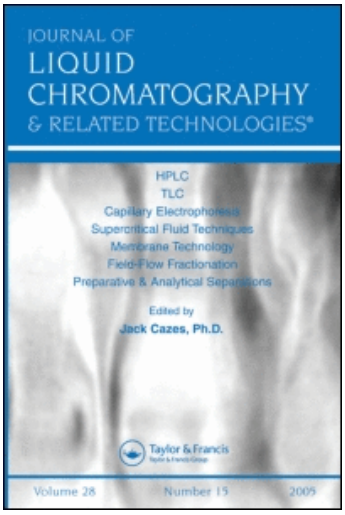

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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Online publication date: 31 May 2001

To cite this Article Indrayanto, Gunawan and Sia, Tan Kiauw(2001) 'DENSITOMETRIC DETERMINATION OF AMPICILLIN IN PHARMACEUTICAL PREPARATIONS, AND ITS VALIDATION', Journal of Liquid Chromatography & Related Technologies, 24: 10, 1501 — 1510
To link to this Article: DOI: 10.1081/JLC-100103926
URL: http://dx.doi.org/10.1081/JLC-100103926

J. LIQ. CHROM. & REL. TECHNOL., 24(10), 1501–1510 (2001)

DENSITOMETRIC DETERMINATION OF AMPICILLIN IN PHARMACEUTICAL PREPARATIONS, AND ITS VALIDATION

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ABSTRACT

A simple and rapid densitometric method has been developed for determination of ampicillin in pharmaceutical preparations. After extraction of the analyte with distilled water, the extracts were spotted on pre-coated silica gel plates, which were then developed with a mixture of butanol-acetic acid-water (4: 1: 1). Quantitative evaluation was performed by measuring the absorbance reflectance of the analyte spots at 485 nm after dipping in 0.2% ethanolic nynhydrin solution. The densitometric method is selective, precise, and accurate, and can be used for routine analysis of the pharmaceutical preparations in pharmaceutical industry quality control laboratories.

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INTRODUCTION

Many pharmaceutical preparations containing ampicillin as the sole active ingredient are marketed in Indonesia.[1] The Indonesian Pharmacopoeia, IV 1995[2] and USP 24 – NF 19,[3] describe a HPLC method for assaying ampicillin preparations. A spectrophotometric method using imidazole-mercury reagent, was described in the BP 2000[4] and the Pharmacopoeia of the People's Republic of China 1997,[5] for determination of ampicillin. Indian Pharmacopoeia 1996[6] describes spectrophotometric and potentiometric methods for assay of ampicillin. In our QC-Laboratory (Bernofarm Pharmaceutical Company), ampicillin was assayed using an HPLC method published by BDH.[7] Some authors have reported TLC analysis of ampicillin with other drugs for qualitative identification.[8] Mrhar and Kozjek reported a HPTLC method for determination of ampicillin in human urine,[8] while Abjean and Lahogue[9] described HPTLC analysis of ampicillin in milk and muscle. Dhanesar[10] reported the RP-HPTLC quantitative analysis of ampicillin and other antibiotics using a direct sample determination (DSD) method (without elution) with very high sensitivity, but this DSD method was only applicable for pure samples.

The aim of this work was to develop a simple and rapid densitometric method for routine analysis of ampicillin in the pharmaceutical preparations.

EXPERIMENTAL

Materials and Reagents

Ampicillin trihydrate (PT Novartis Biochemie, Jakarta Indonesia; Batch 465 L 8N; Assay 99.24%) was pharmaceutical grade. The substance was used as received, for preparing laboratory-made pharmaceutical preparations and standard solutions. Their UV, IR spectra, and melting points were identical to the authentic USP standard and meet the requirements of the Indonesian Pharmacopoeia.[2]

n-Butanol, glacial acetic acid, and ninhydrin (E. Merck) were analytical grade reagents; ethanol 96% (JT Baker) was analytical grade reagent. The solvents and reagents were used without further purification. Excipients for laboratory-made pharmaceutical preparations (CMC-sodium, Aerosil®, nipagin, nipasol, sucrose, talcum, magnesium stearate, Vivapur® type 101, strawberry essence, and distilled water) were pharmaceutical grade.

