Cent. Eur. J. Chem. • 11(2) • 2013 • 133-136
DOI: 10.2478/s11532-012-0151-x

VERSITA

Central European Journal of **Chemistry**

Quantification of sunscreen 2-phenylbenzimidazole-5-sulfonic acid in cosmetic products and water samples by HPTLC/densitometry with fluorescent detection

Short Communication

Anna W. Sobanska*, Karolina Derecka, Jaroslaw Pyzowski

Department of Analytical Chemistry,
Medical University of Lodz, 90-151 Lodz, Poland

Received 6 July 2012; Accepted 28 September 2012

Abstract: The water-soluble sunscreen 2-phenylbenzimidazole-5-sulfonic acid (PBS) was quantified in a sun-care product and water samples by thin layer chromatography followed by densitometric scanning in fluorescence mode (cut-off filter 370 nm, wavelength of excitation – 300 nm). Normal phase TLC was performed on silica gel 60 as stationary phase. Mobile phase used was ethyl acetate-ethanol-water 70:35:30 (v/v/v). The limit of detection (LOD) was 0.0004 μg spot^{-1}, and the limit of quantification (LOQ) was – 0.001 μg spot^{-1} without any sample pre-concentration.

Keywords: *Sunscreen • Fluorescence • Thin-layer chromatography • Densitometry*

1. Introduction

2-Phenylbenzimidazole-5-sulfonic acid (PBS) is a water-soluble sunscreen used in sun-protection cosmetics such as creams, lotions or sprays. Its quantification in cosmetic formulations was achieved by reversed-phase high-performance liquid chromatography [1], UV spectrophotometry [2,3], micellar electrokinetic chromatography [4] or microemulsion electrokinetic chromatography [5,6]. Trace amounts of PBS in biological samples or receptor fluids used in membrane penetration experiments conducted in Franz diffusion cells were quantified by reversed-phase ion-interaction chromatography [7], sequential injection fluorimetry [8] or liquid chromatography [9]. Analysis of PBS in environmental samples (water, sewage sludge) was achieved by liquid chromatography-tandem mass spectrometry [10] or ultra high performance liquid chromatography-tandem mass spectrometry [11]. PBS was traced *in vitro* on *stratum corneum* by ATR-FTIR spectroscopy [9]. Our earlier research was concerned with quantification of PBS in cosmetic formulations in the presence of oil-soluble UV filters by multiple-development normal-phase thin layer chromatography followed by densitometry in absorption mode [12]. Despite interesting photochemistry and photophysics of PBS (pH-dependent fluorescence) [13] very little attention has been paid so far to the analytical applications of PBS fluorescent properties. Apart from the procedure described in [8] in all other methods of PBS quantification (including our earlier work [12]), detection was based on UV absorption [1-7,9,12] or mass spectrometry [10,11].

This paper is focused on the applicability of TLC-densitometry with fluorescent detection to the analysis of PBS in sun-care cosmetic products and in tap water used, *e.g.* for cleaning of manufacturing equipment in the cosmetic industry.

2. Experimental procedure

2.1. Chemicals, materials and solutions

Cosmetic quality 2-phenylbenzimidazole-5-sulfonic acid (Eusolex 232, $C_{13}H_{10}N_2O_3S$), purity > 98% was kindly

* *E-mail: anna.sobanska@umed.lodz.pl*

donated by Merck. Analytical grade ethanol, methanol, isopropanol, dichloromethane and ethyl acetate were from Chempur, Poland. Blank cosmetic cream (o/w emulsion) preserved with parabens and phenoxyethanol and a commercial sun care cream preserved with parabens and phenoxyethanol, containing PBS and oil soluble UV filters (avobenzone, AVO and octyl salicylate, OS) used throughout this study were both from Beiersdorf. Eusolex 232 (500 mg) was weighted accurately into a 100-mL volumetric flask and dissolved in deionized water alkalized with NaOH aq (2 mol L^{-1}, 0.9 mL). Deionized water was added to volume. The stock solution of PBS (prepared separately for each final concentration of the standard solution) was diluted with deionized water to prepare standard solutions containing the following concentrations of PBS: 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 20.0, 40.0, 60.0, 80.0 and 100.0 mg L^{-1}. All flasks were wrapped with aluminum foil and kept refrigerated.

