# SIMULTANEOUS DETERMINATION OF BENZOPHENONE-3, VITAMIN A AND VITAMIN A ACETATE IN PIG SKIN BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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## AIM OF THE WORK

Benzophenone-3 is a broad-band UV filter widely used in sunscreen preparations in concentration of up to 10% in the EU and up to 6% in the USA, alone or in combination with other UV filters. It can also be used in cosmetic products to ensure stability (concentration range between 0.05-0.5%) and in day creams and lipsticks to prevent premature ageing in particular in association with retinoids.

The aim of this work was to develop and validate an analytical method for the simultaneous determination of benzophenone-3, retinol and retinyl acetate in pig ear skin layers. Pig ear skin was used because it is a well accepted model for human skin in percutaneous penetration experiments in vitro.

## METHODS

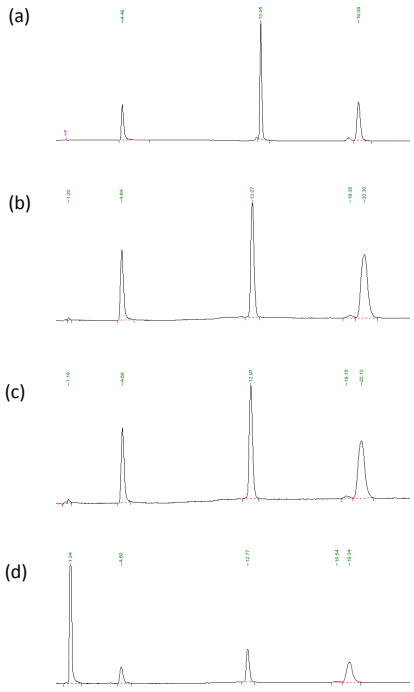
### Skin samples preparation

Full thickness skin was excised from the outer region of pig ear and separated from the underlying cartilage with a surgical blade.
Stratum corneum samples were obtained with the stripping technique. A piece of adhesive tape was applied to the skin surface for 10 seconds under light pressure and then removed. After stripping 16 times, the skin was submitted to a heat-separation process. The skin was heated at 50°C with an hair dryer for 30 seconds and then the epidermis was separated form dermis by scraping with a scalpel. Tape strips, epidermis and dermis were placed in individual plastic test tubes and extracted.

### HPLC conditions

Column: Novapak® C18
UV detection @ 325 nm
Flow rate: 1 ml/min
Mobile phase: Acetonitrile (Solvent A)
Water containing 1% Acetic acid (solvent B)
Gradient: 0-7 min A-B (60:40, v/v)
7-10 min linear change to A-B (85:15, v/v)
22-25 min linear change to initial conditions

## RESULTS




Chromatograms of standard solution (5 µg/ml of BP3, R and RA) in PBS pH 7.4 containing 5% of DMβCB (a) and extracts from skin strips (b), epidermis (c) and dermis (d).

**Extraction conditions tested and recovery of BP3, R and RA from pig stratum corneum, epidermis and dermis (mean value±SD).**

| | Solvent[a] | Temperature °C | Time min | BP3 Recovery (%) | R Recovery (%) | RA Recovery (%) |
|---|---|---|---|---|---|---|
| ***Stratum corneum*** | | | | | | |
| | Methanol | Ambient | 30 | 90.90±2.16 | 89.53±3.29 | 85.02±3.61 |
| | Methanol | Ambient | 60 | 95.29±5.94 | 100.33±2.53 | 97.01±1.86 |
| ***Epidermis*** | | | | | | |
| | Methanol | 45 | 60 | 95.97±5.12 | 95.18±3.46 | 94.06±4.14 |
| ***Dermis*** | | | | | | |
| | Acetone | Ambient | 30 | 76.44±0.70 | 87.73±3.47 | 92.13±5.95 |
| | Acetone | 45 | 60 | 97.56±4.09 | 97.32±3.51 | 99.49±3.67 |

