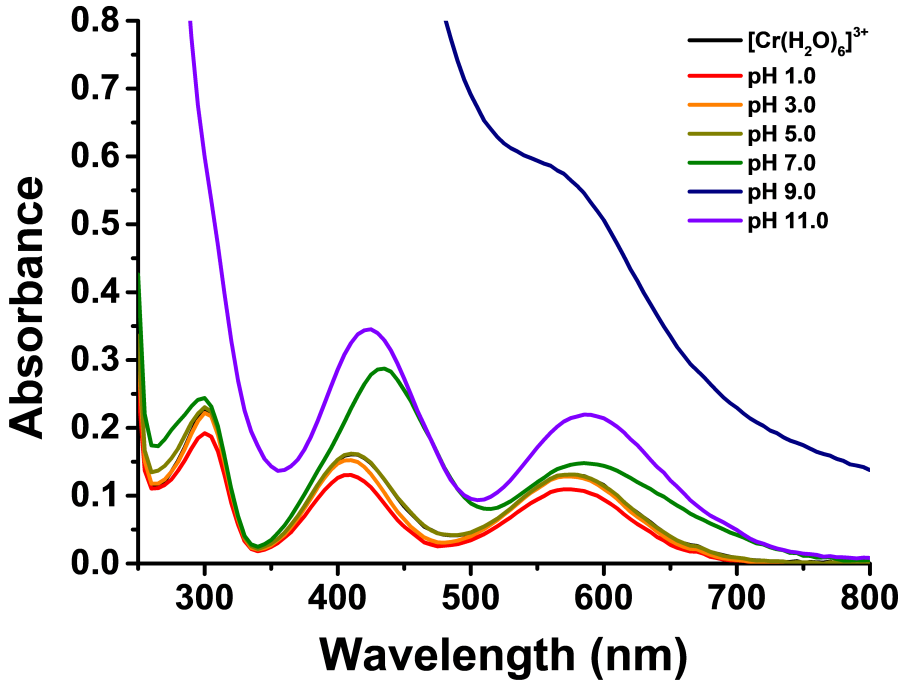


**Fig. S2.** UV-Vis spectra of $[Cr(H_2O)_6]^{3+}$ in DI water (10 mM) at different pH values.

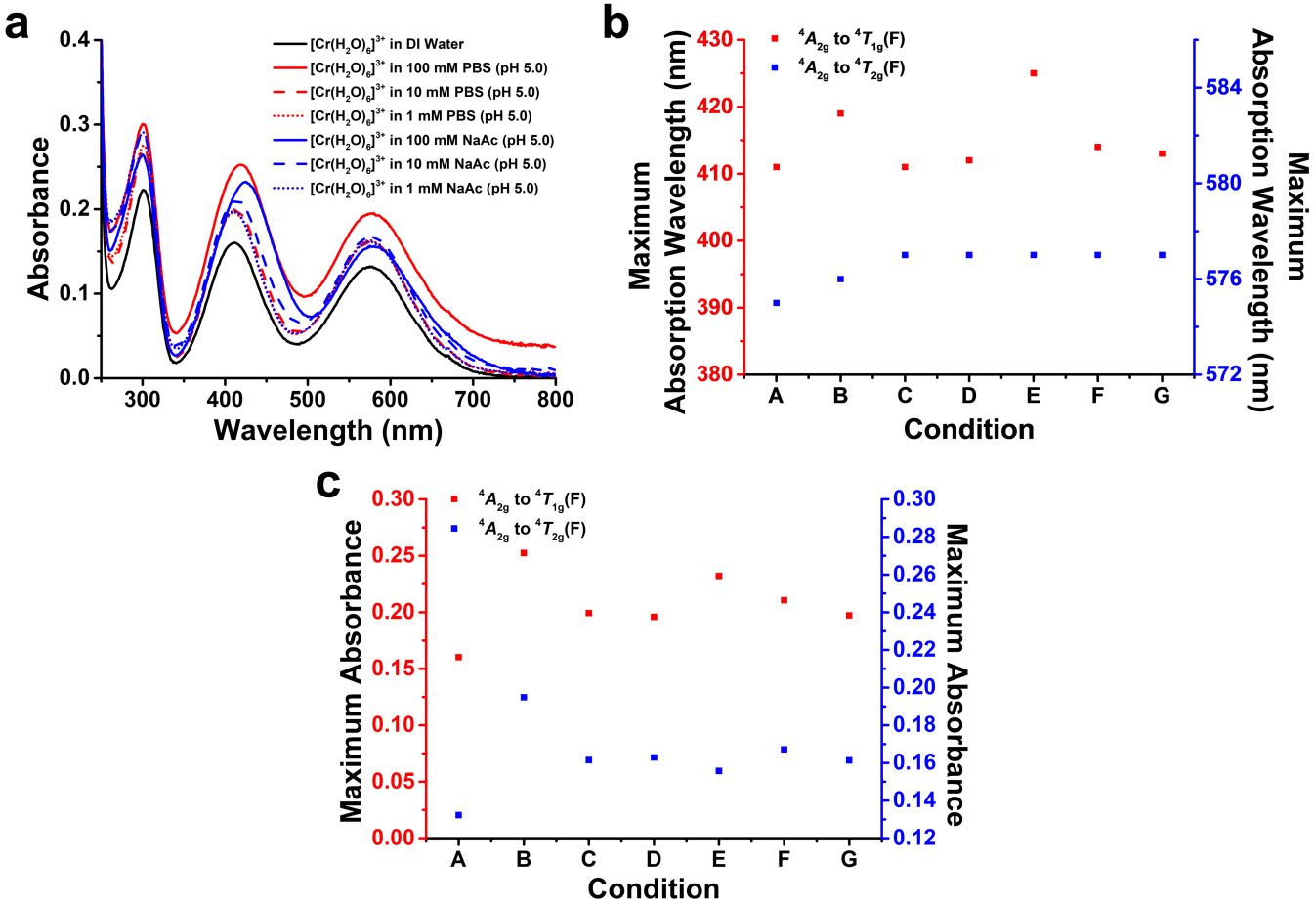


**Fig. S3.** (a) UV-Vis spectra, (b) Maximum absorption wavelength and (c) maximum absorbance of $^4A_{2g} \rightarrow {}^4T_{1g}(F)$ and $^4A_{2g} \rightarrow {}^4T_{2g}(F)$ transition bands of 10 mM $[Cr(H_2O)_6]^{3+}$ in PBS or NaAc buffer (1, 10 or 100 mM) at pH 5.0. Conditions **A**: DI Water; **B**: 100 mM PBS; **C**: 10 mM PBS; **D**: 1 mM PBS; **E**: 100 mM NaAc; **F**: 10 mM NaAc; **G**: 1 mM NaAc buffer.

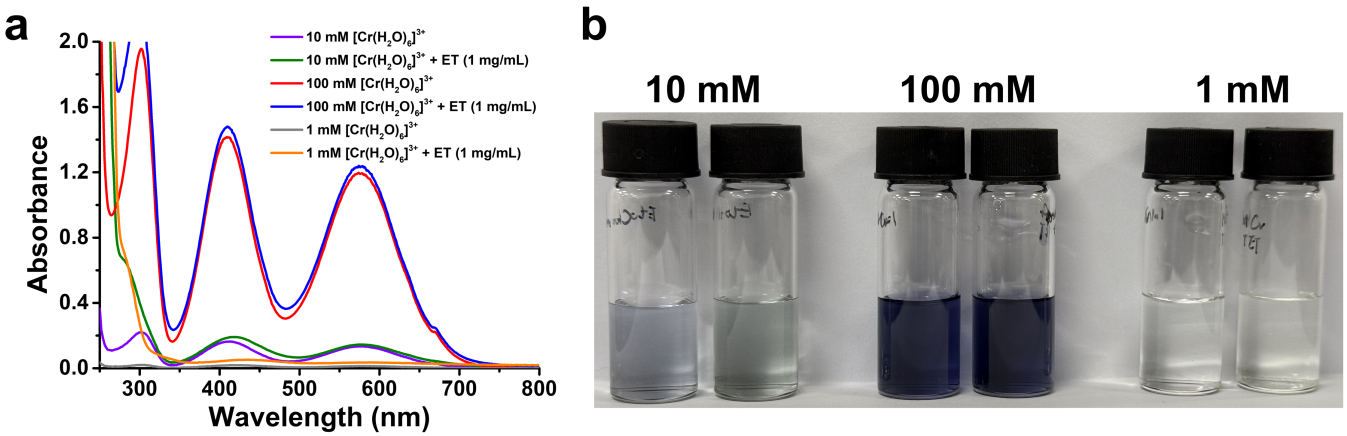


**Fig. S4.** (a) UV-Vis spectra and (b) the colour of 1, 10 or 100 mM $[Cr(H_2O)_6]^{3+}$ in DI water with or without the addition of ET (1 mg/mL).