2.2. Sample preparation

2.2.1. PBS residues in tap water

A commercial sun care cream containing PBS, AVO and OS (50 mg) was weighted accurately into a 500-mL volumetric flask. Sodium lauryl sulfate (SLS)-based, commercially available dishwashing liquid (ca. 200 mg) was added and the flask was filled to volume with tap water, vigorously shaken and left to stand until the foam disappeared.

2.2.2. PBS in sun-care cream

The same commercial sun-care cream (50 mg) was weighted accurately into a 50 mL volumetric flask, isopropanol (ca. 20 mL) was added and the flask was vigorously shaken with the Universal Shaker type 327, Premed, Poland (60 min.). The flask was filled to volume with isopropanol and left to stand.

Samples of the blank cosmetic cream and those of the blank cream spiked with PBS (1, 3 and 6% PBS w/w; each sample was also spiked with AVO (1% w/w) and OS (1% w/w)) were processed in the similar manner (amounts of spiked creams per 500 mL of tap water varied). All flasks were wrapped with aluminum foil and kept refrigerated.

2.3. Thin layer chromatography

Thin layer chromatography was performed on non-activated 10×20 cm HPTLC silica gel 60 plates (layer thickness 0.2 mm) from Merck. Plates were pre-washed with methanol-dichloromethane 1:1 (v/v), dried overnight in ambient conditions and spotted with the Desaga AS 30 sampler equipped with a 10 µL syringe (1 µL $spot^{-1}$ of PBS standard solutions, standards concentrations according to 2.1.), spot-wise, 15 mm from the bottom edge, at 10 mm intervals, starting 10 mm from the plate edge. Plates were developed with ethyl acetate- ethanol-water 70:35:30 (v/v/v) in a vertical chromatographic chamber lined with filter paper and previously saturated with the appropriate mobile phase vapor for 20 min. The development distance was 75 mm from the plate bottom edge. After development, plates were dried at room temperature (20°C), scanned and analyzed in fluorescence mode with the Desaga CD 60 densitometer (cut-off filter 370 nm, wavelength of excitation 300 nm, Mercury lamp). Typical densitograms obtained for PBS as described above are shown in Supplementary Fig. 1

2.4. Quantification of PBS in surfactant-containing water samples and in the commercial cosmetic formulation

The sun-care cream solution in tap water, prepared as described above (Section 2.2), was spotted on silica gel 60 HPTLC plates (1 µL $spot^{-1}$) and chromatographed as described in Section 2.3. The isopropanol extract of the commercial sun-care cream prepared according to Section 2.2 was spotted on silica gel 60 HPTLC plates (1 µL $spot^{-1}$) and chromatographed in the same manner. All samples were spotted in triplicate.

3. Results and discussion

3.1. Method development

According to our earlier research [12,14] water soluble UV filters such as PBS can be chromatographed on silica gel 60 with binary or tertiary aqueous mobile phases to achieve R_f values suitable for densitometric scanning (typically 0.2-0.8). Two mobile phases: acetonitrile-water 9:1 (v/v) and ethyl acetate-ethanol-water 70:35:30 (v/v/v) were selected after preliminary investigations [14] as they were equally effective (both separated PBS from preservatives and oil-soluble UV filters commonly used in sun-care products [12,14] and gave R_f values for PBS 0.80 or 0.82, respectively). At this stage it was decided that chromatographic plates should be developed with the less toxic mobile phase ethyl acetate-ethanol-water 70:35:30 (v/v/v) [12].

The wavelength of excitation and the cut-off filter suitable for PBS were selected on the basis of multiwavelength scanning of chromatograms from 200 to 550 nm at 5 nm intervals (cut-off filters tested during this study – 370, 420, 450 and 550 nm): cut-off filter 370, wavelength of excitation 300 nm. The resulting densitograms are show in Supplementary Figs. 2-5.

3.2. Method validation

3.2.1. Specificity

Purity of PBS peaks obtained during the analysis of the sun-care cream and tap water containing residues of the sun-care cream was confirmed by spectra of PBS acquired directly from chromatographic plates in fluorescence mode (cut-off filter 370 nm). Spectra collected at two different points of particular peaks obtained for the sample solution were identical with the spectrum acquired for PBS standard which was a sufficient proof of sample peak purity (Supplementary Fig. 6).

