

SIMULTANEOUS DETERMINATION OF NINE N-NITROSAMINES PROHIBITED IN COSMETIC PRODUCTS BY VORTEX-ASSISTED DISPERSIVE LIQUID-LIQUID MICROEXTRACTION AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY

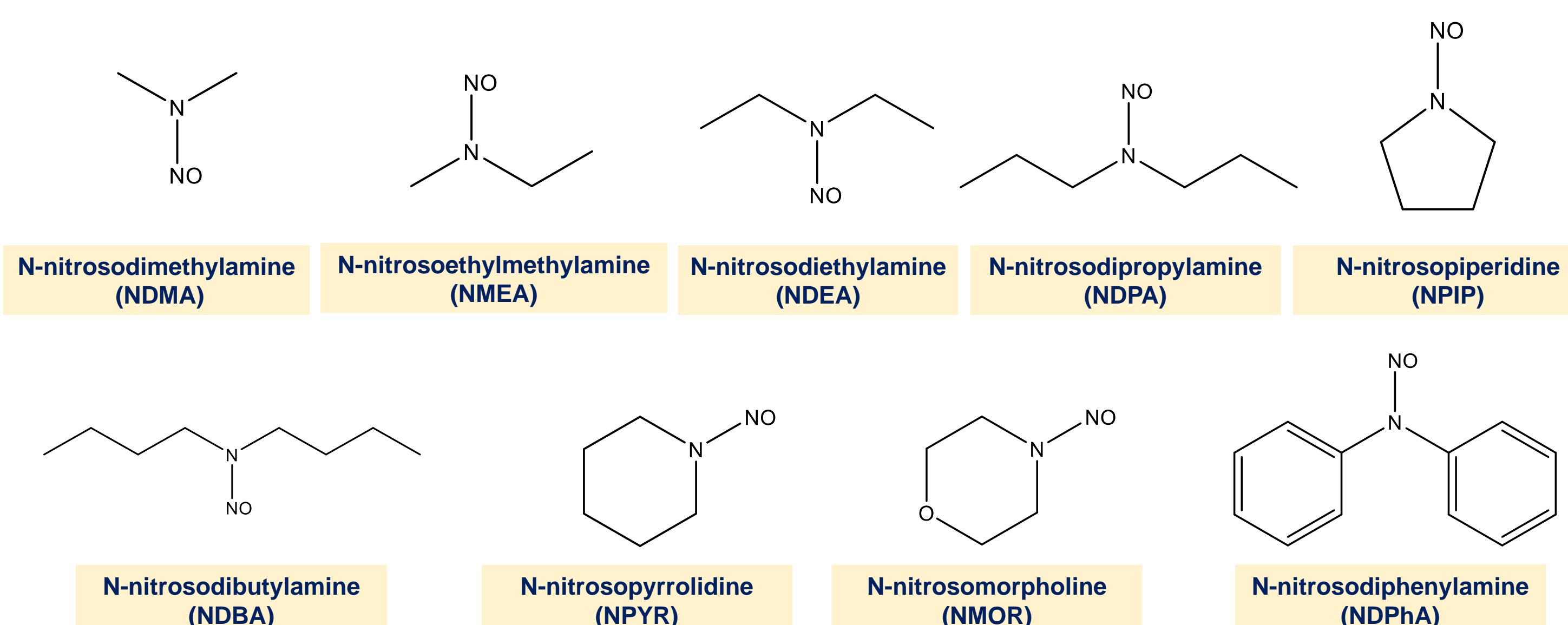
INTRODUCTION

N-nitrosamines are compounds with mutagenic, carcinogenic, and teratogenic effects [1], which can be found in cosmetic products without having been intentionally added during the manufacturing process. To avoid causing a risk to the consumer health, a maximum content limit of $50 \mu\text{g kg}^{-1}$ for traces of N-nitrosamines in cosmetic products has been established [2].