Laboratory-made pharmaceutical preparations were prepared containing five different concentrations of ampicillin trihydrate (462.0, 520.0, 577.5, 635.5, and 693 mg tablet^{-1}; 231.0, 260.0, 288.8, 317.6, and 346.5 mg capsule^{-1}; 1.386,

1.560, 1.773, 1.906, and 2.078 g flask^{-1} for dry syrups); these were used for accuracy determination.

Commercial ampicillin capsules containing 288.8 mg capsule^{-1} ampicillin trihydrate (equivalent to 250 mg ampicillin capsule^{-1}) were provided by the Production Department of the Bernofarm Company.

Stock standard solutions were prepared daily by dissolving accurately weighed ampicillin trihydrate (70.0 mg) in distilled water (25.0 mL). Various standard solutions were prepared from the stock solution by dilution with distilled water. For linearity studies, solutions were prepared containing 560, 840, 1050, 1200, 1400, 1680, and 1870 μg mL^{-1}, and 2 μL of these solutions was spotted on the TLC plate.

Spotting was performed using a Nanomat III equipped with a dispenser magazine containing 2 μL glass capillaries (Camag, Muttenz, Switzerland).

Sample Extraction

Dry Syrups

Three hundred mg (accurately weighed) was transferred into a 25.0 mL volumetric flask and about 20 mL of distilled water was added, ultrasonicated for 15 minutes, diluted to volume, and then filtered through a Millipore filter (0.45 μm, Cat. HVLP 01300). Two μL of this solution was spotted on the TLC plates.

Tablets and Capsules

Twenty tablets or capsules were weighed and their mean weight was determined. The preparations were finely powdered, and an equivalent weight of a tenth of a capsule or a twentieth of a tablet was transferred into a 25.0 mL volumetric flask containing about 20 mL of distilled water, ultrasonicated for 15 min, and diluted to volume with distilled water. The solution was filtered through a Millipore filter (0.45 μm, Cat. HVLP 01300). Two μL of this solution was spotted on the TLC plates

Chromatography

Chromatography was performed on pre-coated silica gel 60 F_{254} aluminium-backed sheets (E. Merck. # 1.05554); a Nanomat III (Camag) was used for sample application. The mobile phase used was n-butanol – glacial acetic

acid – water (4: 1: 1) according to Bardici *et al.*[8] Ascending development was performed in a Camag twin-through chamber (for 20 x 10 cm plates); the mobile phase migration distance in all experiments was 8.0 cm (development time *ca.* 60 min. at 25 ± 1°C). After dipping in 0.2 % ethanolic ninhydrin reagent, the plate was air dried for 15 min. and dark violet spots of ampicillin appeared.

Densitometric scanning was performed with a Camag TLC-Scanner II. The purity and identity of the analyte spots were determined by scanning in the absorbance - reflectance mode from 400 to 800 nm. Quantitative evaluation was performed by measuring the absorbance reflectance of the analyte spots at 485 nm (See Figure 1). The densitometric scanning parameters were: bandwidth 10 nm, slit width 4, slit length 6, and scanning speed 4 mm s^{-1}. Calculations for identity, purity checks ($\mathbf{r}_{S,M}$ and $\mathbf{r}_{M,E}$ where S = start, M = center, E = end spectrum), and quantification of the analyte spots were performed by CATS version 3.17 (1995) software (Camag). Routine quantitative evaluations were performed *via* peak areas with linear regression, using at least four-point calibration on each plate.

