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Simultaneous Quantitative Determination of Nonoxynol-9, Acrylamide, Sorbic Acid and Sodium Cyclamate in Toothpaste by HPLC-MS/MS

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A new method for simultaneous determination and quantitation of nonoxynol-9, acrylamide, sorbic acid and sodium cyclamate in toothpaste has been developed by using high performance liquid chromatography-Tandem mass spectrometry (HPLC-MS/MS). The method is simple, sensitive and rapid. Recoveries were mostly higher than 90 %, method detection limits (MDLs) ranged from 1.69-2.77 ng mL^{-1} and method quantitation limits (MQLs) were included between 5.63-9.22 ng mL^{-1}. Matrix effect was evaluated and quantitation was performed by referring to a matrix matched calibration curve to improve accuracy. This method was also applied to commercial toothpaste samples with good results.

Keywords: Nonoxynol-9, Acrylamide, Sorbic acid, Sodium cyclamate, Toothpaste, HPLC-MS/MS.

INTRODUCTION

Toothpaste is a paste or gel dentifrice used with a toothbrush as an accessory to clean and maintain the aesthetics and health of teeth. It is not for eating but some compounds coming from manufacturing process or additives may be taken to the body during brushing. Such as nonoxynol-9, acrylamide, sorbic acid and sodium cyclamate (Fig. 1).

Nonoxynol-9 is a member of the nonoxynols family of nonionic surfactants, of which the metabolite nonylphenol has been reported to have estrogenic activity[1,2]. Nonoxynol-9 is a ingredient in various cleaning and cosmetic products. It is also widely used in contraceptives for its spermicidal properties[3,4]. However, some studies[5-7] have demonstrated that nonoxynol-9 shows toxicity both *in vitro* and *in vivo*. The directive 2003/53/EC of the European parliament and of the council forbids the use of nonoxynols or nonylphenol as a substance or constituent of preparations in concentrations equal or higher than 0.1 % by mass for cosmetic products and domestic cleaning[8].

Acrylamide is a synthetic monomer that has been found widespread application in industry as a precursor in the production of polyacrylamide, which is widely used for the purification of drinking water and in food packaging. Numerous studies showed that acrylamide is characterized by neurotoxic activity and may cause damage to the central and peripheral nervous systems of both laboratory animals and human beings exposed to this compound[9-11]. In European Union countries, acrylamide has been regulated by the EU 98/83 Drinking Water Directive that stated a minimum quality requirement of 0.1 μg L^{-1} for water intended for human consumption[12].

Sorbic acid and its salts are the permitted chemical preservatives in food, cosmetic and medical products to prevent transformation and degradation by microorganisms during storage. However, sorbic acid may be harmful to consumers in case of excessive addition[13,14]. Many countries have restricted the usage of preservatives in daily food. For example, the upper limit for sorbic acid in foods is in the range of 0.2-2 g kg^{-1} in China[15].

Sodium cyclamate is one of the most common artificial sweeters. It is banned in USA while its usage is permitted in Europe and China[16], in most countries which are using sodium cyclamate, the range of 0-11 mg kg^{-1} is accepted as the daily intake (ADI) value[17]. Martins *et al.*[18] described the toxicity of sodium cyclamate in liver of rat fetuses and it has been reported to induce bladder carcinoma[19,20].

Considering the potential effect of nonoxynol-9, acrylamide, sorbic acid and sodium cyclamate on human body, it is necessary to establish rapid and effective methods for the determination of the four compounds in different kinds of toothpaste. In the last few years, several methods[21-32] have been developed for the determination of nonoxynol-9, acrylamide,

