A METHOD FOR THE DETERMINATION OF PAHs IN MASCARA

Manni A***, Corso A**, Baldassini M*, Croce G*, Dell'Unto E*, Donati P*, Guerranti G*, Lolini M*, Scarpi C*.

** ARPAT, Provincial Department of Florence, Via Ponte alle Mosse, 50144 Italy*
*** Trainee near ARPAT, Provincial Department of Florence,Via Ponte alle Mosse, 50144 Italy*
**** Dept. ICMA, University of Rome, Via Eudossiana,18, 00184 Rome, Italy*

Abstract
The aim of the present work is the presentation of an analytical methodology for the determination of PAHs in mascara. Most of the attention will be paid to the instrumental extraction and the purification by column of silica gel as the liquid-liquid extraction has been already detailed in Croce et al, 2005[3]. Each congener is identified and quantified individually by mass spectrometry. Analytical results for 22 different mascara sample will also be described.

Introduction
Face make up products are some of the most used cosmetics; eye make up – in particular mascara – are largely diffused. Basic components of mascara are different kind of waxes (between 30 and 40%), a triethanolamine soap (between 45% and 50%) and no soapable fat portions (elements) (5%)[1]. Pigment colours may vary from chestnut to brown, to black brown, to black; they are chosen mostly according to hair colour. In order to increase natural pigments covering power, small amounts of oil soluble colourants are often added[2]. Fat content in analyzed samples vary, by weight, between 30% and 50%.

Analytical Method
Analytical method is applied to a sample portion between 0,5 and 1,5 g, and it is based on two different sequential extractions. Final extract is cleaned up by a silica-gel chromatographic column, then analyzed by mass spectrometry. Extracting process procedures have been developed by using certified internal deuterated standards added.

Extraction of the fat portion from mascara
First extraction phase aims to recover the fat portion of the sample, which contains PAHs; the sample can be extracted alternatively by soxhlet extractor, fast solvent extraction and ultrasonic extraction. These three extraction techniques are monitored by recovering labelled congeners added to the sample. The comparison between these three extraction techniques shows that Soxhlet extractor did provide the best performance allowing better recovery of sample fat portions by weight.
Sample was put in a hexane pre-washed glass fiber or cellulose thimble together with 100uL of a labelled mix standard (tab.2) (Chiron, Norway). As suitable extraction solvent 90% penthane/10% acetone mix was used.
The resulting organic extract was concentrated at 15ml by bubbling of N_2, then was transferred in a 100ml split-funnel where was extracted four times with dimethylsulfoxide (DMSO). The two phases were constituted by penthane, the lighter and DMSO, the heavier; At the interface besides the two, during the cooling, even at ambient temperature of the extract, a fat portions thicken.
Each DMSO sample was than transferred to another 250ml split funnel, paying attention they were clear. If an emulsion take place, they were resolved by stirring lightly with a glass stick or with a flame closed capillar. If the emulsion was not resolved, it was than transferred in a glass which has to undergo a 15 minutes ultrasonic bath; in this way any emulsion was completely disappeared. 100 ml of cold (4 °C) distilled water is added to DMSO as the reaction between water and DMSO is slightly exothermic; water and DMSO become a lonely phase. Then, four aqueous phase extractions with cyclohexane were performed: 50 ml of cyclohexane were added and the split funnel was strongly stirred for 20 seconds. As the aqueous phase has a higher density, it stays in the lower part of the split funnel, while organic phase stays in the upper part. Lower phase was transferred to another 250ml split funnel, where 50 ml of cyclohexane are newly added. Then it was re-extracted. The cyclohexane used in each of the four extraction was collected in a 500 ml split funnel, then washed with 100ml of distilled water. Water phase is discharged; the organic phase is filtered over an anhydrous Na_2SO_4 layer, then concentrated to 1 ml and purified over a silica gel column.

Purification of the extract

The silica used for the purification needs to be activated one night long in oven at 130 °C, then cooled in an drier (containing P_20_5); It was then transferred in a glass ball and finally hexane was added until the silica was completely covered. The glass ball is applied to a rotating evaporator until hexane evaporation begins, in order to take out the air between the silica particles. Silica and hexane suspension is loaded in the glass column, 2 cm internal diameter fitted and with a tap; some glass wool is putted over the tap, so that the hexane-silica suspension lays on it, reaching 11 cm height. An at least 2 cm layer of Na_2SO_4 anhydrous is putted on it; then the column undergoes a 60 ml of hexane pre-washing. The resulting organic extract, concentrated to 1 ml, is then transferred to the column head and eluted as follows:

- 40 ml hexane (pre-eluate),
- 40 ml hexane (1^{st} eluate),
- 80 ml hexane/CH_2Cl_2 at 50% w/w (2^{nd} eluate) containing PAHs analytes.

