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# Application of modified cloud point extraction method for the chromium speciation in artificial saliva extracts of different snuff products

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ABSTRACT

A modified cloud point extraction method (*m*-CPE) was developed for the speciation of chromium species ($Cr^{3+}$ and $Cr^{6+}$) in artificial saliva extracts (ASE) of snuff products. In this method, $Cr^{3+}$ was complexed with 8-hydroxyquinoline, which was trapped in nonionic surfactant (Triton X-114), prior to analyzed by electrothermal atomic absorption spectrometer (ETAAS). Whereas, the total extractable Cr was determined by reducing $Cr^{6+}$ to $Cr^{3+}$ using $Na_2SO_3$ as a reducing reagent. Several parameters were optimized for the developed *m*-CPE. Under the most favorable conditions, enrichment factor, enhancement factor and limit of detection for the proposed method were 60, 134 and 0.04 $\mu g\,L^{-1}$, respectively having relative standard deviation <5%. Health risks associated with the intake of total Cr in snuff products was also assessed. Estimated daily intake of Cr via sniffing 10 g/day of dry and moist snuff products was found below the maximum tolerable daily intake whereas the calculated risk for cancer due to Cr was observed in the acceptable range of 10 $E^{-6}$–$E^{-4}$.


## Introduction

Non-combustible or smokeless tobacco (SLT) products are getting more popularity than smoked tobacco products among several communities. These items are frequently consumed by the general population from various regions of the world.

Variety of snuff products are usually composed of finely minced tobacco, available in loose as well as packed forms. The moist snuff is usually dipped/sandwiched in between the cheek and gum, whereas the dry snuff is ingested via oral or nasal route [1,2]. The International Agency for Research on Cancer (IARC) has declared SLT as carcinogenic product, as it contains around 30 cancer causing agents [3]. The moist and dry snuffs are commonly used in United States and other European countries. The snuff and other SLT products have also been communally called as spit tobacco products [4]. The tobacco products might contain different toxic organic and inorganic constituents, which release while chewing and attack/absorb across the epithelial tissues of oral cavity. During chewing the toxic ingredients might become soluble in saliva and initially absorbed directly into oral mucosa, whereas the remaining portion of SLT products could be absorbed in the digestive tract [3]. Excessive consumption of these products has been related with several kinds of lesions in oral cavity. The different type of tobacco-related abrasions comprises of melanosis, tooth stains, periodontal conditions such as acute necrotizing ulcerative gingivitis, epithelial dysplasia and squamous-cell carcinoma and leukoplakia [4]. Tobacco consumption is associated to cause oral cancer, which is the fifth utmost common malignancy across the globe. Tobacco and its products have been supposed to share 30% of the global cancer load. Several factors like climatic conditions, soil physiognomies and plant variety affects the elemental level of the tobacco plants, so in tobacco products [5–7].

Numerous toxic metals have been found in SLT products including chromium (Cr), lead (Pb), nickel (Ni) and cadmium (Cd). The IARC have confirmed Cr as a carcinogenic element (Group 1) [8]. Heavy metals are usually absorbed by tobacco plant from polluted air and soil [9]. Other sources of these heavy metals in such products are lime and ash which are used as alkalinizing and binding agents, respectively [10].

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The Cr species have contrasting effects on human health because hexavalent chromium ($Cr^{6+}$) is considered as carcinogenic while $Cr^{3+}$ is nutritionally crucial and its deficiency can cause immune system sensitization [11]. Elemental analysis at trace level has attained great interest of scientists due to strong ecological contaminating influence and involvement in several physical processes. Actually, the assessment of the influence of human activity on environment is one of the chief objectives of modern analytical chemistry. The analysis of biological fluids (blood, saliva or urine) or bio-monitoring is the most effective means for evaluating chemical exposure. Modern analytical chemists design optimized, advance, reliable and validated methods for the routine environmental analysis presenting high recoveries, good reproducibility, low matrix effect and high aptitude to determine total metal content and metal species at trace levels [12].

Instrumental methods including flame and/or graphite furnace atomic absorption spectrometry (FAAS/GFAAS) are not sufficient for the analysis of Cr species at trace levels. To resolve this issue several separation and preconcentration steps including liquid–liquid extraction, membrane filtration, cloud point extraction, ion-exchange and solid phase extraction have been employed, prior to analysis of Cr species by atomic absorption spectrometry [13–21]. Cloud point extraction (CPE) has been proven to be a greener approach for the enrichment of heavy metals in several media [22,23]. A study proposed a procedure for preconcentration of $Cr^{3+}$ in lake water, complexed with 8-hydroxyquinoline and trap in a surfactant (TX-100) solution [24]. In another study, same group of researchers have determined total chromium, $Cr^{3+}$ and $Cr^{6+}$ in cigarettes and cigarette ash [25]. The hydrated chromium ion ($Cr^{3+}$) is inert, and make complex very slowly in aqueous solutions. On the other hand, these reactions considerably occur at faster rate in nonionic surfactant rich media. In non-ionic surfactant solutions, reactions between $Cr^{3+}$ and 8-hydroxyquinoline follow first-order kinetics [26]. The $Cr^{6+}$ assay was based on its reduction to $Cr^{3+}$ by sulphite [27]. In the current study, the total and artificial saliva extractable Cr was determined in snuff products (dry and moist). The $Cr^{3+}$ in ASE was chelated with 8-hydroxyquinoline, before and after the reduction of $Cr^{6+}$. The resulted complex was subsequently entrapped in the micelles rich phase. The effect of several factors, like pH, Triton X-114, ligand concentration, equilibration time and temperature was studied in detail. The daily intake and risk assessments of total Cr contents in different types of snuff samples were also calculated.

