Rapid HPTLC Quantification of *p*-Aminobenzoic Acid in Complex Pharmaceutical Preparations

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Key Words

***p*-Aminobenzoic acid**
Determination
HPTLC
Spectrodensitometry
***Bratton–Marshall* reaction**
ChromaJet DS20

Summary

A particularly rapid HPTLC method has been established for chromatographic separation and quantification of *p*-aminobenzoic acid (PABA) in complex dietary supplement tablets. After chromatography, PABA was determined by spectrodensitometry at 270 nm. PABA spots were then visualized by a novel staining procedure involving the *Bratton–Marshall* coupling reaction after spraying with 8-hydroxyquinoline in situ on the chromatographic plates. After visualization, spectrodensitometric analysis was repeated at 500 nm. Linearity, intermediate precision, sensitivity, accuracy, and precision were compared for both methods. Results from tablet analysis were verified with the modified *Bratton–Marshall* spectrophotometric procedure.

1 Introduction

p-Aminobenzoic acid (PABA) is a compound of great biological significance, occurring naturally in plant and animal tissues [1, 2]. It is used in dietary supplements (vitamin B-complex preparations) [1–5] and as a UV-filter in sun-care cosmetic products [6–13]. It is also a metabolite of some drugs, e.g. of *N*-benzoyl-L-tyrosyl-*p*-aminobenzoic acid, Bz-Ty-PABA [14], and the local anesthetic procaine (*p*-aminobenzoic acid 2-(diethylamino)ethyl ester) [15, 16]. Apart from its advantages, PABA can also have adverse effects. Administered orally or produced in the organism as the result of enzymatic hydrolysis of drugs *p*-aminobenzoic acid esters interferes with sulfonamides [16, 17] and in excess it causes liver damage [2]. When used in cosmetic formulations, free PABA reduces their stability or leads to allergic reactions [12, 13].

Analysis of PABA in pharmaceuticals, dietary supplements, or cosmetic preparations has been achieved by titration [17], UV–visible spectrophotometry [9, 10, 16, 18], by measurement of room-temperature phosphorescence [19], Raman spectroscopy [1], micellar electrokinetic capillary chromatography [8], HPLC [3, 5–7, 11, 13, 20], electrophoresis [4], voltammetry [2], and kinetic [21] and microbiological [22] methods. In the past the most widely used spectrophotometric method for quantification of PABA was based on the *Bratton–Marshall* reaction that consists in diazotization of primary aromatic amines and subsequent coupling of the resulting diazonium ions with phenols or other aromatic amines to form red or orange azo dyes that may be measured colorimetrically [23]. Advantages of the *Bratton–Marshall* procedure include sensitivity but its drawbacks are lack of selectivity (the *Bratton–Marshall* reaction is generally typical of primary aromatic amines) and relatively long duration of analysis, involving multi-step preparation of colored solutions.

To improve the selectivity of the *Bratton–Marshall* procedure the reaction was performed in an automated analytical system in SI (sequential-injection) mode [9]. Apart from much better selectivity, quantities of reagents and the duration of analysis were significantly reduced by this approach compared with the classical colorimetric method. Another idea that may potentially enhance the selectivity of the *Bratton–Marshall* reaction with regard to PABA consists in performing the color reaction after chromatographic separation, TLC being the technique of choice because of its relatively low cost and convenient timing.

TLC analysis of PABA in the presence of other B-group vitamins has been achieved by scraping sample and standard spots of PABA from the plates and measurement of their UV reflectance in the solid state [24]; this method was tedious and impractical, however. Much more convenient TLC procedures for analysis of *p*-aminobenzoic acid involve direct spectrodensitometric evaluation of the chromatographic plates. This approach has been applied to cell cultures of *Solanum laciniatum* [25] and used as a quality-control tool in an industrial process [26], with UV-absorbance and fluorescence detection, respectively.

Visualization of PABA spots by color reaction has been reported in just one paper concerned with analysis of PABA in the presence of its ester novocain [27]. In this case the authors processed the chromatograms with apparatus of their own design that, unfortunately, was not described in detail.