The **aim** of this work was to develop a new analytical method for the simultaneous determination of nine prohibited N-nitrosamines in cosmetic products, obtaining detection limits that allow the determination of these analytes below the limit established by the European Regulation [3], reducing the consumption of organic solvents and the process time.

Vortex-assisted dispersive liquid-liquid microextraction (VA-DLLME) was selected as microextraction technique because vortex agitation helps the formation of the cloudy solution, reducing the consumption of additional organic solvents beyond the extraction solvent.

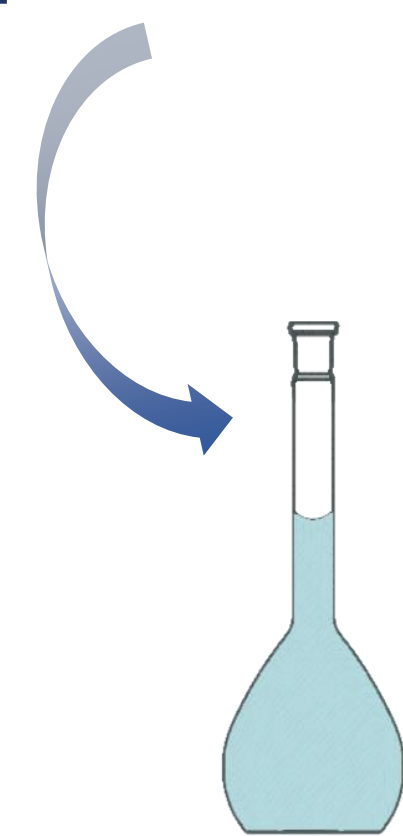
ANALYTES



EXPERIMENTAL

PRETREATMENT

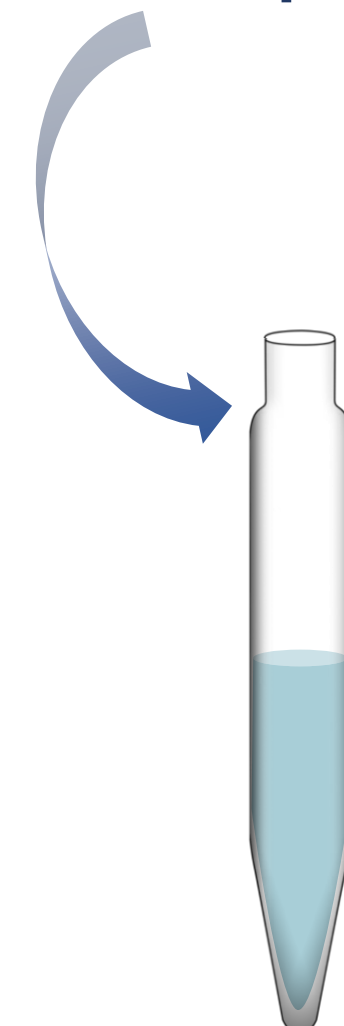
0.05 g of sample
+ an aliquot of a 100 ng mL^{-1}
standard aqueous solution
+ ultrapure water up to 5 mL



Vortex (1 min)

