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No. 122, March 2019
CAMAG Bibliography Service
Planar Chromatography
Edited by Gertrud Morlock
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published by CAMAG Switzerland

IN THIS ISSUE

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Printed on FSC paper

Validated method for fast quantification of glycine in cosmetics

Caroline Petitti

The main activity of the analytical laboratory at the Development Center of Bayer Healthcare, Gaillard, France, is to develop, optimize and validate methods for analysis of new Over The Counter products in the field of nutritionals and skin care brands, such as Berocca® and Bepanthen®. Caroline Petitti has previously reported on the quantification of amino-propanol in dermatological products (CBS 98, 2007, 2–4). These methods are transferred to the quality control laboratories at production sites all over the world, as has the following HPTLC method to a German production site. For quantification, HPLC is the most used technique; however, HPTLC is the top choice for QC laboratories already equipped with HPTLC.

Introduction

The goal of this study was to develop a new method for the quantification of glycine at a low quantity (<1% assay) in a gel in oil formula. As an amino acid, glycine is a small polar molecule, which makes its analysis difficult for RP-HPLC due to low sensitivity and retention.

Hence, HPTLC had been considered due to its decisive advantages. HPTLC is accurate, precise and reproducible, as proven by the method validation. The sample preparation is simple and the development time is short (4 min). The derivatization agent is simply included in the mobile phase (advantageous for safety), and the costs of analysis are low due to low solvent consumption and parallel analysis of 12 samples on one HPTLC plate.

Standard solution

Aqueous glycine solution (0.25 mg/mL)

Sample preparation

Liquid-liquid extraction of the sample (600 mg) with dichloromethane – water 1:2.5 (35 mL); aqueous phase was taken

Chromatogram layer

HPTLC plates silica gel 60 F_{254} (Merck), 20 × 10 cm, prewashed with methanol, followed by drying on the TLC Plate Heater at 80 °C for 15 min

Sample application

Automatic TLC Sampler (ATS 4), bandwise application, band length 8.0 mm, delivery speed 50 nL/s, application volumes 2.0 µL for sample solutions and 1.0 to 2.0 µL for standard solution (250 to 500 ng/band; note that this narrow calibration range is justified due to the targeted known sample content)

Chromatography

In the Twin Trough Chamber (saturated with mobile phase) with 0.5% (m/V) ninhydrin (derivatization reagent added to the mobile phase) in ethanol – water – glacial acetic acid 14:5:1 up to 2 cm

Postchromatographic plate treatment for derivatization

Drying on the TLC Plate Heater at 100 °C for 2 min until orange-violet zones appear at hR_F 50

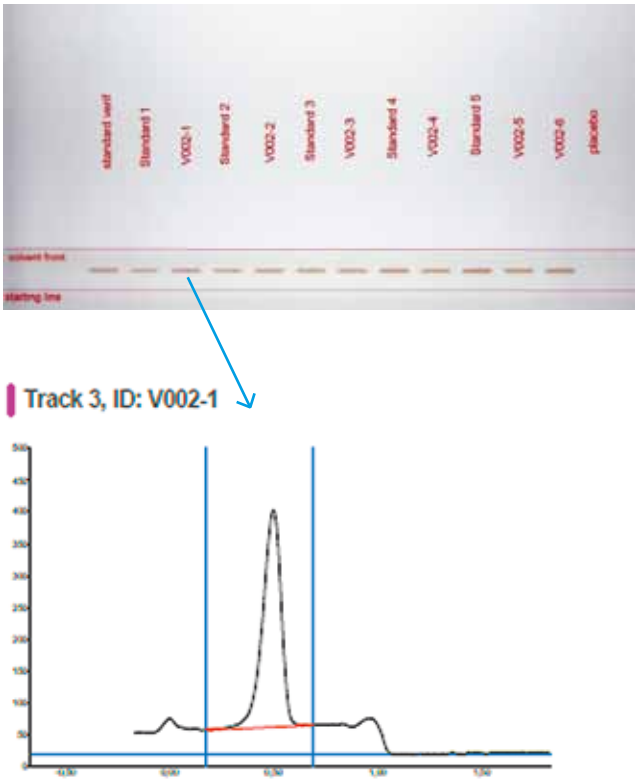
Densitometry

Absorbance measurement at 386 nm with TLC Scanner 3 and winCATS

Results and discussion

The developed method is fast and specific. It takes only 4 min for separation of up to 12 samples in parallel. The method's specificity was verified by applying 2.0 µL of a prepared placebo sample (last track). As no colored zone appeared, the method was proven to be specific (specific derivatization).

The repeatability of the method was checked by applying 6 preparations of the same sample, resulting in a relative standard deviation of 1.5%, proving a good precision. The analytical response was demonstrated by applying 5 different levels of the glycine solution (50 to 150% of the targeted sample content), whereby each level was prepared twice. For the resulting linear regression, the coefficient of correlation was ≥0.990 (sdv 3%) and the axial intercept was close to 0 (-1.6%), which proved that the sample did not degrade. A Michaelis Menten 1 function showed the best performance as calibration curve (sdv 0.6%). The mean recovery



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HPTLC-Vis chromatogram of cosmetic samples, standards and placebo as well as densitogram at 386 nm as example

was 100% showing the high accuracy of the method. The intermediate precision was verified by analyzing the same batch on 2 different days by 2 different analysts with different reagents and plate batches. Each analyst prepared the sample 6 times. The resulting intermediate precision (*%RSD*) was 2.5%. As the relative standard deviation of each series corresponded to the acceptance criteria, the method was considered to be precise.

Further information is available on request from the author.

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