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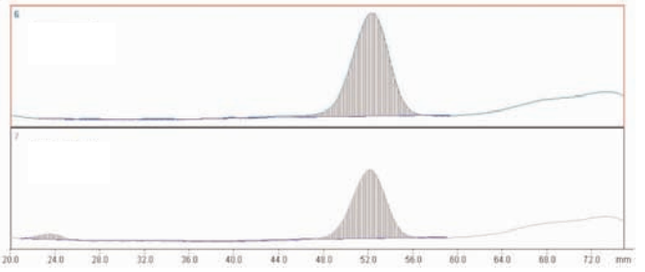

Figure 1

Densitograms (at 270 nm) of PABA standard (upper) and Vitapil extract (lower).

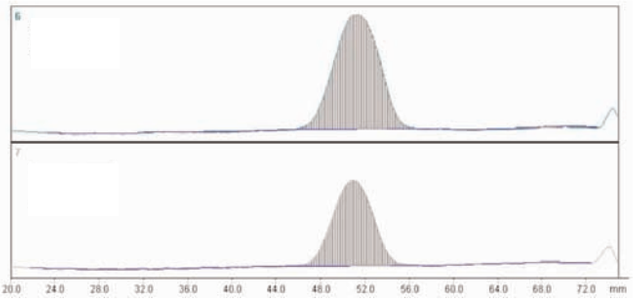

Figure 2

Densitograms (at 500 nm) of PABA standard (upper) and Vitapil extract (lower).

The objective of this study was to develop a method for rapid analysis of PABA in complex dietary supplements or pharmaceuticals. Such products usually contain, apart from PABA, various amounts of other vitamins, e.g. B_1 (λ_{max} at acidic pH ca 243 nm, broad-band absorption), B_2 (λ_{max} 223, 267, 375, and 444 nm), B_3 or PP (λ_{max} 262 nm), B_6 (λ_{max} in dilute aq. HCl 288–296 nm, in phosphate buffer 248–256 and 320–327 nm), and B_{12} (λ_{max} 278, 361, and 547–559 nm) [28]. The presence of these vitamins makes analysis of PABA, especially by absorption spectroscopy, a complicated task. The direct spectrophotometric method developed for PABA tablets by *Paul* [18] (based on simple measurements of absorption in solutions, analytical wavelength 268 nm) is obviously not feasible in the presence of compounds whose absorption ranges overlap with that of *p*-aminobenzoic acid. In such circumstances the spectra must be resolved either by means of derivative UV spectrometry [16] or by converting PABA into a derivative with a completely different absorption range.

For the latter purpose the *Bratton–Marshall* reaction seems to be the procedure of choice although, when applied to B-complex preparations, it may have disadvantages. Caution is necessary if the formulation contains vitamin B_1, which may undergo coupling with diazotized primary aromatic amines [29]. If vitamin B_{12} or red dyes are present their absorption bands at approximately 500 nm may overlap with those of PABA-derived azo compounds. In such circumstances chromatographic separation of the components before the color reaction is advantageous.

It was assumed that an approach consisting in TLC separation of formulation components followed by staining of PABA spots by use of a Desaga ChromaJet DS20 automated spraying device would combine the selectivity of the color reaction with that of the chromatographic separation, with the added benefits of simplicity and significantly reduced analysis time resulting from use of the computer-controlled spraying instrument.

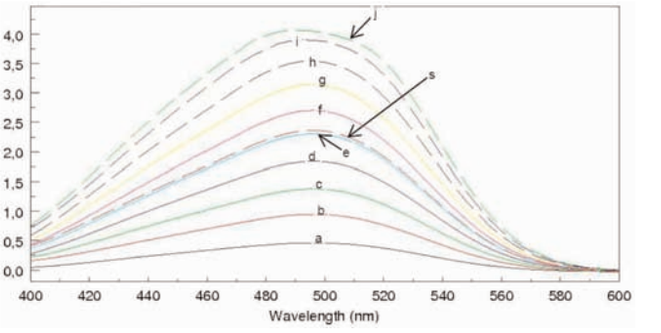

Figure 3

Absorption spectra of PABA after diazotization and coupling with 8-hydroxyquinoline. Initial PABA concentrations [µg mL^{-1}]: a, 2.0; b, 4.0; c, 6.0; d, 8.0; e, 10.0; f, 12.0; g, 14.0; h, 16.0; i, 18.0; j, 20.0. s, Vitapil sample.