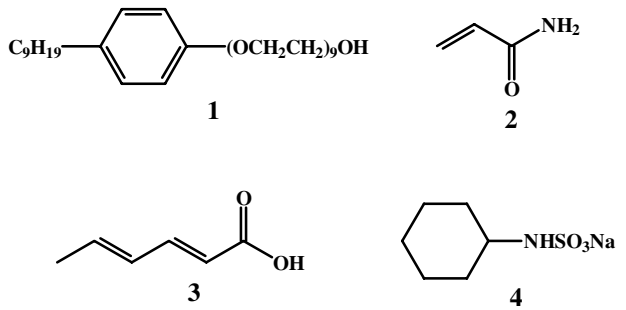

Fig. 1. Chemical structures of nonoxynol-9 (1), acrylamide (2), sorbic acid (3) and sodium cyclamate (4)

sorbic acid or sodium cyclamate, using capillary electrophoresis (CE), liquid chromatography (LC), gas chromatography (GC), gas chromatograph-mass spectrometry (GC-MS) or liquid chromatography-tandem mass spectrometry (LC-MS/MS). Hence, this paper reports a sensitive, dependable and simple method, based on reversed-phase HPLC-MS/MS to simultaneously determine nonoxynol-9, acrylamide, sorbic acid and sodium cyclamate in toothpaste. No solid-phase extraction (SPE) column is needed during extraction procedure and a matrix-matched calibration curve was used to optimize the entire method. The method was finally tested on commercial toothpaste samples.

EXPERIMENTAL

Chemicals, reagent and working solutions: HPLC-grade methanol were purchased from TEDIA (Ohio, USA). Analytical standards used were obtained from the following sources: nonoxynol-9, Toronto Research Chemicals (Toronto, Canada); acrylamide, Bai-lingwei technology Co., Ltd., (Beijing, China); sorbic acid and sodium cyclamate, Dr. Ehrenstorfer GmbH (Augsburg, Germany). All other chemicals were analytical-reagent grade. Deionized water was obtained from a Milli-Q water system (Millipore, Bedford, MA, USA) and was used throughout the study.

Standard stock solutions of nonoxynol-9, acrylamide, sorbic acid and sodium cyclamate were prepared at 100 µg mL^{-1} level in methanol and stored at 4 °C in glass vials.

Test samples were purchased from local supermarkets in Kunming, PR China and stored at 4 °C.

About 1 g (accurately weighed to ± 0.01 g) of toothpaste sample was weighed into a 50 mL plastic centrifuge tube and 20 mL of 50 % methanol in deionized water was added, which was then vortex-mixed for 1 min. The mixed sample was ultrasonicated for 15 min and then centrifuged at 3500 rpm for 5 min. The supernatant was filtered through a 0.22 µm membrane and transferred to an auto-sampler vial. Finally, 5 µL aliquot was injected into the a HPLC-MS/MS system.

UPLC-MS/MS instrumentation and conditions: The liquid chromatography Tandem mass spectrometry system was comprised of an API 4000 MS/MS system equipped with an electrospray ionization (ESI) probe and a syringe pump (AB Sciex, Foster City, CA, USA) and an Ultra Performance LC system was equipped with a binary pump and an autosampler (Waters, Milford, MA, USA). The system was connected by PEEK tubing (1/16 in. o.d. × 0.01 in. i.d.). Data was acquired and processed using AB Sciex Analyst software (version 1.5.1).

Samples (5 µL) of the final extracts were separated on an Atlantis dC18 column (4.6 × 150 mm; 3 µm particles) at a flow rate of 0.8 mL min^{-1} with a split ratio of 4:6 and eluted with a linear binary gradient of 5 mmol L^{-1} ammonium acetate in water (A) and methanol (B) (Table-1). The temperature of the analytical column was maintained at 40 °C.