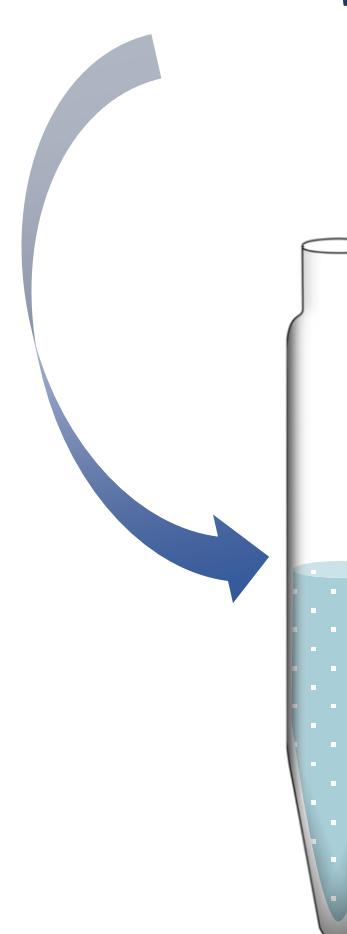
MICROEXTRACTION

+ 120 μL CHCl_3

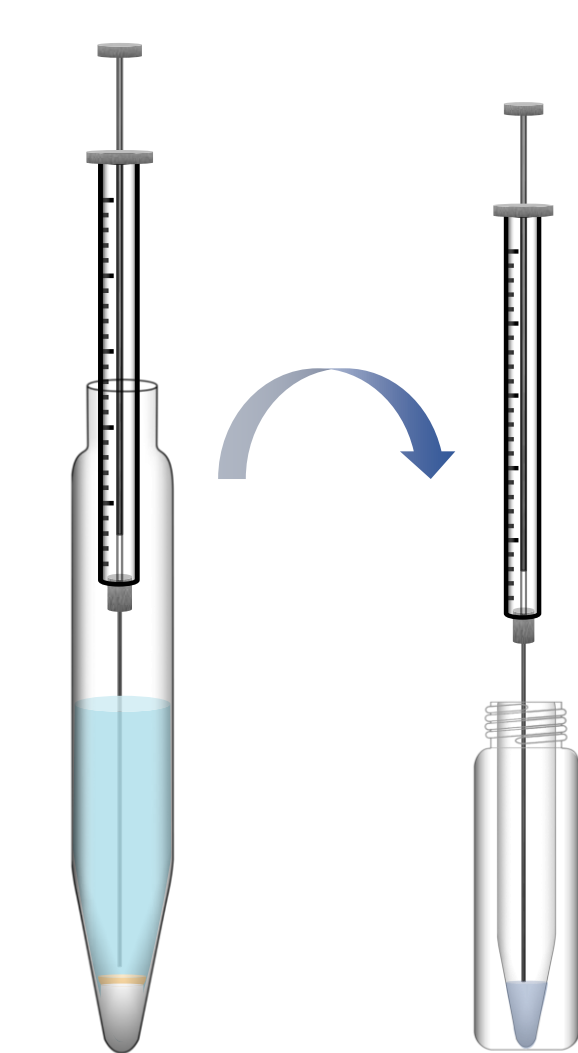


Vortex (1 min)

Formation of the
cloudy solution



Centrifuge
(6000 rpm, 5 min)