2 Experimental

2.1 Chemicals, Materials, and Solutions

The dietary supplement Vitapil was manufactured by Puritan's Pride (Bohemia, NY 11716, USA).

p-Aminobenzoic acid was purchased from Merck. 8-Hydroxyquinoline was obtained from Serva (Germany). Sodium nitrite, sodium hydroxide, hydrochloric acid, diethyl ether, ethyl acetate, chloroform, ethanol, and cyclohexane were supplied by Polskie Odczynniki Chemiczne (POCh Poland). Methanol was purchased from Lach-Ner (Czech Republic), 2-propanol from Chemed (Poland). All chemicals were of analytical grade.

PABA standard (500 mg) was accurately weighed into a 100-mL volumetric flask, dissolved in an adequate amount of 2-propanol and diluted to volume with the same solvent to give a stock solution of concentration 5 mg mL^{-1}. This stock solution was diluted with 2-propanol to furnish standard solutions of concentrations 50, 200, 400, 800, 1200, 1600, and 2000 µg mL^{-1}. The same stock solution of PABA was diluted with 2-propanol to give a solution of concentration 500 µg mL^{-1}, used to prepare standards for spectrophotometric determination of PABA. All dilutions were prepared in standard volumetric flasks.

Solutions of hydrochloric acid (0.1 mol L^{-1}), sodium nitrite (0.1 mol L^{-1}), 8-hydroxyquinoline (0.03 mol L^{-1}), and sodium hydroxide (10%) were prepared to perform staining of PABA spots on thin layer chromatograms. 8-Hydroxyquinoline was dissolved in ethanol, other reagents were used as aqueous solutions. The same reagent solutions were used to determine PABA by the spectrophotometric procedure.

2.2 Sample Preparation

Six Vitapil tablets were powdered and the powder was stirred with ca 70 mL 2-propanol at room temperature for 1 h. The

resulting slurry was filtered through Filtrak no. 389 filter paper. The filtrate was collected in a 200-mL volumetric flask and diluted to volume with 2-propanol. The solution was refrigerated overnight, decanted, filtered through Filtrak no. 390 filter paper (with no extra solvents added) and used for chromatographic and spectrophotometric analysis without further processing.

2.3 Thin-Layer Chromatography

Thin-layer chromatography was performed on 10 cm × 10 cm glass HPTLC plates coated with 0.2 mm layers of silica gel 60 (Merck, Germany). Before use, plates were washed with the mobile phase and dried at ambient temperature. Samples (1 µL) were spotted 20 mm from the bottom edge of the plate, at 10 mm intervals, starting 10 mm from the plate edge. The mobile phase used throughout the study was diethyl ether–cyclohexane 5:1 (*v*/*v*). Plates were developed to a distance of 85 mm from the plate bottom edge (development time 20 min) in an unsaturated vertical chromatographic chamber. After development plates were dried at room temperature (20°C).

Plates were scanned and quantified in reflectance mode with a Desaga CD 60 densitometer at 270 nm. The spots were then visualized by spraying with the reagent solutions prepared as described above, by means of the Desaga ChromaJet DS 20 automated spraying device. The solutions were applied separately in the order: $NaNO_2$, HCl, 8-hydroxyquinoline, NaOH. For each solution 2 spraying cycles were applied. Plates were then dried in a current of warm air (very carefully to avoid overheating), scanned at 500 nm with the Desaga CD 60 densitometer and quantified.

Typical densitograms obtained from a standard solution and an extract of the dietary supplement Vitapil prepared as described above are shown in **Figures 1** and **2**.

2.4 Modified *Bratton–Marshall* Method

To 25-mL volumetric flasks containing PABA dissolved in methanol the reagent solutions prepared as described above were added in the order: HCl (5.0 mL), $NaNO_2$ (1.0 mL), 8-hydroxyquinoline (1.0 mL), NaOH (0.2 mL), at 5-minute intervals. The resulting red solutions were diluted to volume with methanol and their spectra were recorded after 15 min with the PY 4 UV–visible spectrophotometer, in 1.0 cm quartz glass cuvettes, against blank solution containing HCl, $NaNO_2$, 8-hydroxyquinoline, and NaOH in methanol at concentrations given above. The analytical wavelength selected on the basis of the spectra was 500 nm (**Figure 3**).