TABLE-1
MOBILE PHASE GRADIENT PROGRAM OF HPLC-MS/MS
(A: 5 mmol L^{-1} AMMONIUM ACETATE IN WATER AND B: METHANOL)

Time (min)	Methanol (%)	5 mmol L^{-1} Ammonium acetate in water (%)
0	20	80
1	20	80
3.5	70	30
5	99	1
8	99	1
9	20	80
10	20	80

Detection of analytes were operated in the positive ion mode. Optimization of the operation conditions, infusing diluted stock solutions of each analyte into the mass spectrometer were as follows: source temperature 600 °C, curtain gas 30 psi (83 kPa of max. 99.5 % nitrogen), ion source gas 1 (nebulizer gas) 60 psi (414 kPa of nitrogen), ion source gas 2 (auxiliary gas) of 60 psi (276 kPa of nitrogen), spray voltage 5.5 kV. Other MS parameters are shown in Table-2.

Method validation: A standard calibration line was constructed by analyzing mix solutions at seven concentration levels in the ranges of 5-500 ng mL^{-1}. A matrix matched calibration curve was also performed by spiking the extracts of toothpaste. The curve was constructed by addition of appropriate volumes of the standard mix working solution at blank toothpaste sample extracts in order to have the same concentration levels of the standard working solution. The analyst peak area *versus* analyst concentration in toothpaste samples were plotted to get the calibration curve.

Signal suppression or enhancement on ESI-MS/MS response due to matrix effect was evaluated, for each analyte, by comparing the slope of the standard calibration curve with the slope of the matrix matched calibration curve.

TABLE-2
OPTIMIZED MS PARAMETERS OF NONOXYNOL-9, ACRYLAMIDE, SORBIC ACID AND SODIUM CYCLAMATE

Analyte	Precursor ion (*m/z*)	Product ion (*m/z*)	Declustering potential (U/V)	Collision energy (U/eV)
Nonoxynol-9	634.5 $[M + NH_4]^+$	617.4*/291.3	56/55	25/35
Acrylamide	72.0 $[M + H]^+$	55.0*/44.0	40/42	16/26
Sorbic acid	113.2 $[M + H]^+$	95.2*/67.2	25/26	12/20
Sodium cyclamate	202.2 $[M + H]^+$	122.4*/140.3	31/36	12/14

*Quantitative ion

Accuracy was evaluated in terms of percentage of recovery. For recovery studies blank toothpaste were spiked prior to the extraction step. A weighted sample was added of a small and suitable volume of working solutions of the analytes and then extraction was carried out, as previously described.

For each analyte, five replicates of three levels of concentration, corresponding to 10, 50 and 250 ng mL^{-1}, were investigated. The averaged recovery, the relative standard deviation (RSD) and relative error (RE) were calculated.

To calculate the detection limits and quantitation limits of each analyte, seven replicates of blank toothpaste sample extracts spiked with an appropriate volume of the standard mix working solution in order to have the same concentration level of the lowest level of the calibration curve were analyzed and the MDL and MQL were expressed as 3 × SD and 10 × SD , respectively.

RESULTS AND DISCUSSION

Extraction: For the development of an appropriate extraction procedure for the determination of nonoxynol-9, acrylamide, sorbic acid and sodium cyclamate in toothpaste, the efficiency of different concentrations of methanol in water as extraction solvent was studied and quantitation was calculated by using the standard calibration curve. The result is shown in Fig. 2. Hence, 50 % methanol in water was used for the extraction of analytes. Ultrasonic extraction time was tested at 5, 10, 15, 20, 25, 30 min and 15 min was only for four analytes.

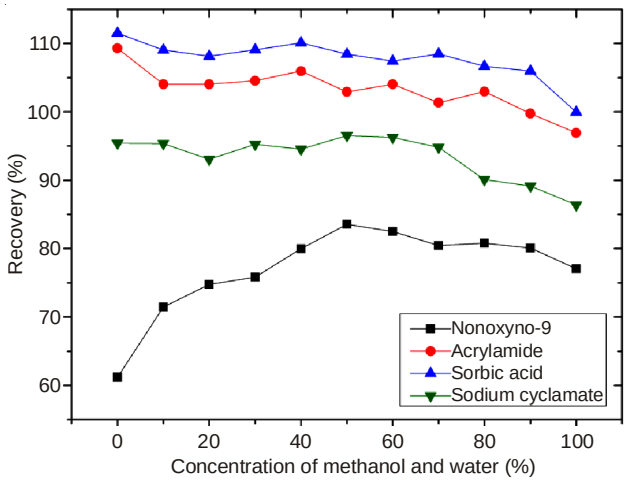

Fig. 2. Recovery of nonoxyno-9, acrylamide, sorbic acid and sodium cyclamate using different concentrations of methanol in water (from 100 % water to 100 % methanol) as extraction solvent