The eluate was then put into a neck buffed flask, concentrated with rotavapor and transferred (hexane) in a 4 ml vial. The washings (hexane) were added to it. After evaporation of hexane, by bubbling of N_2, the eluate was reconstituted with syringe standards (tab. 3) (Chiron, Norway) and analyzed.

Instrumental analysis

IPA identification and quantification were carried out by GC instrument (model 6890, Hewlett Packard, USA) with a DB-17 ms (or equivalent) gaschromatographic column, coupled with a quadrupolar mass spectrometer (model 5793, Hewlett Packard, USA). SIM acquisition mode was performed. After the verification of the correct working conditions of the instrument, 1 µl of an external IPA referring mixture "RRF IPA" was injected (Tab.4) (Chiron, Norway). The product injected appear in the areas that are used to calculate relative answer factors, retention times and relative abundance ratio between monitored fragments in order to identify the resulting congeners peaks. Moreover, the injection gives information about chromatographic GC column efficiency. Finally, 1 µl of purified sample extract was injected; the quantification of the congeners were carried out according to the isotopic dilution method principles.

Conclusions

Deuterated recovery percentage were normally over 70% (Fig.1). Chromatograms resulted to be mainly free from interferences; it was a critical factor as mascara is an complex matrix. The positive results reached did allowed to be extremely precise in peaks identification and quantification. This consideration do overcome any problem due to long manual extraction (liquid – liquid) and purification procedure.

An other positive result was obtained with the demonstration that the gascromatographic column used clearly separates different benzo(x)fluoranthenes (where x = b,k,j) and benzo[e]pyrene from benzo[a]pyrene peaks avoiding any mis-interpretation of the results, as shown in figure 3.

Analytical results from 22 sample are reported in figure 2; it shows the PAH's total amount for each single analysed sample. Their average concentration is between 200-400 ng/g.

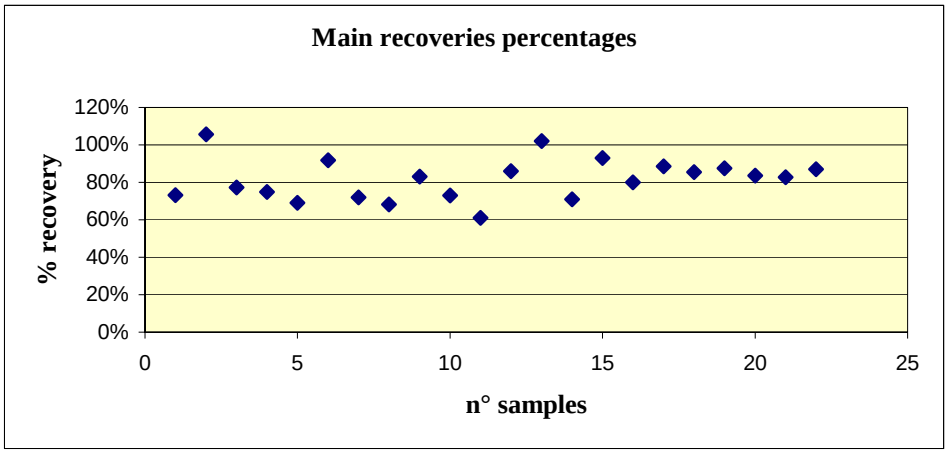

Figure 1 – Recoveries results.

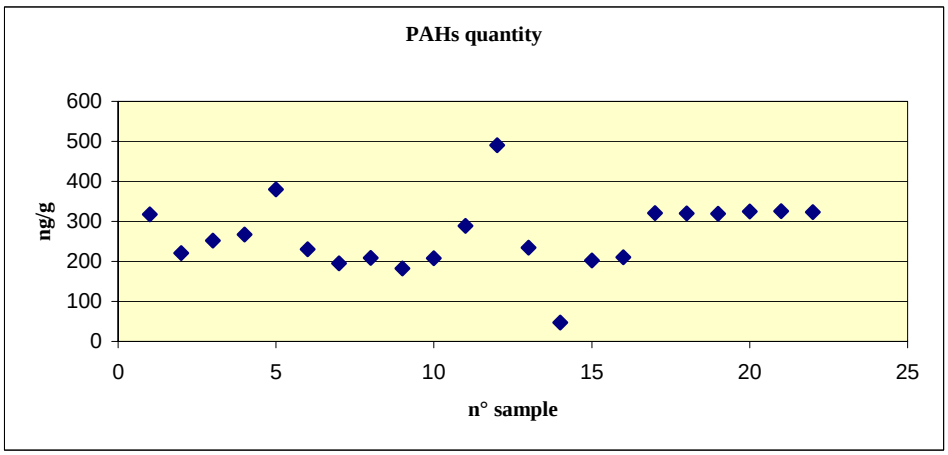

Figure 2 – Samples results, PAHs quantity.