3 Results and Discussion

3.1 Reference Method – Spectrophotometric Analysis of PABA

The dietary supplement analyzed in the course of this study is manufactured as coated tablets containing, apart from PABA (30 mg per tablet), choline, calcium panthotenate, soya proteins, inositol, niacin, zinc gluconate, copper gluconate, manganese gluconate, yeast, iron(II) sulfate, folic acid, iodine, and cyanocobalamin. As may be concluded from its composition, Vitapil contains substances that can interfere with the direct spectrophotometric quantification of PABA according to Ref. [18] (e.g. niacin). On the other hand the product contains neither substances that absorb visible light at approximately 500 nm (apart from the negligible amount of vitamin B12) nor compounds other than PABA that could undergo the *Bratton–Marshall* reaction to yield azo dyes absorbing within this range. The procedure involving the *Bratton–Marshall* reaction was therefore selected as a reference method for analysis of PABA.

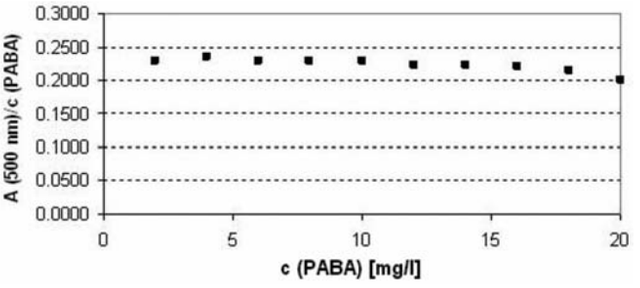

Figure 4

Results from study of linearity of the modified *Bratton–Marshall* spectrophotometric procedure for PABA.

The classic version of the *Bratton–Marshall* colorimetric procedure involves the following steps:

– diazotization of a primary aromatic amine with sodium nitrite in the acidic environment;

– destruction of excess nitrous acid with sulfamic acid; and

– coupling of the diazotized primary aromatic amine with a suitable phenol or another aromatic amine [23].

During a study on the sequential-injection method for analysis of PABA in sunscreens [9] various phenols were investigated as potential coupling reagents. When 8-hydroxyquinoline was used, the reaction proceeded similarly both with and without application of sulfamic acid. It was therefore decided in the course of this work to modify spectrophotometric assay of PABA by the *Bratton–Marshall* reaction using just this coupling reagent, without the sulfamic acid addition step.

The modified *Bratton–Marshall* procedure was validated for linearity using initial concentrations of PABA of 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 µg mL^{-1} [30]. The ratio of the color product absorbance (at 500 nm) to the initial concentration of PABA, shown in **Figure 4**, can be regarded as constant for PABA concentrations up to 18 µg mL^{-1} ($A_{(500\ nm)}/c_{PABA}$ = 0.226 ± 0.006). The ratio A/c_{PABA} corresponding to 20 µg mL^{-1} PABA is clearly lower (0.200), it may be therefore concluded that the absorbance recorded for this concentration should not be included in the calibration plot.

The linear regression data for the calibration plot $A = f(c_{PABA})$ for PABA analyzed in accordance with the modified *Bratton–Marshall* procedure are presented in **Table 1**. Limits of detection and quantification were calculated by use of the equations $LOD = 3.3 \times SD_a/b$ and $LOQ = 3 \times LOD$ [30].

Table 1

Linear regression data for analysis of PABA by the modified *Bratton–Marshall* spectrophotometric procedure.

Slope, $b \pm S_b$	0.215 ± 0.003
y-Intercept, $a \pm S_a$	0.088 ± 0.034
Linear range [μg mL^{-1}]	0–18
LOD [μg mL^{-1}]	0.5
LOQ [μg mL^{-1}]	1.5
Residual standard deviation S_{xy}	0.046
Correlation coefficient, *R*	0.9993

Table 2

R_F values for PABA on silica gel plates developed with different mobile phases. Comparison with other tablet constituents.