3.2.2. Calibration

Unlike in the case of spectroscopy of solutions, in TLC/densitometry in reflectance mode Lambert-Beer's law does not necessarily have to be observed so calibration plots generated in densitometric experiments are not perfectly linear ([15] and some references cited therein). This refers to densitometry with UV/VIS absorption detection, but there is no reason why non-linear calibration should not be used for fluorescence detection as well.

A linear and a non-linear (second-degree polynomial) calibration plots were obtained by plotting peak areas against the amount of PBS in the range 0.001 – 0.1 µg spot^{-1}:

$y = 46743\ x + 534.8$ R = 0.9874, n = 11

$y = -304782\ x^2 + 74911\ x + 302.9$ R = 0.9994, n = 11

The plots quality was compared by means of their R values and non-numerical analysis of residuals according to [15]. Residuals for both calibration models are compared in Supplementary Fig. 7. The quadratic plot was found superior – its R value was much higher, and the residuals were lower and did not show any trend compared to those obtained for the linear calibration function.

3.2.3. Precision

Repeatability of the method was tested according to [15,16] by replicating the entire method on the same day, using the same commercially available cosmetic preparation, batches of solvents and chromatographic plates, by the same analyst (Day 1, Analysis A and B). Intermediate precision was verified according to [15,16] by repeating the procedure on the same cosmetic preparation but on a different day, by different analysts, using other batches of solvents and chromatographic plates (Day 2). The results of these experiments (Tables 1 and 2) prove that the method precision is sufficient for the analysis of sun-care preparations and water samples containing PBS.

Table 1. Repeatability and intermediate precision tests for quantification of residual PBS in tap water samples containing SLS (n=3). Cream content in tap water – 50 mg per 500 mL, application volume – 1 µL spot^{-1}.

	Day 1, Analyst 1		Day 2
	Analysis A	Analysis B	Analyst 2
Received [µg spot^{-1}]	0.0039	0.0040	0.0040
RSD [%]	6.57	6.36	3.71

Table 2. Repeatability and intermediate precision tests for quantification of PBS in the sun-care cream (n=3). Cream samples extracted with isopropanol (50 mg per 50 mL^{-1}), application volume – 1 µL spot^{-1}.

	Day 1, Analyst 1		Day 2
	Analysis A	Analysis B	Analyst 2
Received [µg spot^{-1}]	0.042	0.041	0.043
PBS content in cream [%]	4.2	4.1	4.3
RSD [%]	4.64	3.80	2.70

3.2.4. Limits of detection and quantification

The limits of detection and quantification for PBS were determined experimentally by calculating the signal-to-noise ratio according to [17]: LOD – 0.0004 µg spot^{-1}; LOQ – 0.001 µg spot^{-1}. Densitograms for PBS concentrations covering the concentration range slightly below and above LOD and LOQ are presented in Supplementary Fig. 8.

3.2.5. Accuracy

Blank cosmetic cream was spiked with PBS at three concentrations: 1, 3 and 6% w/w. Each sample was also spiked with AVO (1% w/w) and OS (1% w/w). Samples were processed according to Section 2.2. The chromatographic procedure described in Section 2.3 was performed on the samples and the recoveries are presented in Tables 3 and 4. The results of recovery tests are sufficiently good to accept the proposed procedure for PBS quantification in sun-care preparations and in water samples containing residues of such preparations.

3.2.6. Storage and stability of standard solutions

Standard solutions of PBS were refrigerated between the experiments, and not exposed to light except for time needed for plates spotting. The stability of PBS stock solutions was monitored by UV/VIS spectroscopy over the period of 2 weeks [12]. Spectra acquired before and after this period were identical.

Table 3. Recovery tests (n=3) for residual PBS in tap water samples containing SLS. Application volume – 1 μL spot^{-1}.