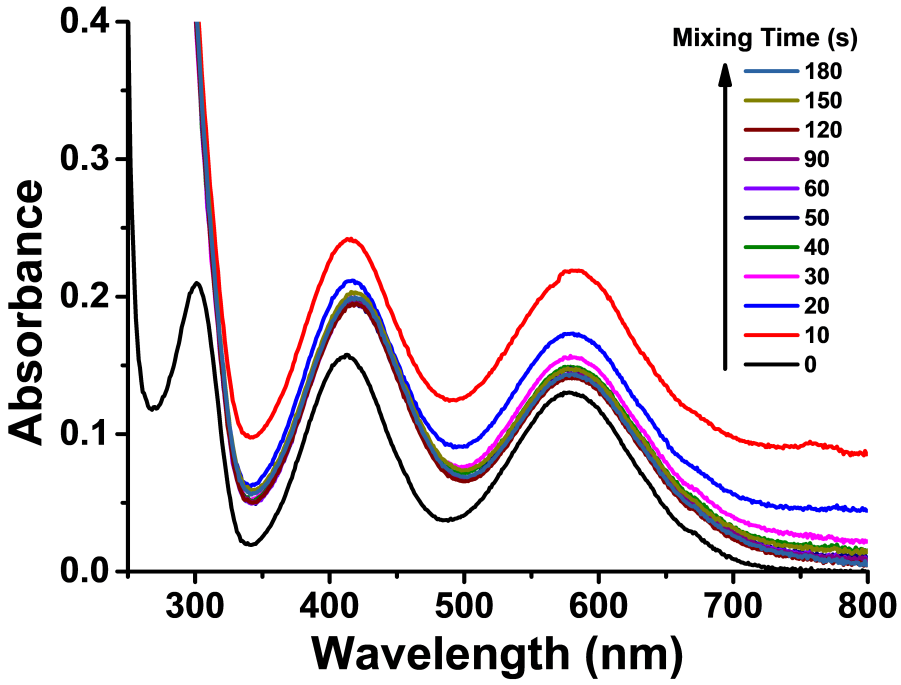


**Fig. S5.** UV-Vis spectra of **EtoChrom** (10 mM) upon the addition of ET (1 mg/mL) at different time points over a period of 3 min.

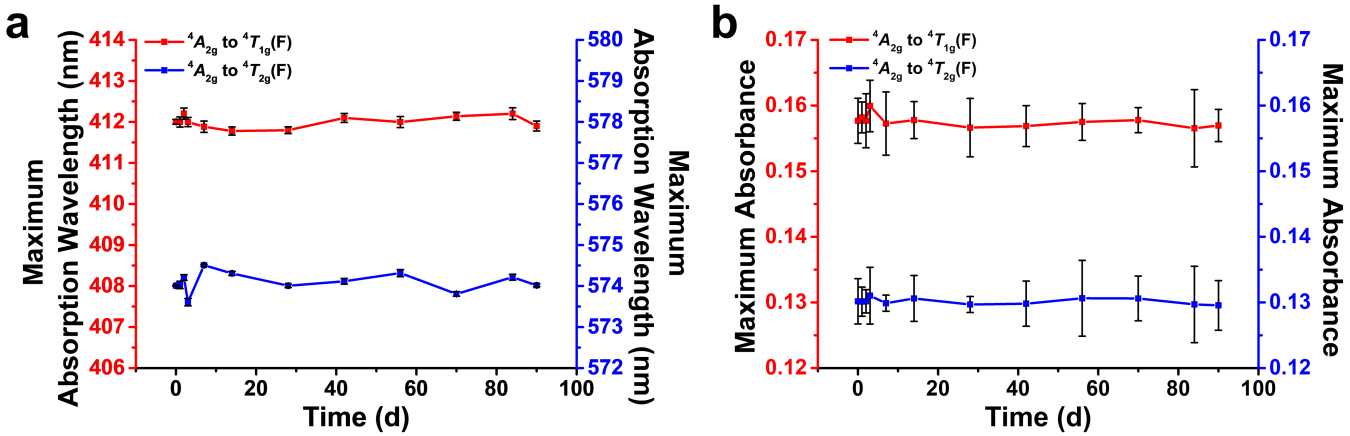


**Fig. S6.** (a) Maximum absorption wavelength and (b) maximum absorbance of $^4A_{2g}\rightarrow{}^4T_{1g}$(F) and $^4A_{2g}\rightarrow{}^4T_{2g}$(F) transition bands of **EtoChrom** (10 mM) over a period of 90 d.