Mobile phase	R_F (PABA)	R_F (vitamin B_{12})	R_F (niacin)
n-PrOH–H_2O–$CHCl_3$ 5:2:1	0.93	0.11	0.69
n-PrOH–H_2O–$CHCl_3$ 5:1:2	0.81	0.03	0.64
$CHCl_3$–AcOEt 1:1	0.50 (tailing)	0	0.06
AcOEt–EtOH–H_2O 14:7:6	0.99	0.07	0.69
n-PrOH–$CHCl_3$ 5:3	0.78	0	0.58
n-PrOH–$CHCl_3$ 3:5	0.80	0	0.55
Et_2O–cyclohexane 1:1	0.12	0	0
Et_2O–cyclohexane 5:1	0.65	0	0.04
Et_2O (neat)	0.83	0	0.05

3.2 Chromatographic Conditions

According to the literature TLC separation of *p*-aminobenzoic acid can be achieved on cellulose [26] or silica gel [24, 25, 27]. Mobile phases used for separation of PABA on silica gel were: glacial acetic acid–acetone–methanol–benzene 5:5:20:70 [24]; ethyl acetate–methanol–water 77:13:10 [25]; and I, acetone, II, acetone–cyclohexane–30% ammonia 9:1:0.1 [27]. When separation was performed on cellulose, the bottom layer produced after shaking isoamyl alcohol with citrate buffer was selected [26].

Although detailed optimization of conditions for chromatographic separation of PABA was not the main objective of this study, it seemed useful to propose a different mobile phase, involving no toxic or irritating solvents. It was also preferred to avoid gradient development of plates and complex solvent mixtures. Compositions of the mobile phases tested in the course of this investigation are listed in **Table 2**, with R_F values for PABA and other sample components.

Of all the mobile phases tested, diethyl ether–cyclohexane 5:1 (*v*/*v*) was found to give the best separation and was subsequently used as the mobile phase. To confirm the quality of chromatographic separation UV spectra were recorded directly from the chromatographic plate for PABA standard and the analyzed sample peak (**Figure 5**).

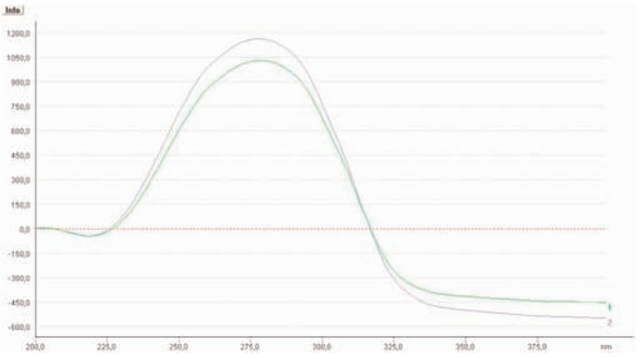

Figure 5

Absorption spectra of: 1, PABA standard solution; 2, PABA (Vitapil sample) recorded directly from the chromatographic plate in remission mode.

3.3 Spraying Conditions

The ChromaJet DS20 automatic spraying device used in this work may be programmed to spray plates with up to four solutions (A–D) in any order. The following spraying sequences were tested to select an option leading to the best repeatability, linearity of calibration plots, and limits of detection:

– $NaNO_2$, HCl, 8-hydroxyquinoline + NaOH

– $NaNO_2$ + HCl, 8-hydroxyquinoline

– $NaNO_2$ + HCl, 8-hydroxyquinoline, NaOH

– $NaNO_2$, HCl, 8-hydroxyquinoline

– $NaNO_2$, HCl, 8-hydroxyquinoline, NaOH

It was found that detection limits and linearity were by far the best when the solvents are applied separately in the order indicated by sequence ‘e’. When the other spraying arrangements were used the linearity of response and repeatability of spraying were compromised, the lower detection limit increased significantly in some cases or no color reaction was observed.

It was established that the optimum number of spraying cycles for each solution was two – enough to ensure uniformity of spraying and, on the other hand, to avoid diffusion of spots. Particular caution is necessary to ensure flushing of appropriate ports of the ChromaJet device when swapping between solutions, to avoid their mutual contamination which, according to this study, led to accelerated degradation of the solutions, effecting the yield of the color reaction.

3.4 Spectrodensitometric Analysis

Analytical wavelengths for densitometric analysis of chromatograms were selected on the basis of multi-wavelength scans of chromatographic plates both before and after visualization of spots. The values most suitable for densitometric analysis were 270 and 500 nm, respectively.