Collect the clean
sedimented phase



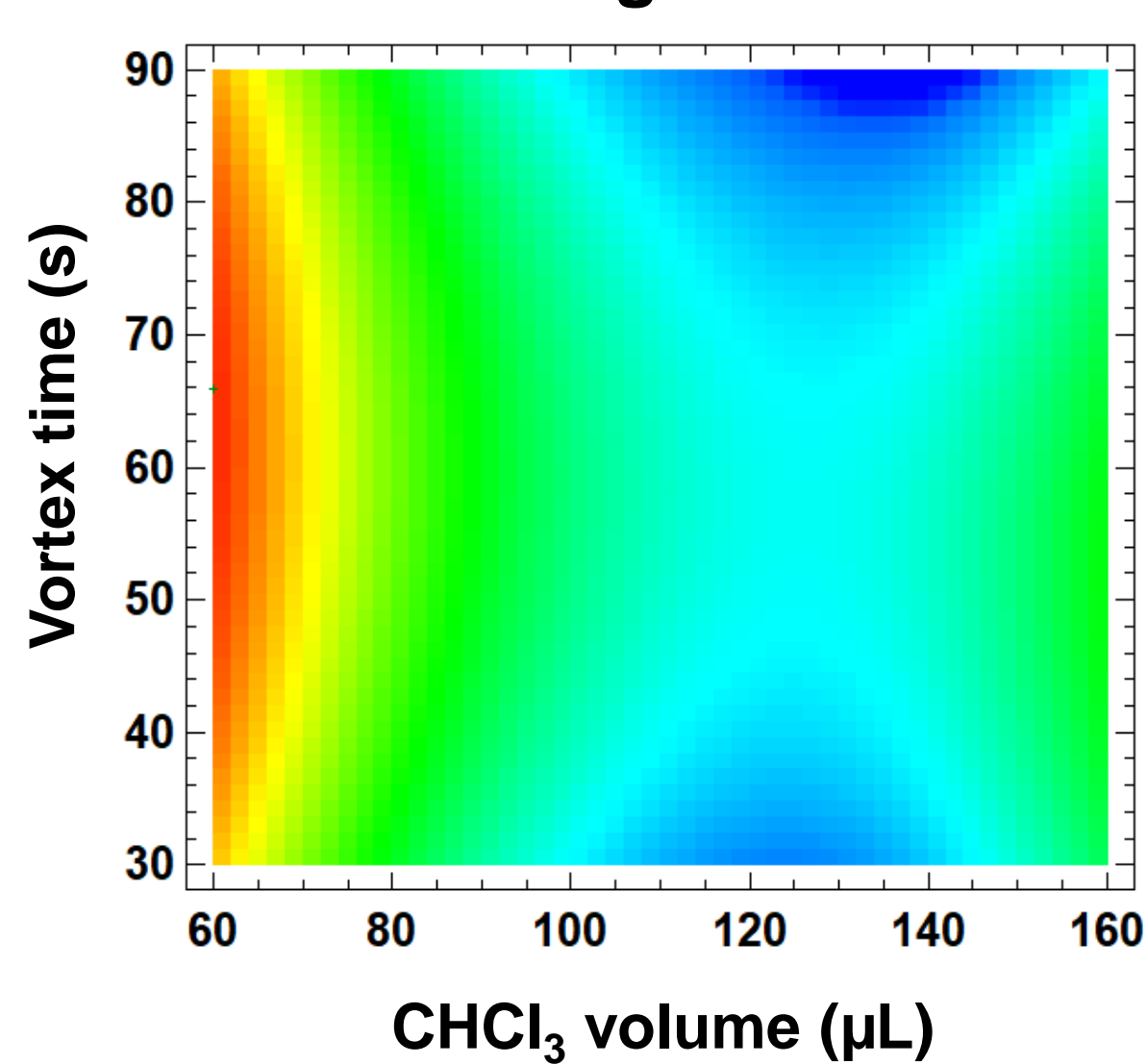
GC-MS

Instrumental variable	
Injection volume	2 μL
Injection mode	Splitless
Injection temperature	230 $^{\circ}\text{C}$
Carrier gas	Helium
Carrier gas flow	1 mL min^{-1}
Column	VF-WAXms (30m x 0.25 mm I.D.; 0.25 μm film thickness)
Oven temperature	60 $^{\circ}\text{C}$ (2min) \rightarrow 10 $^{\circ}\text{C/min}$ \rightarrow 160 $^{\circ}\text{C}$ \rightarrow 40 $^{\circ}\text{C/min}$ \rightarrow 240 $^{\circ}\text{C}$ (4min)
Ionization mode	Electron impact (EI)
Mass range	Start mass: 40; end mass: 200
Electron energy	70 eV
Ion source temperature	230 $^{\circ}\text{C}$
Transfer line temperature	230 $^{\circ}\text{C}$
Quad temperature	150 $^{\circ}\text{C}$