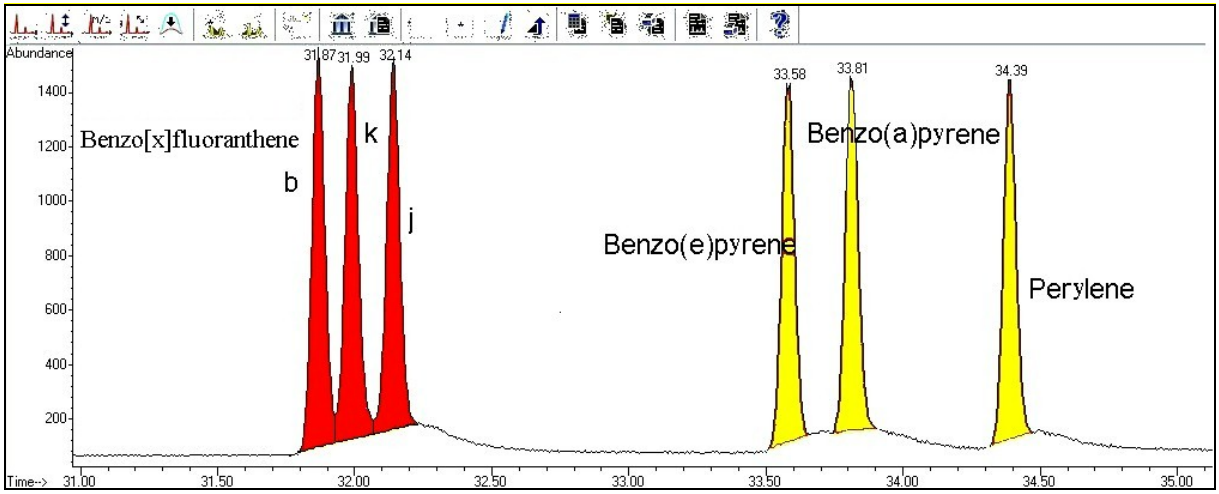

Figure 3 – Benzo[x=b,k,j]fluoranthene separation using the proposed column

Table 1. "Extraction standards"

Acenaphthene-d10
Phenanthrene-d10
Fluoranthene-d10
Benzo[a]anthracene-d12
Benzo[a]pyrene-d12
Dibenzo[a,h]anthracene-d14
Dibenzo[a,i]pyrene-d14

Table 2. "Syringe standards"

Acenaphthylene-d8
Chrysene-d12
Indeno[1,2,3-cd]pyrene-d12

Table 4. Native (RRF IPA) and labelled mixes with the reference mass for each PAH

MR	Nativi	MR	STD estrazione	MR	STD siringa
128	Naphthalene				
152	Acenaphthylene			160	Acenaphthylene-d8
154	Acenaphthene	164	Acenaphthene-d10		
166	Fluorene				
178	Phenanthrene	188	Phenanthrene-d10		
178	Antracene				
202	Fluoranthene	212	Fluoranthene-d10		
202	Pyrene				
228	Benzo[a]anthracene	240	Benzo[a]anthracene-d12		
228	Chrysene			240	Chrysene-d12
252	Benzo[b]fluoranthene				
252	Benzo[k]fluoranthene				
252	Benzo[j]fluoranthene				
252	Benzo[e]pyrene				
252	Benzo[a]pyrene	264	Benzo[a]pyrene-d12		
252	Terilene				
276	Indeno[1,2,3-cd]pyrene			288	Indeno[1,2,3-cd]pyrene-d12
278	Dibenzo[a,h]anthracene	292	Dibenzo[a,h]anthracene-d14		
276	Benzo[ghi]perilene				
302	Dibenzo[a,l]pyrene				
302	Dibenzo[a,e]pyrene				
302	Dibenzo[a,i]pyrene	316	Dibenzo[a,i]pyrene-d14		
302	Dibenzo[a,h]pyrene				

References

1. Cosmetic a toiletry formulations, Ernest W. Flick, 1992, Noyes Pubblications, Park Ridge, N.J., USA
2. Turco A, 1990. *Nuovissimo ricettario chimico. Hoepli editore (3° ed.), MI, pp. 844-845.*
3. Croce G, Donati P, Lolini M, Alfani A, Baldassini M, Guerranti G, Marsico AM, (2005). *Bollettino die Chimici Igienisti volume. 56, pp 17-22.*