HPTLC plates were spotted with standard solutions of PABA (50, 200, 400, 800, 1200, 1600, 2000, and 2400 μg mL^{-1}) applied as described above (1 μL per spot). After development the plates were scanned at 270 nm. The same plates were than stained by spraying with reagents specified above and scanning was repeated at 500 nm. Peak areas and heights were plotted against amounts of PABA in the corresponding spots. Because it

Table 3

Linear regression data for PABA (n = 6, λ = 270 nm, 50–1600 ng per spot) before staining of the chromatographic plate.

	Peak area	Peak height
Slope, $b \pm S_b$	1452.6 ± 82.4	360.8 ± 29.8
y-intercept, $a \pm S_a$	192.3 ± 74.0	71.5 ± 26.8
LOD (calculated) [ng per spot]	170	250
LOD (estimated visually) [ng per spot]	50	50
LOQ (calculated) [ng per spot]	510	750
LOQ (estimated visually) [ng per spot]	150	150
S_{xy} (Residual SD)	111.5	40.3
Correlation coefficient, R	0.9936	0.9866
Linear range [ng per spot]	200–1800	200–1800

Table 4

Linear regression data for PABA (n = 6, λ = 500 nm, 50–1600 ng per spot) after staining of the chromatographic plate.

	Peak area	Peak height
Slope, $b \pm S_b$	1554.6 ± 151.6	297.3 ± 46.8
y-intercept, $a \pm S_a$	223.9 ± 136.2	85.6 ± 42.1
LOD (calculated) [ng per spot]	290	470
LOD (estimated visually) [ng per spot]	50	50
LOQ (calculated) [ng per spot]	870	1410
LOQ (estimated visually) [ng per spot]	200	200
S_{xy} (Residual SD)	205.1	63.4
Correlation coefficient, R	0.9815	0.9538
Linear range [ng per spot]	200–1800	200–1800

was obvious that the points corresponding to 2000 and 2400 µg PABA per spot tended to deviate from the straight line it was decided to keep PABA concentrations below this level. Linear regression data from calibration plots for PABA concentrations 50–1600 ng per spot are given in **Tables 3** and **4**.

The quality of calibration plots drawn for peak areas and heights was compared for spectrodensitometric analysis of TLC plates before and after their visualization (270 and 500 nm, respectively). On the basis of the calibration data (Tables 3 and 4) it was concluded that use of peak areas resulted in slightly better linearity and precision than peak heights, further work was therefore based on peak areas.

Limits of detection (*LOD*) and quantification (*LOQ*) were calculated from calibration plots both at 270 and 500 nm (Tables 3 and 4), in accordance with Ref. [30].

Calculated levels of *LOD* and *LOQ* were regarded as clearly too high, because the spot corresponding to 50 ng PABA is visible on chromatograms both before and after the color reaction (270 and 500 nm, respectively) and the spot containing 200 ng PABA is most definitely within the linear range of the calibration plots.

Table 5

Linear regression data for PABA (n = 6, λ = 270 nm, 200–1600 ng per spot, peak area) before and after staining of the chromatographic plate.

	270 nm	500 nm
Slope, $b \pm S_b$	1374.2 ± 73.3	1406.1 ± 129.9
y-intercept, $a \pm S_a$	284.5 ± 72.2	398.0 ± 127.7
S_{xy} (Residual SD)	84.02	148.7
Correlation coefficient, R	0.9958	0.9874

Table 6

Results of intermediate precision study before and after chromatographic plate staining.

	270 nm		500 nm	
	Day 1 (n = 6)	Day 2 (n = 6)	Day 1 (n = 6)	Day 2 (n = 6)
Peak areas	1530.4	1503.2	1651.7	1672.3
RSD [%]	2.81	2.59	8.01	6.00

The *LOD* estimated experimentally was, therefore, ca 50 ng per spot and the *LOQ* was at the level of ca 200 ng per spot. Calibration plots based on densitometric peak areas were drawn again for concentrations of PABA in the range 200–1600 ng per spot. The linear regression data for these plots are given in **Table 5**.

Intermediate precision of PABA analysis at 270 and 500 nm was evaluated by comparing average peak areas for PABA in Vitapil samples spotted as above on two different days. Results from this comparison are given in **Table 6**.

The accuracy and precision of the densitometric methods were evaluated by conducting recovery tests for three different concentrations of PABA. The recoveries and relative standard deviations are given in **Table 7**.