PBS content in cream [%]	Cream content in water samples (mg per 500 mL)	Expected [μg spot^{-1}]	Received [μg spot^{-1}]	Recovery [%]	RSD [%]
1.0	90	0.0018	0.0017	94.4	5.78
3.0	45	0.0027	0.0027	100.0	4.96
6.0	35	0.0042	0.0040	95.2	6.36

Table 4. Recovery tests for quantification of PBS in sun-care creams (n=3). Cream samples extracted with isopropanol (50 mg per 50 mL^{-1}), application volume – 1 μL spot^{-1}.

PBS content in cream [%]	Expected [μg spot^{-1}]	Received [μg spot^{-1}]	Recovery [%]	RSD [%]
1.0	0.010	0.010	100.0	2.66
3.0	0.030	0.030	100.0	2.59
6.0	0.060	0.064	106.7	5.40

3.3. Discussion of results

The results of method validation were considered acceptable. The method was found effective and very cost-efficient as well as relatively environment-friendly (involving un-modified silica gel 60 chromatographic plates and solvents such as isopropanol, ethanol, ethyl acetate and water). The detection limit of the procedure without any pre-concentration step is by ca. 2 orders of magnitude lower than that of the procedure based on densitometric scanning in absorption mode [12].

4. Conclusion

PBS may be effectively detected and quantified in cosmetic creams by NP HPTLC on silica gel 60 by densitometric scanning in fluorescent mode at 300 nm (cut-off filter 370 nm). The same method may be applied to rapid quantification of PBS residues in tap water containing surfactants and used for washing of laboratory and manufacturing equipment in a cosmetic lab or plant. Substances present in tap water and typical components of cosmetic creams do not interfere.

Acknowledgements

This work was supported by an internal grant from the Medical University of Lodz, Poland (no. 503(3-016-03)503-01.

Thanks are due to Merck for free samples of sunscreens used through this investigation.

References

[1] A. Salvador, A. Chisvert, Anal. Chim. Acta 537, 1 (2005)
[2] A. Chisvert, M.T. Vidal, A. Salvador, Anal. Chim. Acta 464, 295 (2002)
[3] J.S. Azevedo, N.S. Viana Jr, C.D. Vianna Soares, Il Farmaco 54, 573 (1999)
[4] P.G. Pietta, A. Bruno, P.L. Mauri, C. Gardana, R. Maffei-Facino, M. Carini, J. Pharm. Biomed. Anal. 13, 229 (1995)
[5] C.W. Kampfl, T. Leitner, J. Sep. Sci. 26, 1259 (2003)
[6] C.W. Klampf, T. Leitner, E.F. Hilder, Electrophoresis 23, 2424 (2002)
[7] Z. Leon, A. Balaguer, A. Chisvert, A. Salvador, M. Herraez, O. Diez, Anal. Bioanal. Chem. 391, 859 (2008)
[8] M.T. Vidal, A. Chisvert, A. Salvador, Talanta 59, 591 (2003)
[9] G. Yener, J. Hadgraft, W.J. Pugh, Acta Pharm. Turcica 40, 27 (1998)
[10] R. Rodil, J.B. Quintana, P. Lopez-Mahia, S. Muniategui-Lorenzo, D. Prada-Rodriguez, Anal. Chem. 80, 1307 (2008)
[11] A. Nieto, F. Borrull, R.M. Marce, E. Pocurull, J. Chromatogr. A 1216, 5619 (2009)
[12] A.W. Sobanska, J.Pyzowski, J. Planar Chromatogr. 25, 344 (2012)
[13] J.J. Inbaraj, P. Bilski, C.F. Chignell, Photochem. Photobiol. 75, 107 (2002)
[14] A.W. Sobanska, E. Brzezinska, Acta Pol. Pharm. 69, 791 (2012)
[15] K. Ferenczi-Fodor, B. Renger, Z. Vegh, J. Planar Chromatogr. 23, 173 (2010)
[16] ICH Harmonized Tripartite Guideline: Validation of Analytical Procedures: Text and Methodology, Q2 (R1) (Geneva, 2005) http://www.ich.org
[17] P. Konieczko, J. Namiesnik (Eds.), Ocena i kontrola jakości wyników pomiarów analitycznych (Evaluation and quality control of analytical results) (Wydawnictwo Naukowo-Techniczne, Warszawa 2007) pp. 269-288 (In Polish)