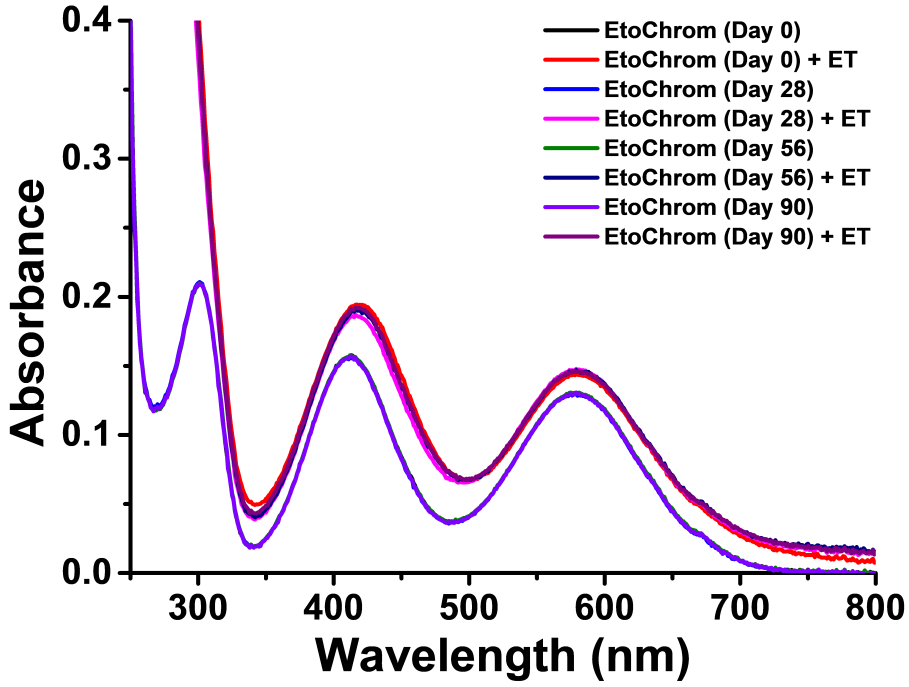


**Fig. S7.** UV-Vis spectra of **EtoChrom** (10 mM) with or without the addition of ET (1 mg/mL) at days 0, 28, 56, and 90.