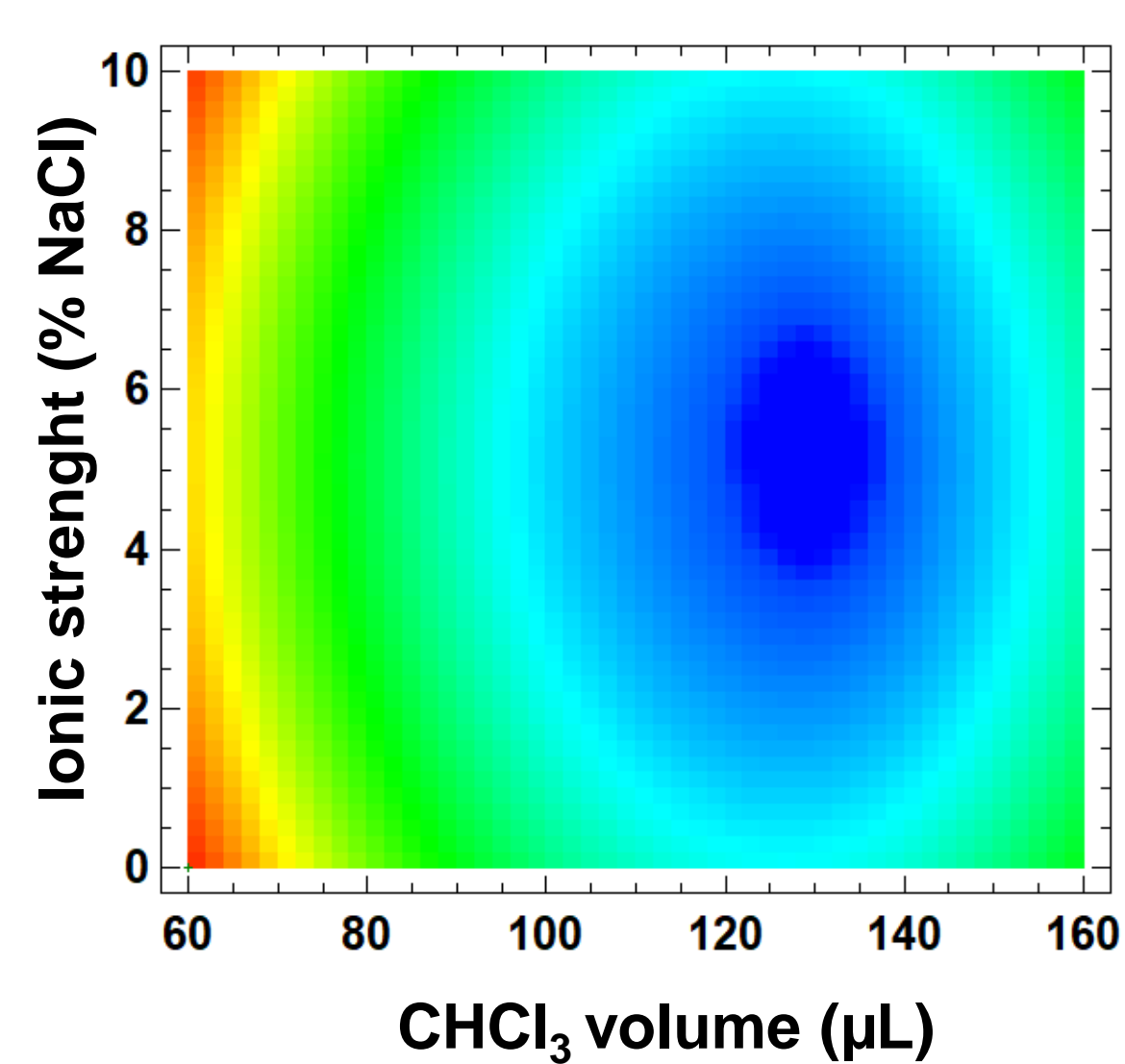
RESULTS AND DISCUSSION

Response Surface Methodology for the extraction procedure (Box-Behnken design)