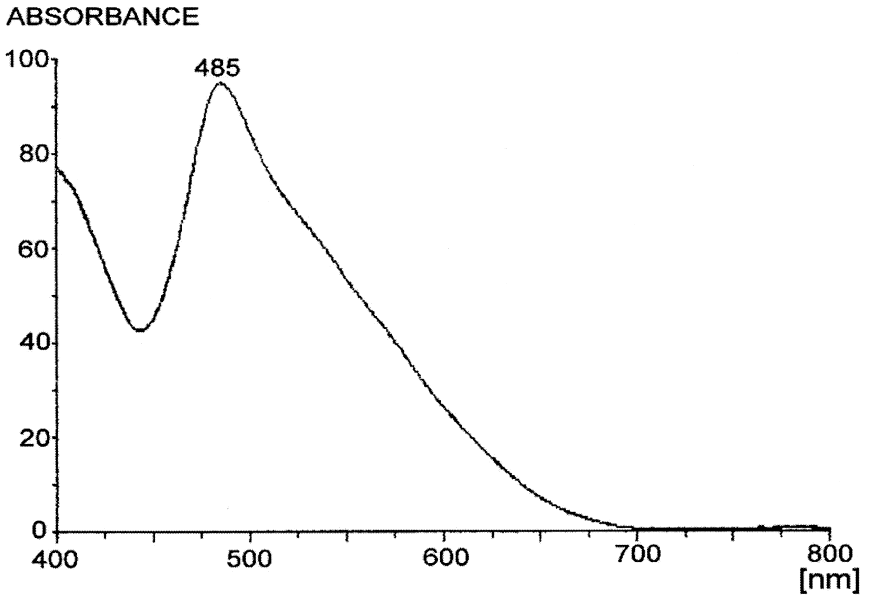

Figure 1. *In situ* absorbance reflectance spectrum of ampicillin trihydrate from 400 to 800 nm, with its maximum at 485 nm. Stationary phase: pre-coated plate silica gel 60 F_{254} (E. Merck); mobile phase: n-butanol – glacial acetic acid – water (4: 1: 1), detection with 0.2 % ethanolic ninhydrin reagent.

Validation

The method was validated for linearity, homogeneity, detection limit (DL), and accuracy by the method of Funk *et al.*[11] The selectivity of the method was proven by identification and purity checks of the analyte spots. A five-point accuracy study (80 - 120 % of the expected value) was performed for the pharmaceutical preparations. The precision was evaluated by analyzing six different extract aliquots from laboratory-made pharmaceutical preparations containing ampicillin trihydrate 288.8 mg capsule^{-1}, 577.5 mg tablet^{-1}, and 1.73 g flask^{-1}, according to a modified method of Renger *et al.*[12]

RESULTS AND DISCUSSION

After the TLC plate was developed and reacted with the ninhydrin reagent, the densitograms (Figure 2) showed a single spot of ampicillin trihydrate, which

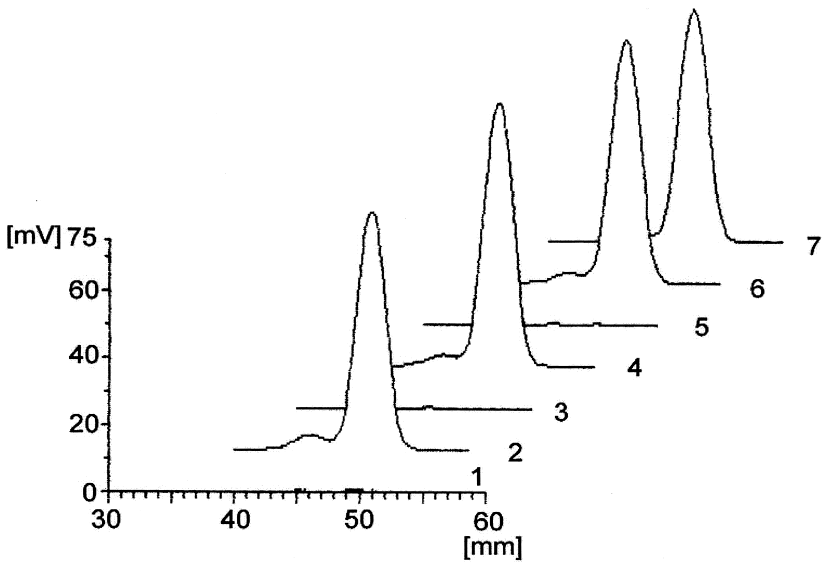

Figure 2. Densitograms (λ = 485 nm) obtained from: (1) extract from the excipients of a laboratory-made capsule, (2) extract of a laboratory-made capsule, (3) extract from the excipients of a laboratory-made tablet, (4) extract of a laboratory-made tablet, (5) extract from the excipients of laboratory-made dry syrup, (6) extract of a laboratory-made dry syrup, and (7) solution of standard ampicillin trihydrate (2.1 μg spot^{-1}).