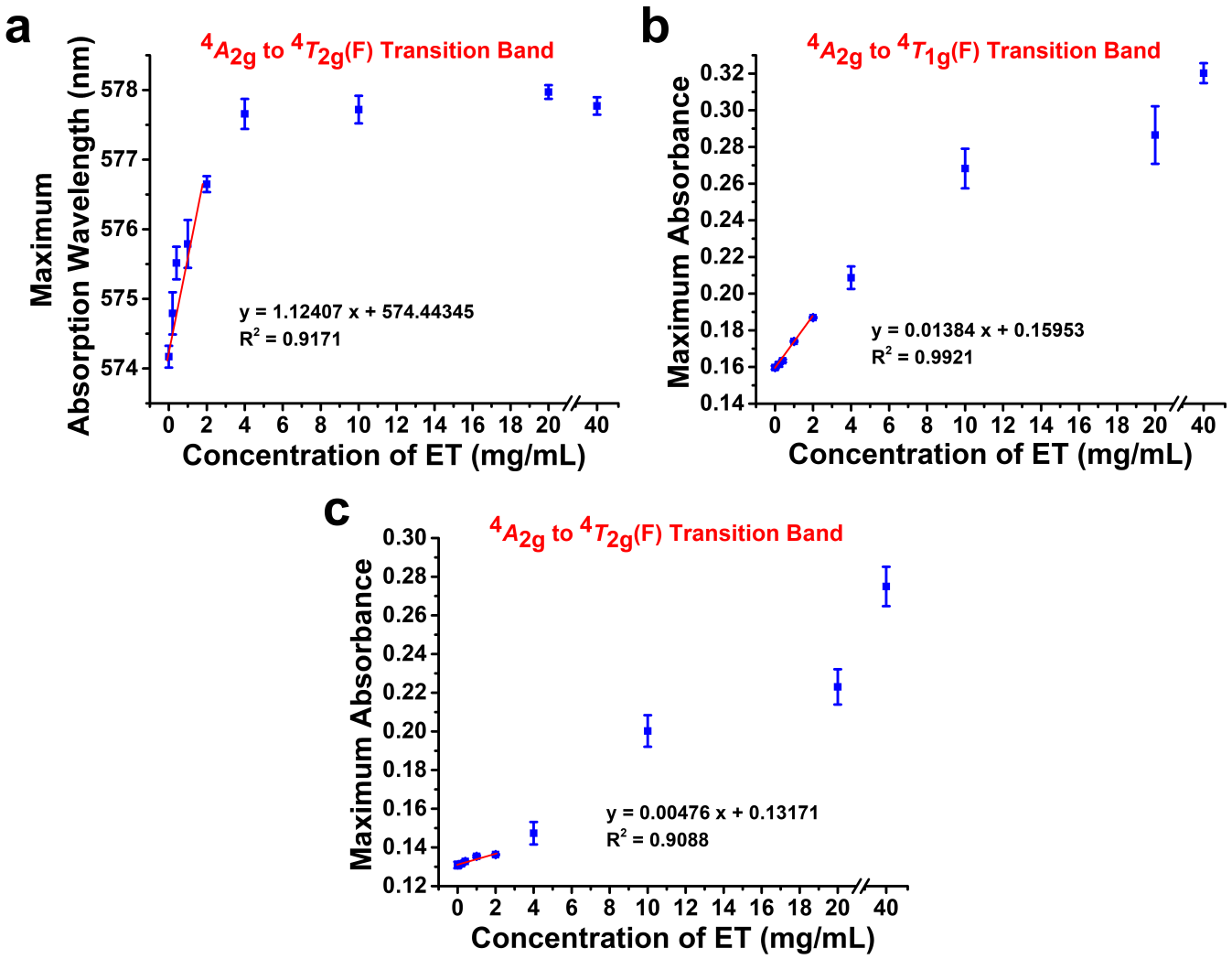


**Fig. S8.** The relationship between the (a) $\lambda_{abs}$ of $^4A_{2g} \longrightarrow {}^4T_{2g}(F)$ transition band, maximum absorbance of (b) $^4A_{2g} \longrightarrow {}^4T_{1g}(F)$, or (c) $^4A_{2g} \longrightarrow {}^4T_{2g}(F)$ transition band and **EtoChrom** (10 mM) and the concentration of ET.

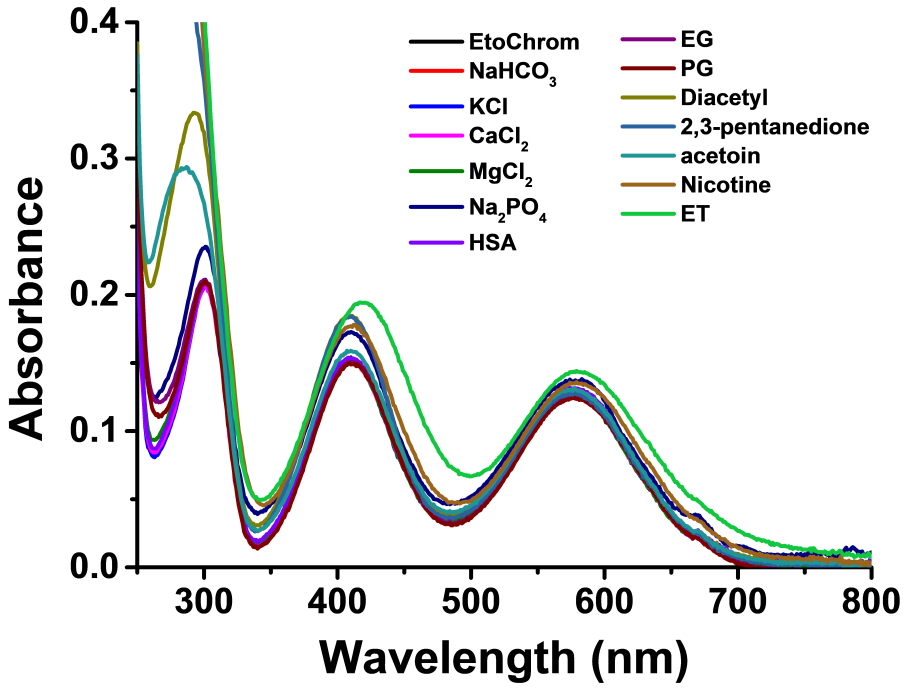


**Fig. S9.** UV-Vis spectra of **EtoChrom** (10 mM) after the treatment with ET or different potential interfering species in the vape juice & saliva matrices with the corresponding concentrations.