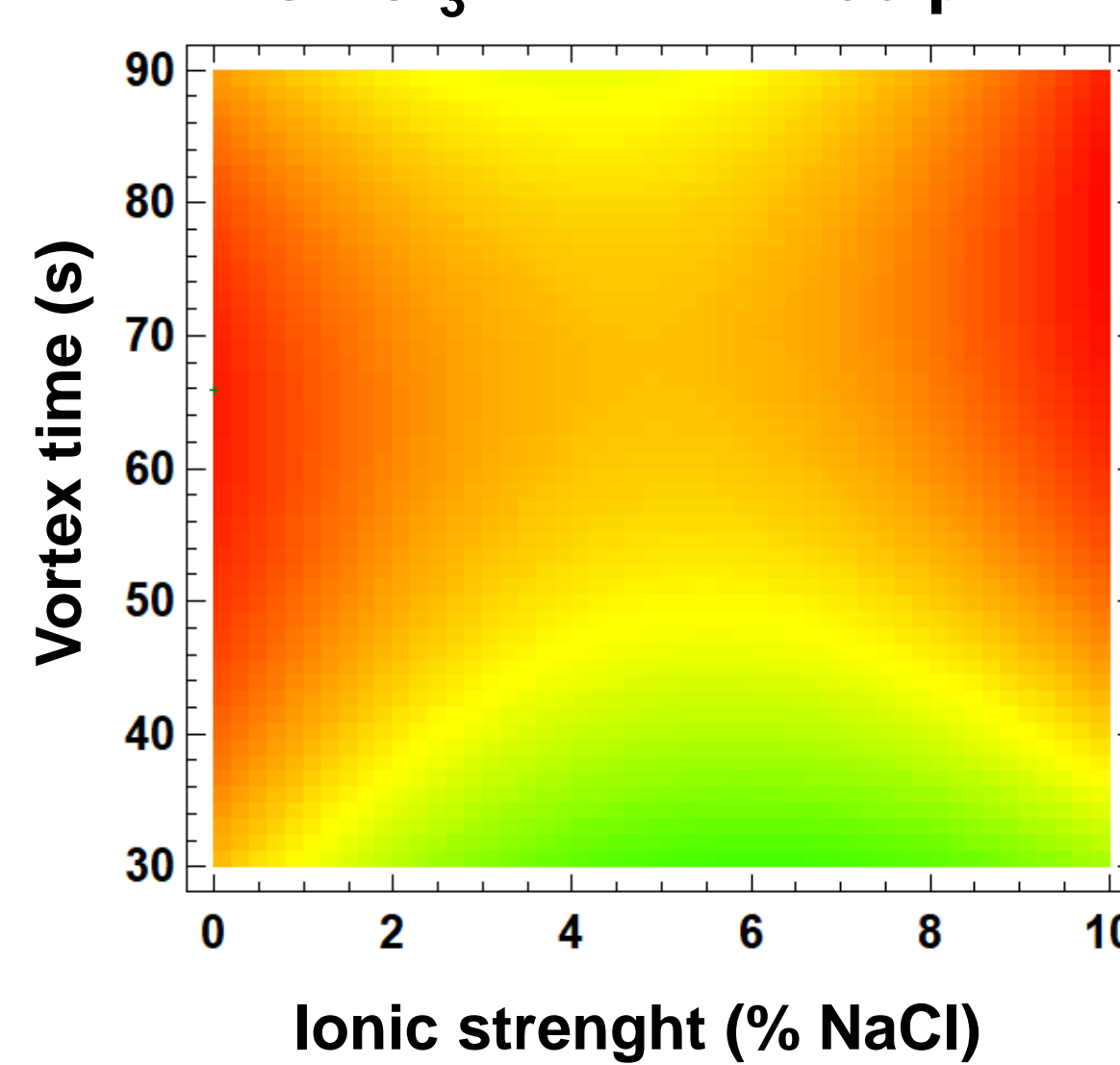
a) Ionic strength = 0% NaCl



b) Vortex time = 60 s

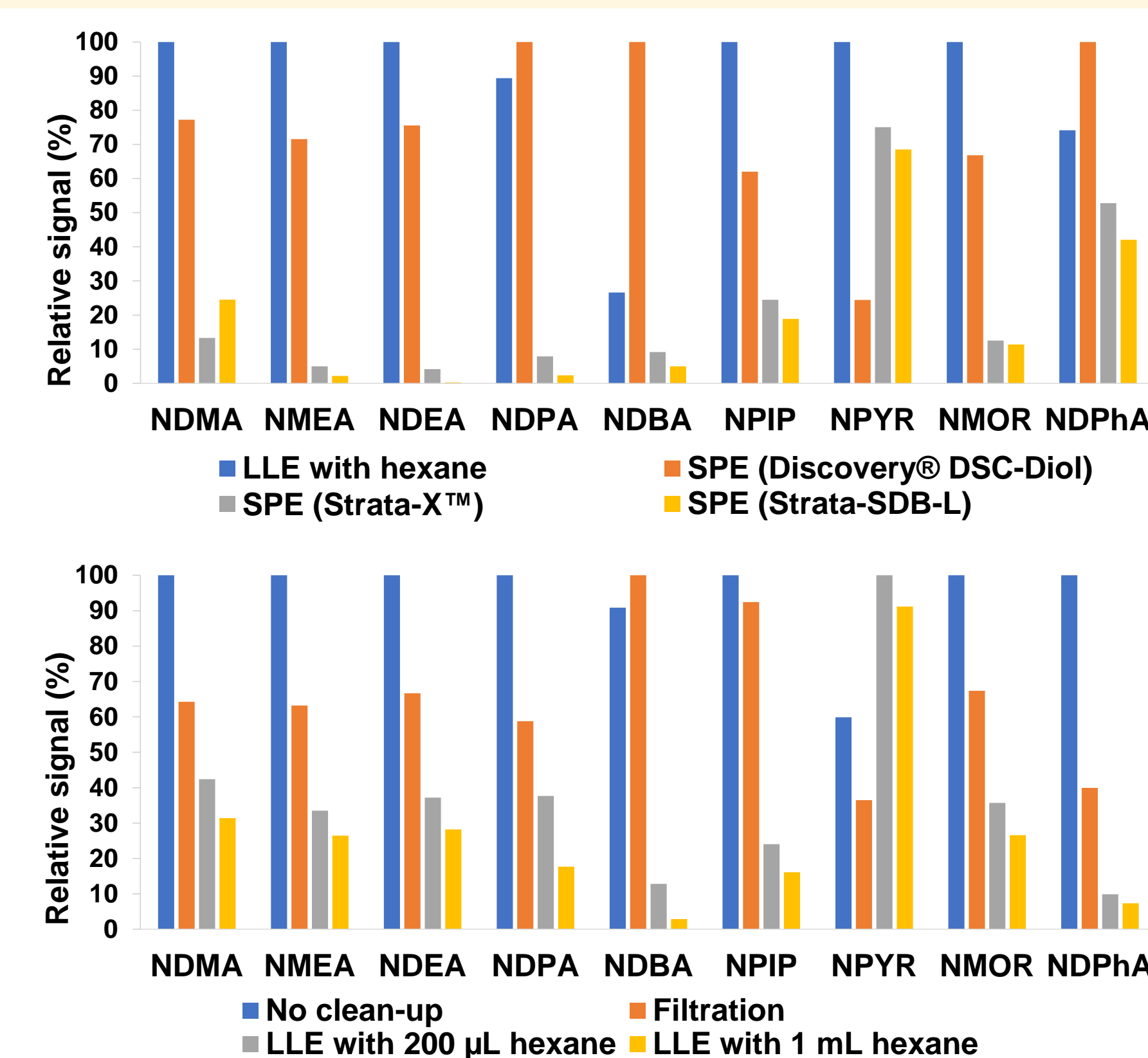


c) CHCl_3 Volume = 60 μL



When samples were analyzed, it was necessary a volume of CHCl_3 of 120 μL to obtain a clean drop after the extraction, easy to be collected and injected into the chromatographic system