3.5 Determination of PABA in a Commercial Pharmaceutical Formulation

3.5.1 Spectrophotometric Procedure

Vitapil tablet extract prepared as described above (0.3 mL) was subjected to the spectrophotometric procedure described above for PABA standards and the absorbance of the resulting solution was recorded at 500 nm. The PABA contents of the tablets, calculated on the basis of the calibration plot described in Table 1, were in good agreement with the manufacturer's claim (**Table 8**).

3.5.2 Spectrodensitometric Procedure

Samples of Vitapil tablet extract prepared as described above (1 µL) were subjected to the chromatographic procedure described above for PABA standards. Peak areas were recorded with the densitometer at 270 nm (before the color reaction) and at 500 nm (after the staining procedure described above). The PABA contents of the tablets, calculated on the basis of the calibration plots presented in Table 5, were in good agreement with the manufacturer's claim and with the PABA concentration in tablets determined spectrophotometrically (Table 8).

Table 7

Results of recovery tests for the spectrodensitometric procedures at 270 nm (*n* = 6) and 500 nm (*n* = 6) (peak areas).

Spiked amount	Before staining (270 nm)			After staining (500 nm)		
[ng per spot]	Found [ng]	Recovery [%]	RSD [%]	Found [ng]	Recovery [%]	RSD [%]
600	600	100.0	6.70	590	98.3	8.10
1000	1000	100.0	2.23	1020	102.0	6.31
1400	1410	100.7	5.47	1440	102.9	4.70

Table 8

Results from analysis of PABA in Vitapil tablets (PABA concentrations expressed in mg PABA mL^{-1} tablet extract prepared as above; peak areas, *n* = 8.

Method	C_{PABA}	RSD [%]
HPTLC, 270 nm	0.90	4.4
HPTLC, 500 nm	0.89	6.7
Spectrophotometric	0.88	1.9
Expected (label claim)	0.90	

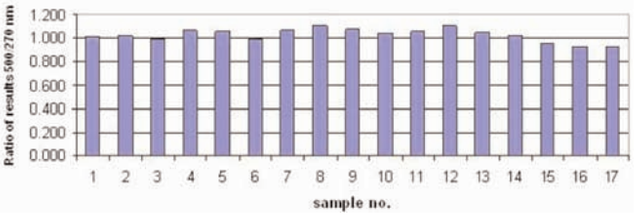

Figure 6

Ratio of peak areas for chromatograms before and after staining of plates.

Results from analysis of PABA in commercial tablets were found to be similar for spectrodensitometric analysis at 270 nm (without visualization of the spots) and at 500 nm (after visualization), although the precision of the analysis after the color reaction was slightly worse than that for analysis at 270 nm. It was established that the ratio of PABA concentrations obtained on the basis of the same spots before and after the color reaction was 1.028 ± 0.055 (n = 17, **Figure 6**). On the other hand, it seemed from comparison of data presented in Table 5 that the quality of calibration plots (correlation coefficients, residual standard deviations) is slightly better for plates scanned at 270 nm before the staining reagents were applied.

4 Conclusion

PABA can be separated from other components of complex pharmaceutical preparations by normal-phase thin-layer chromatography on silica gel plates with diethyl ether–cyclohexane 5:1 (*v*/*v*) as mobile phase. Quantification of PABA can be achieved by spectrodensitometry with UV detection (270 nm) and after conversion of PABA into a colored derivative (visible range). PABA TLC spots can be visualized with the modified *Bratton–Marshall* reaction. Diazotization of PABA and coupling of the resulting diazonium ions with 8-hydroxyquinoline can be performed in situ on developed chromatographic plates, by use of the ChromaJet DS20 automatic spraying device, to yield a red dye that can be determined densitometrically at 500 nm.

If PABA is analyzed by use of the modified *Bratton–Marshall* reaction performed in situ on chromatographic plates the duration of analysis is substantially reduced compared with the conventional colorimetric procedure. The selectivity of the color reaction, typical of primary aromatic amines, is at the same time much improved by chromatographic separation of formulation components before quantification.

Spectrodensitometric analysis of PABA by use of the color reaction proposed in this paper may be especially useful for rapid, routine analysis of complex pharmaceutical preparations when TLC separation of particular sample components is difficult.