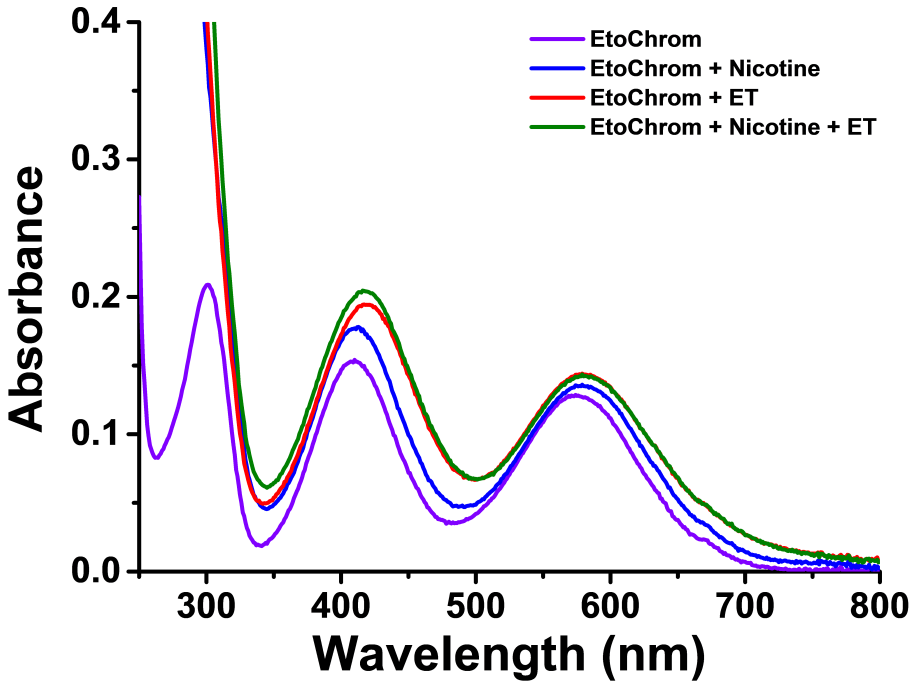


**Fig. S10.** UV-Vis spectra of **EtoChrom** (10 mM) in the absence or presence of nicotine (18 mg/mL) and ET (1 mg/mL).

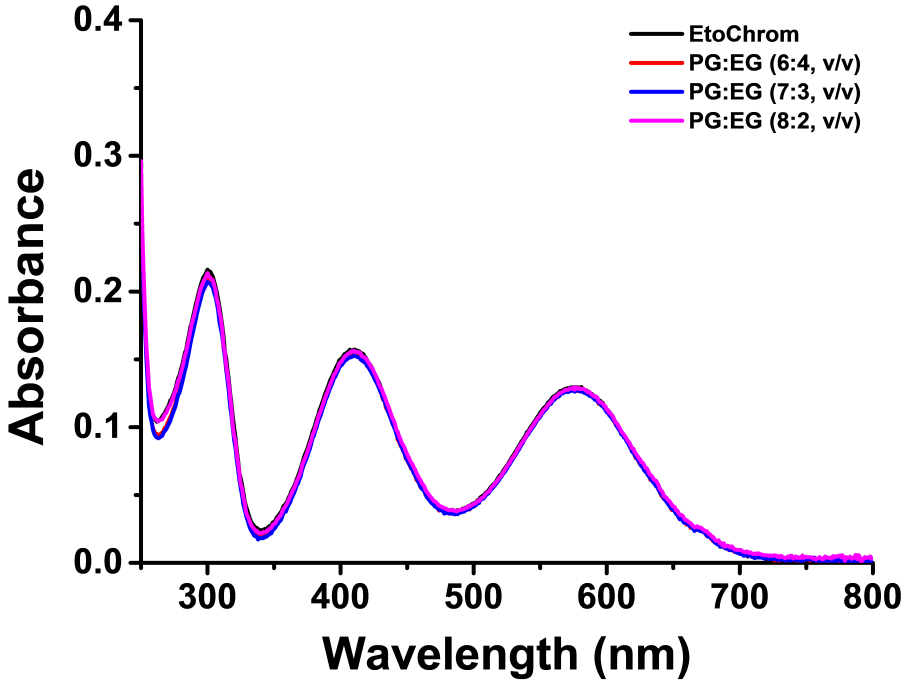


**Fig. S11.** UV-Vis spectra of **EtoChrom** (10 mM) after the treatment with different ratios of propylene glycol/glycerol (PG/EG).