Sample pretreatment studies



Figures of merit

Analytes	EF	MLOD ($\mu\text{g kg}^{-1}$)	MLOQ ($\mu\text{g kg}^{-1}$)	Repeatability (%RSD)					
				Intra-day (N = 5)			Inter-day (N = 5)		
				0.1 ng mL^{-1}	0.5 ng mL^{-1}	5 ng mL^{-1}	0.1 ng mL^{-1}	0.5 ng mL^{-1}	5 ng mL^{-1}
NDMA	2	7.4	24.8	n.a. ^e	6.7	3.3	n.a.	10.9	4.3
NMEA	18	9.2	30.5	n.a.	4.6	2.1	n.a.	7.2	9.1
NDEA	38	1.9	6.4	3.0	3.4	3.3	7.3	4.6	4.6
NDPA	100	0.4	1.5	4.6	1.1	4.5	6.0	7.6	12.4
NDDBA	73	0.02	0.06	6.0	4.4	3.7	6.6	8.4	12.5
NPIP	91	1.9	6.2	5.3	4.2	4.8	9.7	12.5	7.6
NPYR	25	3.3	11.1	7.4	2.2	3.8	6.1	11.1	5.2
NMOR	10	8.4	27.9	n.a.	2.0	2.7	n.a.	8.4	5.5
NDPhA	66	3.5	11.7	4.3	2.4	6.9	9.9	6.7	7.7

Analysis of cosmetic samples

Analytes	N-nitrosamines contents found applying the developed method		
	Found amount ($\mu\text{g kg}^{-1}$)		
	Aftersun gel	Body cream 1	Body cream 2
NDMA	< LOD	< LOD	< LOD
NMEA	770 \pm 90	< LOD	560 \pm 20
NDEA	< LOD	< LOD	< LOD
NDPA	1.19 \pm 0.01	< LOD	< LOD
NDDBA	16.5 \pm 0.1	< LOD	< LOD
NPIP	50.6 \pm 0.3	< LOD	< LOD
NPYR	114 \pm 5	< LOD	< LOD
NMOR	< LOD	870 \pm 60	< LOD
NDPhA	10.57 \pm 0.03	< LOD	< LOD

AGREEprep



Green sample preparation

CONCLUSIONS

- The proposed method is based on a reduced pretreatment of the sample, which consists of simple leaching of the analytes in water, followed by VA-DLLME and GC-MS analysis
- The variables involved in the microextraction stage were optimized, and comparative studies were carried out on the pretreatment of samples to find the best methodology that would allow the analysis of the greatest number of nitrosamines at the same time with the required sensitivity, favoring their extraction from the complex cosmetic matrices without losing analytes during the procedure
- The proposed method is suitable for the quality control of cosmetics in order to guarantee the safety of users and compliance with the European Regulation on cosmetic products [3]

REFERENCES

- Fernández-Alba, A. R., Agüera, A., Worsfold P., Townshend A., Poole C. (Eds.), Nitrosamines. Encyclopedia of Analytical Science. Second Edition, Elsevier, Amsterdam, 6 (2005) 197–202.
- Scientific Committee on Consumer Safety, Opinion on Nitrosamines and Secondary Amines in Cosmetic Products, 2012.
- Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on Cosmetic Products, and its Successive Amendments.

ACKNOWLEDGEMENTS

This article is based upon work from the National Thematic Network on Sample Treatment (RED-2018-102522-T) of the Spanish Ministry of Science, Innovation and Universities, and the Sample Preparation Study Group and Network supported by the Division of Analytical Chemistry of the European Chemical Society.

ONLINE VERSION