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References

[1] *V.A. Narayanan, J.M. Bello, D.L. Stokes, T. Vo-Dinh,* J. Raman Spectrosc. **22** (1991) 327–331.

[2] *R.M. Kotkar, A.K. Srivastava,* Sens. Actuat. B **119** (2006) 524–530.

[3] *D. Ivanović, A. Popović, D. Radulović, M. Medenica,* J. Pharm. Biomed. Anal. **18** (1999) 999–1004.

[4] *X.-Y. Li, Q.-L. Zhang, H.-Z. Lian, J.-J. Xu, H.-Y. Chen,* J. Sep. Sci. **30** (2007) 2320–2325.

[5] *M.C. Gennaro,* J. Chromatogr. Sci. **29** (1991) 410–415.

[6] *L. Gagliardi, A. Amato, A. Basili, G. Cavazzutti, D. Tonelli,* J. Chromatogr. **362** (1986) 450–454.

[7] *L. Gagliardi, A. Amato, A. Basili, G. Cavazzutti, D. Tonelli,* J. Chromatogr. **408** (1987) 409–415

[8] *S.-P. Wang, W.-J. Chen,* Anal. Chim. Acta **416** (2000) 157–167.

[9] *A. Chisvert, J.-V. Izquierdo, A. Salvador,* Anal. Bioanal. Chem. **374** (2002) 963–967.

[10] *A. Salvador, A. Chrisvert, A. Rodriguez, J.G. March,* Anal. Chim. Acta **493** (2003) 233–239.

[11] *D.J. Schakel, D. Kalsbeek, K. Boer,* J. Chromatogr. A **1049** (2004) 127–130.

[12] *S.J. Herman,* Cosmet. Toiletr. **109** (1994) 71–75.

[13] *K.L. Granger, P.R. Brown,* J. Liq. Chromatogr. Related Technol. **24** (2001) 2895–2924.

[14] *P.W. Pemberton, P. Gagjee, C. Chaloner, J.M. Braganza, R.W. Lobley,* Clin. Chim. Acta **199** (1991) 253–262.

[15] *M.R. Dhananjeyan, C. Bykowski, J.A. Trendel, J.G. Sarver, H. Ando, P.W. Erhardt,* J. Chromatogr. B **847** (2007) 224–230.

[16] *I. Badea, D. Moja, L. Vladescu,* Anal. Bioanal. Chem. **374** (2002) 51–53.

[17] *L. Vladescu, I. Badea, D. Moja,* Roum. Biotechnol. Lett. **7** (2002) 665–660.

[18] *W.L. Paul,* J. Pharm. Sci. **62** (1973) 660–662.

[19] *W.J. Long, R.C. Norin, S.Y. Su,* Anal. Chem. **57** (1985) 2873–2877.

[10] *A.R. Khan, K.M. Khan, S. Perveen, N. Butt,* J. Pharm. Biomed. Anal. **29** (2002) 723–727.

[21] *S. Panadero, A. Gómez-Hens, D. Perez-Bendito,* Talanta **45** (1998) 829–834.

[22] *J. Tautt,* Acta Polon. Pharm. **21** (1964) 189–192.

[23] *H.W. Eckert,* J. Biol. Chem. **148** (1943) 197–204.

[24] *M.M. Frodyma, V.T. Lieu,* Anal. Chem. **39** (1967) 814–819.

[25] *T.S. Panjaitan, A. Syahrani, G. Indrayanto,* J. Planar. Chromatogr. **13** (2000) 114–118.

[26] *D. White, P. Varlashkin, D.N. Rusch,* J. Pharm. Sci. **81** (1992) 1204–1209.

[27] *M.J. Pormalie, I.N. Rozental, N.A. Kaszkina,* Chim.–Pharm. Zh. **10** (1976) 141–144.

[28] European Pharmacopoeia, 5th edn., vol. 5.0, 2005.

[29] *D. Melnick, H. Field Jr,* J. Biol. Chem. **127** (1939) 505–514.

[30] *P. Konieczko, J. Namiesnik* (eds.), Ocena i kontrola jakości wyników pomiarów analitycznych (Evaluation and quality control of analytical results, in Polish), Wydawnictwo Naukowo-Techniczne, Warszawa, 2007.

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