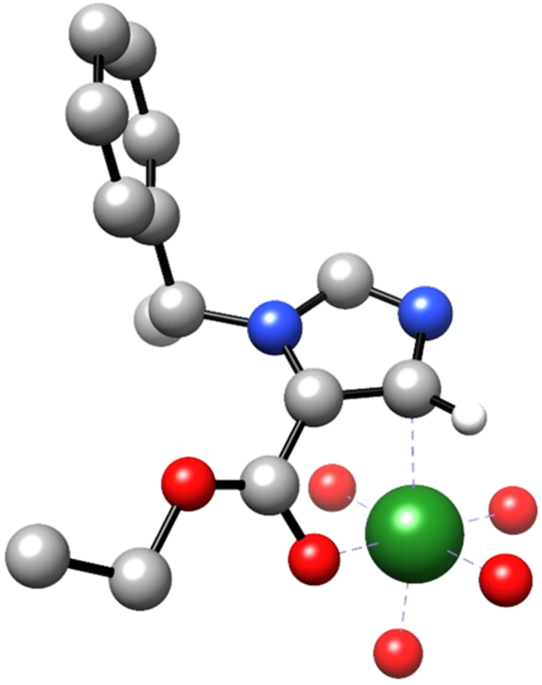

**Fig. S12.** The optimized bidentate coordinated structure between ET and $Cr^{3+}$ showing C and C=O coordination despite attempting N and C=O coordination, showing the infeasibility of N/C=O bidentate coordination. Green: Cr; Grey: C; Red: O; Blue: N. Hydrogens are omitted for clarity.

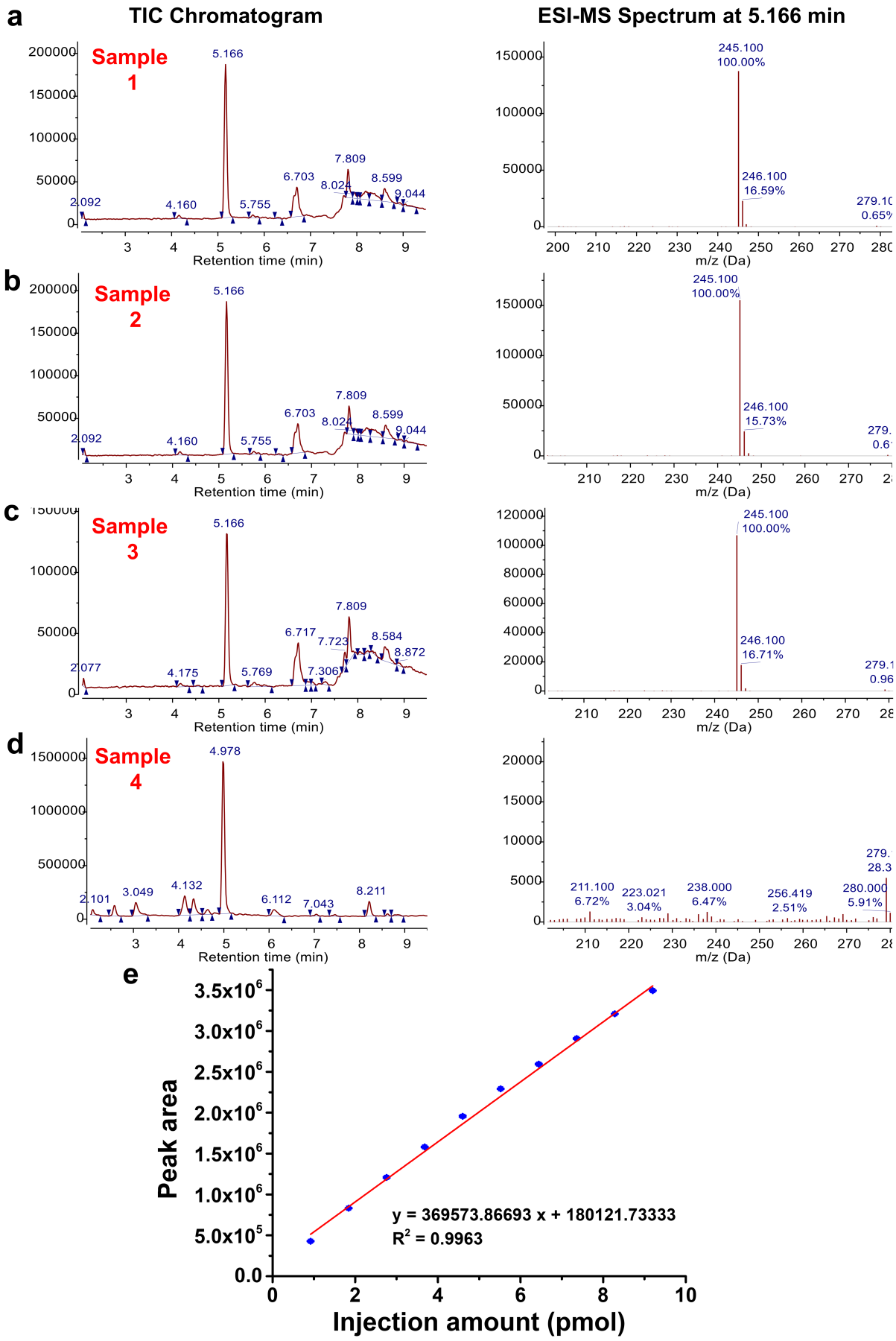


**Fig. S13.** LC/MS Chromatograms of Sample (a) 1, (b), 2, (c) 3, (d) 4, and (e) calibration curve of ET from 0.92 to 9.2 pmol based on the integrated peak area of the TIC chromatogram.