Optimization of chromatographic and MS/MS conditions: Analytes were mass-selected and fragmented. For each compound two ion pairs were chosen for acquisition in multiple reaction monitoring (MRM) mode. The mass spectra of nonoxynol-9, acrylamide, sorbic acid and sodium cyclamate revealed base peaks at *m/z* 634.5 $[M + NH_4]^+$, *m/z* 72.0 $[M + H]^+$, *m/z* 113.2 $[M + H]^+$ and *m/z* 202.2 $[M + H]^+$. Product ions were *m/z* 617.4/291.3, *m/z* 55.0/44.0, *m/z* 95.2/67.2, *m/z* 122.4/120.2, respectively. Tuning parameters are summarized in Table-2.

HPLC separation was performed using reversed phase chromatography and satisfactory separation was obtained with methanol and ammonium acetate in water as mobile phases. Typical HPLC-ESI-MS/MS chromatogram of four analysts was shown in Fig. 3. The retention time of nonoxynol-9, acrylamide, sorbic acid and sodium cyclamate are 6.72, 2.53, 3.85 and 4.19 min, respectively.

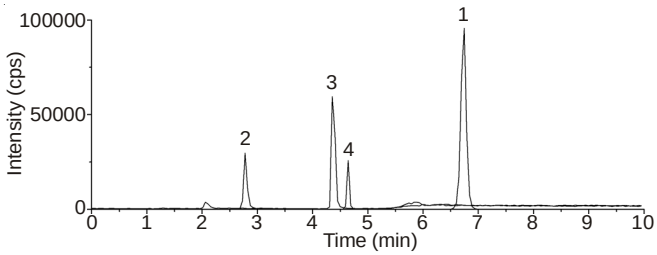

Fig. 3 Typical extracted ion chromatogram (XIC) of nonoxynol-9 (1), acrylamide (2), sorbic acid (3) and sodium cyclamate (4) by injecting a matrix matched mix solution

Method validation: As illustrated above, linear calibration curves were obtained both by standard calibration and by matrix matched procedures. The linearity ranges of all the analytes were evaluated. For each analyte the calibration curves and its linear regression analysis are shown in Table-3. All calibration curves showed good linear regression (R = 0.9990) within liner range.

The matrix effect was calculated and shown in Table-3. For nonoxynol-9 it was less than 1, showing signal suppression. For acrylamide, sorbic acid and sodium cyclamate it was more than 1, showing signal enhancement. Due to these differences between standard and matrix matched calibration, we chose to carry out the evaluation of method performances on the matrix curve, in order to improve the accuracy of the evaluation.

The evaluation of accuracy, expressed as percentage of recovery, was carried out on blank sample extracts, spiked

TABLE-3
CALIBRATION CURVES

Analyte	Standard equation[a]	Matrix-matched equation[b]	Matrix effect[c]
Nonoxynol-9	y = 1550x + 7990 (R = 0.9995)	y = 1300x + 8960 (R = 0.9996)	0.84
Acrylamide	y = 215x + 925 (R = 0.9990)	y = 226x-170 (R = 0.9992)	1.05
Sorbic acid	y = 455x + 33.8 (R = 0.9995)	y = 509x-544 (R = 0.9994)	1.12
Sodium cyclamate	y = 134x + 503 (R = 0.9999)	y = 140x + 516 (R = 0.9997)	1.04