The risk assessment methodology established by the US Environmental Protection Agency (USEPA), associated with intake of Cr in selected snuff products, was also studied. The possible health hazards related with Cr contents by sniffing selected snuff (dry or moist) products was estimated using the daily intake, target hazard quotient, and carcinogenic risk to assess possible alert regarding adverse effects.

## Experimental work

### Sampling

Four different varieties of snuff including green moist (GM = 7), brown moist (BM = 7), dry brown (DB = 5) and dry black (DBk = 4) snuff were selected for the present study. Samples were purchased from different markets in Hyderabad, Pakistan during September–February 2016. In order to get a representative sample of a specific product, homogenized mixture was prepared of the same brand. The names of brands for each SLT have not been mentioned in the manuscript due to authorized necessities. The samples were dried out at 80 °C and were pulverized in powder form. Powdered form of the samples was passed through a nylon sieve with mesh width of 125um. Finally, the samples were stored in the sealed plastic bags.

### Reagents and glassware

The deionized water was used during the experimental work, taken from the ELGA labwater system (Bucks, UK). Analytical grade chemicals, ethanol, 8-hydroxyquinoline (oxine), concentrated $HNO_3$ (65%), HCl (37%), $H_2O_2$ (30%) and α-amylase were used. The Triton X-114 was acquired from Sigma (St. Louis, MO, USA). The 0.1 M of acetate and phosphate buffer solution was used to control the desired pH (3–9) of the reaction mixture with adjustment made by the addition of 0.1 M $HNO_3$/NaOH solutions. The certified standard solution of $Cr^{3+}$ (1000 mg $L^{-1}$) was obtained from Fluka Kamica (Bush, Switzerland). Stock solutions (1000 mg $L^{-1}$) of $Cr^{6+}$ were prepared by dissolving of $K_2Cr_2O_7$ (Sigma Aldrich, Germany) in 0.1 mol $L^{-1}$ of nitric acid. The artificial saliva was made in accordance with McKnight-Hanes and Chou formula [28,29]. Detailed composition of artificial saliva has been reported in our previous study [30]. Glass wares and polyethylene containers were soaked for 24 h in 10% (v/v) $HNO_3$; finally rinsed with de-ionized water and dried to avoid any contamination. The 8-hydroxyquinoline solution was made in 10 mL of ethanol and diluted up to 100 mL with 0.01 mol $L^{-1}$ acetic acid and kept in a refrigerator (4 °C).

### Instrumentation

A PEL domestic microwave oven (Osaka, Japan) of power (900 W) was utilized for the digestion of samples. An electrical shaker (220/60 Hz, Gallenkamp, England) was used for the dispersion of the test solution. The pH of the samples was measured by using a pH meter (Ecoscan Ion 6, Malaysia). ROWKA Laboratoryjna type WE-1, nr-6933 centrifuge (Mechanika Phecyzyjna, Poland) was used to achieve the phase separation. The determination of total and extracted Cr in artificial saliva was carried out, while utilizing A Analyst 700 PerkinElmer flame atomic absorption spectrometer (Norwalk, CT, USA). The instrumental parameters have been shown in Table 1. The instrument was equipped with the graphite furnace HGA-400, pyrocoated graphite tubes with integrated platform, auto sampler AS-800 and deuterium lamp as background correction system.

### Digestion of snuff samples

Digestion of the snuff samples was carried out by following microwave assisted digestion method (MAD). 0.2 g of CRM (n = 6) and each snuff samples (n = 3) were taken in separate PTFE flasks (25 mL). Then added $HNO_3$ and $H_2O_2$ in a ratio of 2:1 to the samples

**Table 1**
Instrumental conditions for extraction method.

| Step | Temperature (°C) | Ramp time (s) | Hold time (s) | Gas flow rate (mL/min) |
|---|---|---|---|---|
| Drying | 200 | 15 | 15 | 300 |
| Ashing | 1400 | 10 | 20 | 300 |
| Atomization | 2800 | 0 | 3 | Stop |
| Cleaning | 2700 | 1 | 3 | 300 |

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