appeared as a dark violet-spot in each of the extracts. All the excipients did not react with this reagent. This TLC system demonstrated that all the analyte spots of the laboratory-made pharmaceutical preparation extracts furnished *in situ* visible absorption spectra, identical with the standard (**r**>0.9999). Purity checks using the CATS software also showed that all of the analyte spots of the laboratory-made pharmaceutical preparation extracts were pure. The values of $\mathbf{r}_{S,M}$ and $\mathbf{r}_{M,E}$ were >0.9999, demonstrating that the proposed TLC method is highly selective. Our work showed that using ethanolic ninhydrin reagent yielded clearer spots, compared to using UV absorption alone, as the detection method.

The basic calibration plot of peak area against amount of analyte, was constructed within the ranges 50 - 180 % of the expected values in the pharmaceutical preparations. Under this condition, linearity of ampicillin trihydrate was achieved from 1.12 to 3.72 μg spot^{-1} with line equation Y = 795.09 + 577.86X. The relative process standard deviation (V_{XO}) and X_p values[11] of ampicillin trihydrate were 4.11 % and 0.58 μg spot^{-1}, respectively (n = 7; r = 0.9950). ANOVA

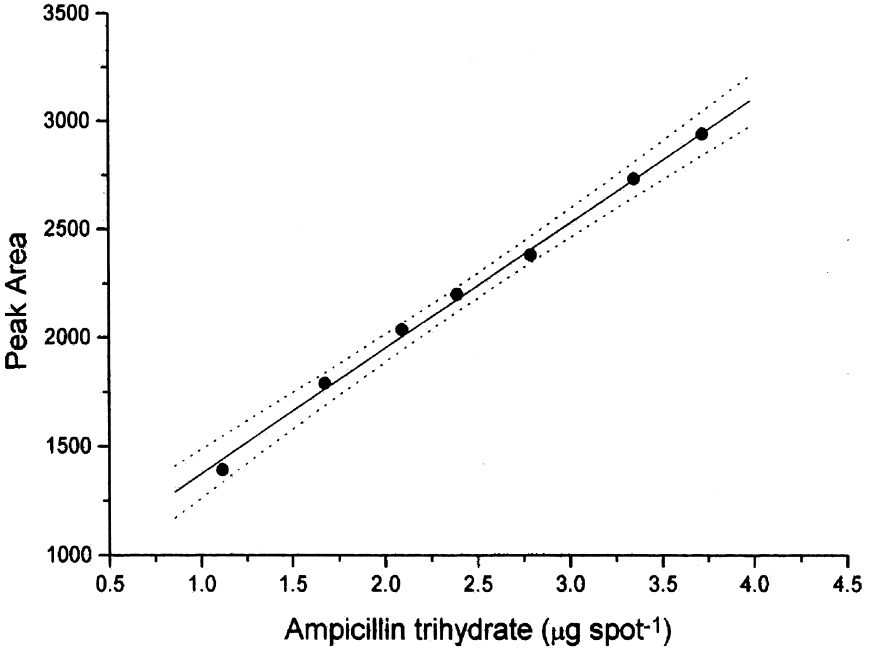

Figure 3. Basic calibration curves and their confidence bands (α = 0.05) for ampicillin trihydrate (regression equation Y = 795.09 +577.86 X).

regression-test for testing linearity of the regression line showed significant calculated F-values (497.65 for $p<0.0001$). The basic calibration curve is presented in Figure 3. The plots of the residuals against the quantities of the analyte, confirmed the linearity of the basic calibration graphs (Figure 4). The residuals were distributed at random around the regression line; neither trend nor uni-directional tendency was found.

The basic calibration curve showed variance homogeneity over the whole range. The calculated parameter PW[11] was 0.174. The PW-value was less from the F_{table}-value (5.35; for $f_1=9$, $f_2=9$; $\alpha=0.01$).