[a] y = Analyte peak area, and x = concentration of analyte expressed as ng ml^{-1}. Standard calibration lines were constructed by analyzing mix standard solutions at seven concentration levels in the ranges of 5-500 ng mL^{-1}. All the solutions were prepared three times for each level, once injected and the results were averaged; [b]y = Analyte peak area, and x = concentration of analyte expressed as ng mL^{-1}. Matrix matched calibration lines were constructed by addiction of appropriate volumes of the standard mix working solution at blank toothpaste sample extracts in order to have the same concentration levels of the standard working solution. Each sample was three times analyzed, and data were averaged in order to assure a representative matrix matched curve; [c] Matrix effect was evaluated for each analyte by comparing the slope of the standard calibration curve with the slope of the matrix-matched calibration curve

TABLE-4
ACCURAY AND PRECISION

Analyte	Spiking level (ng mL^{-1})	Average recovery (%)	RSD (%)[a]	RE[b]
Nonoxynol-9	10	104.38	6.16	4.38
	50	98.11	2.43	-1.89
	250	101.38	4.21	1.38
Acrylamide	10	103.45	8.39	3.45
	50	97.46	5.11	-2.54
	250	96.68	2.75	-3.32
Sorbic acid	10	96.12	4.89	-3.88
	50	104.57	5.08	4.57
	250	105.61	1.76	5.61
Sodium cyclamate	10	83.44	2.18	-16.56
	50	89.05	7.61	-10.95
	250	98.7	4.12	-1.3

[a]Relative standard deviation, [b]Relative error

with a known amount of the analytes. Recoveries (Table-4) were evaluated at three different levels of concentration for each analyte, corresponding to a low, a high and an intermediate value of the evaluated range. Experimental data showed the overall good accuracy of the method for nonoxynol-9, acrylamide, sorbic acid and sodium cyclamate.

The detection limits and quantitation limits were evaluated as described above and data are listed in Table-5. Results showed that detection limit was 1.69-2.77 ng mL^{-1} and quantitation limit was 5.63-9.22 ng mL^{-1}.

TABLE-5
METHOD DETECTION LIMIT (MDL) AND
METHOD QUANTITATION LIMIT (MQL)

Analyte	MDL (ng mL^{-1})[a]	MQL (ng mL^{-1})[b]
Nonoxynol-9	1.69	5.63
Acrylamide	2.15	7.17
Sorbic acid	2.12	7.06
Sodium cyclamate	2.77	9.22

[a]Method detection limit was calculated as 3 × SD of 7 replicates of blank toothpaste sample extracts spiked with standard mix working solution to have the concentration of 5 ng mL^{-1} of each analyte.
[b]Method quantitation limit was calculated as 10 × SD of 7 replicates of blank toothpaste sample extracts spiked with standard mix working solution to have the concentration of 5 ng mL^{-1} of each analyte

Real sample analysis: The method was finally applied to analyze nonoxynol-9, acrylamide, sorbic acid and sodium cyclamate in commercial samples of toothpaste. Each sample was three times analysed and in order to assure an accurate determination, quantitation was calculated by using the matrix matched calibration curve. All the four analytes were not found in those real samples, as it is declared on the label of the samples.

Conclusion

The presented HPLC-MS/MS method allows for the rapid, efficient and simple determination of nonoxynol-9, acrylamide, sorbic acid and sodium cyclamate in toothpaste. The sample pretreatment methods avoided the difficult and time-consuming procedures, such as cleanup and derivatization. A relevant matrix effect was observed and by applying the matrix matched calibration curves, the method showed good recoveries of four analytes added to toothpaste, always above 83 % and RSDs were less than 8.39 %. MDL and MQL were 1.69-2.77 ng mL^{-1} and 5.63-9.22 ng mL^{-1}.

The method was tested against commercial samples, to confirm its reliability, with results in line with their respective labels.

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