[a] 1 ml

**Recovery of BP3, R and RA from pig stratum corneum, epidermis and dermis (mean value±SD).**

| Compound | Amount Added (µg) | Strips Recovery (%) | Strips CV (%) | Epidermis Recovery (%) | Epidermis CV (%) | Dermis Recovery (%) | Dermis CV (%) |
|---|---|---|---|---|---|---|---|
| **BP3** | 0.4 | 98.87 | 5.66 | 102.77 | 5.98 | 101.90 | 5.95 |
| | 2.0 | 102.76 | 1.71 | 99.64 | 3.01 | 95.43 | 4.54 |
| | 5.0 | 91.60 | 8.63 | 97.29 | 3.89 | 97.57 | 4.19 |
| | | y=0.914x+0.062 (R=0.99875) | | y=0.969x+0.027 (R=0.99994) | | y=0.972x+0.006 (R=0.99991) | |
| **R** | 0.4 | 92.14 | 8.18 | 98.14 | 3.94 | 102.34 | 8.94 |
| | 2.0 | 97.44 | 3.83 | 95.49 | 3.94 | 92.60 | 6.26 |
| | 5.0 | 95.07 | 9.56 | 95.96 | 3.63 | 97.40 | 3.61 |
| | | y=0.953x+0.005 (R=0.99991) | | y=0.955x+0.014 (R=0.99996) | | y=0.970x+0.025 (R=0.99926) | |
| **RA** | 0.4 | 100.51 | 6.33 | 102.99 | 3.19 | 101.62 | 10.31 |
| | 2.0 | 97.34 | 6.18 | 96.98 | 3.76 | 93.77 | 5.52 |
| | 5.0 | 97.07 | 1.92 | 94.61 | 3.87 | 99.40 | 3.69 |
| | | y=0.903x+0.046 (R=0.99935) | | y=0.944x+0.026 (R=0.99991) | | y=0.989x+0.010 (R=0.99959) | |

**Data for calibration curves, precision and accuracy of HPLC analysis (mean values±SD).**

| Compound | Nominal value (µg/ml) | Fitted value (µg/ml)[a] | RE %[b] | RSD%[c] | Regression equation | Correlation coefficient |
|---|---|---|---|---|---|---|
| **BP3** | 0.052 | 0.056±0.002 | 8.12 | 3.10 | y=23313x+215.9 | 0.9998 |
| | 0.23 | 0.23±0.01 | 1.91 | 2.35 | | |
| | 2.33 | 2.33±0.04 | 1.22 | 1.68 | | |
| **R** | 0.058 | 0.057±0.003 | 3.81 | 5.82 | y=65597x-793.1 | 0.9997 |
| | 0.22 | 0.22±0.003 | 0.83 | 1.34 | | |
| | 2.21 | 2.21±0.05 | 1.58 | 2.10 | | |
| **RA** | 0.045 | 0.044±0.001 | 2.18 | 2.46 | y=25121x+101.7 | 0.9999 |
| | 0.22 | 0.21±0.003 | 4.68 | 1.55 | | |
| | 2.20 | 2.20±0.05 | 1.80 | 2.36 | | |

[a] Obtained from regression equation
[b] Relative error
[c] Relative standard deviation

The stock solution was prepared by dissolving weighted amount of BP3, R and RA in methanol. Five working solutions were obtained with appropriate dilution of the stock solution with phosphate buffer pH 7.4 containing 5% (w/w) of DMβCD. This solution was used as receptor phase in in-vitro permeation studies.

## CONCLUSIONS

The method proposed is suitable for the determination of BP3, R and RA in pig skin layers and in percutanoeus penetration samples.
The method developed and validated is relatively rapid and simple and showed good linearity, precision, accuracy and sensitivity. To further improve sensitivity, HPLC system equipped with diode array detector could be used to analyze BP3 at 285 nm and retinoids at 325 nm in the same run.