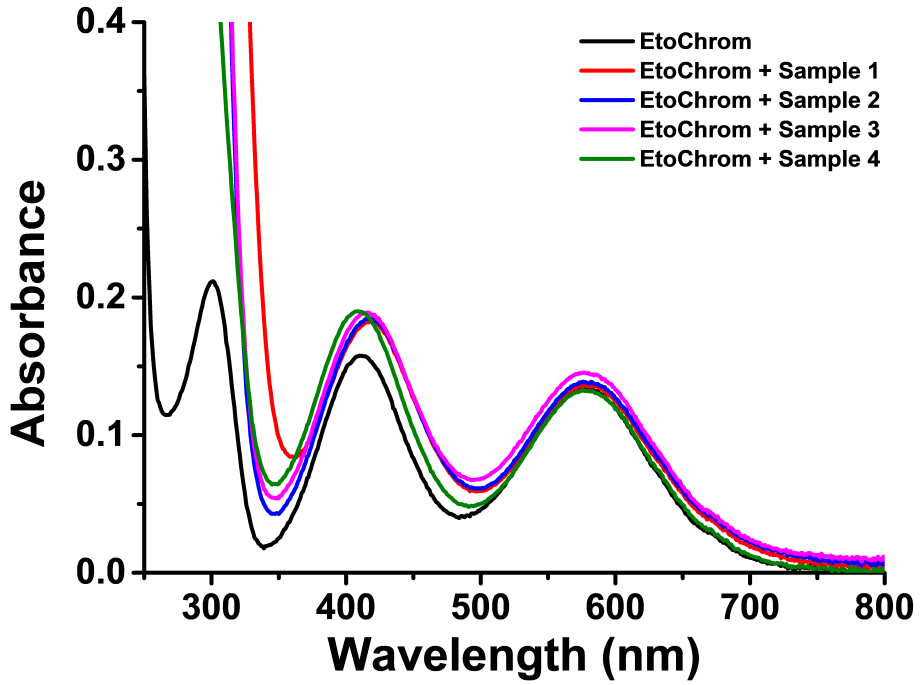


**Fig. S14.** UV-Vis spectra of **EtoChrom** (10 mM) with or without the addition of Samples 1-4.

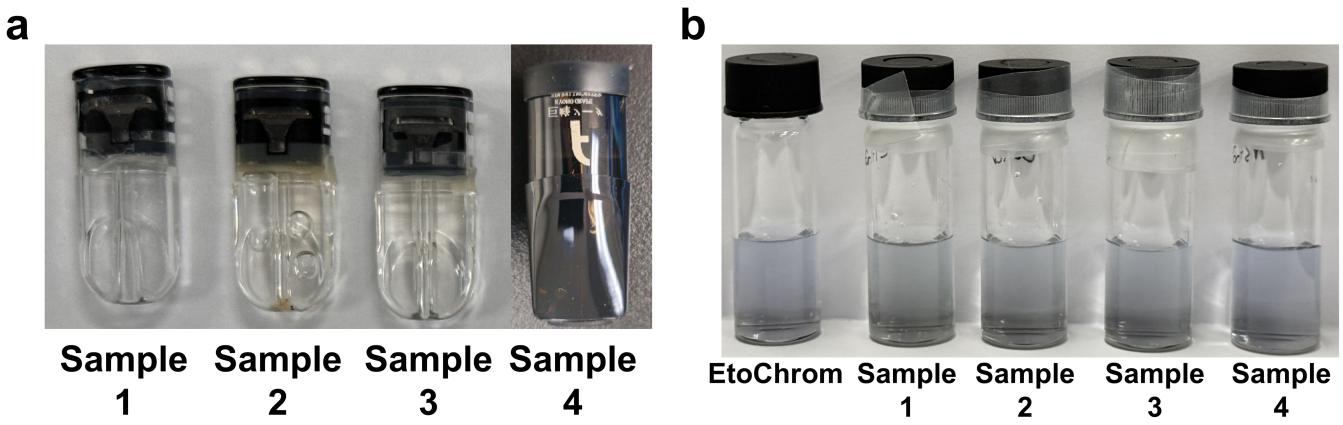


**Fig. S15.** (a) Photo of the E-cigarette pods containing Sample 1-4. (b) Change in colour of **EtoChrom** (10 mM) upon the addition of Sample 1-4.