DL was determined by making a linear regression of relatively low concentrations of ampicillin trihydrate (0.22 to 2.11 μg spot^{-1}; n=7; V_{XO} = 4.35 %; r = 0.9985; line equation Y = 25.44 + 1246.55X).[11] By this method, the DL value was 0.20 μg spot^{-1}. According to Carr and Wahlich,[13] the value of quantitation limit (QL) could be estimated as 3 times the DL-value (0.61 μg spot^{-1}).

Table 1 demonstrates the high accuracy as revealed by the percentage of mean recovery data (100.2 to 101.5%). To prove whether systemic errors occurred, linear regression of recovery curves of $\mathbf{X}_f$ (concentration of the analyte measured by the proposed method) against $\mathbf{X}_c$ (nominal concentration of the analyte) were constructed.[11] The confidence range data ($\alpha=0.05$) of the intercept {VB(a_f)} and slope {VB(b_f)} from the recovery curves did not reveal the occurrence of constant- and proportional-systematic errors.

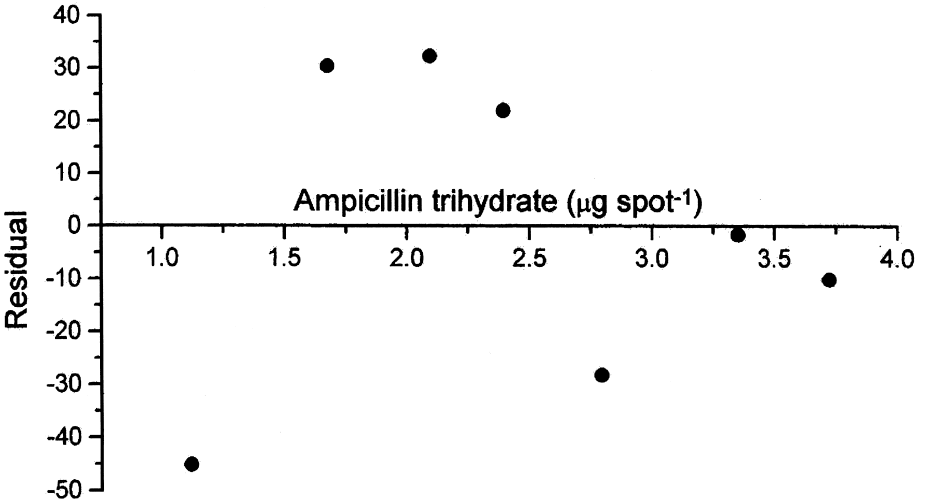

Figure 4. Residual plot (peak area) in the linear working range of ampicillin trihydrate.

Table 1. Results from Determination of Accuracy Using Laboratory-Made Preparations

Laboratory-made preparation	% Recovery (Mean ± SD, n = 5)	Recovery Curve	$VB_{(af)}$ [a]	$V_{B(bf)}$ [a]
Capsule	100.24 ± 0.38	$X_f = -00076 + 1.003\ X_C$	−0.0076 ± 0.095	1.003 ± 0.044
Tablet	101.46 ± 0.77	$X_f = 0.0096 + 1.010\ X_C$	0.0096 ± 0.212	1.01 ± 0.0.098
Dry syrup	100.65 ± 0.83	$X_f = -0.01116 + 1.012\ X_C$	−0.01116 ± 0.189	1.011 ± 0.087

X_f = measured concentration of the analyte in the spotted solution (μg $spot^{-1}$).
X_C = Nominal concentration of the analyte in the spotted solution (μg $spot^{-1}$).
[a] For $\alpha = 0.05$[11].

Table 2. Results from Evaluation of Precision of Laboratory-Made Preparations

	RSD Value (%, n = 6)[b]		
Measurement[a]	Capsule	Tablet	Dry Syrup
1	1.09	0.78	0.94
2	1.20	0.74	0.74
3	1.05	0.73	1.05

[a]Each measurement was performed by a different analyst on different days and plates.
[b] Evaluated on one plate by one analyst (repeatability).

All the RSD values of the repeatability and intermediate precision evaluations were less than 2% (see Table 2). The three measurements were performed within one laboratory by different analysts on the different plates and days. These results demonstrated that the accuracy and precision of the proposed method were satisfactory.

Although, the concentration range for quantitative analysis that is reported here (1.12 to 3.72 μg $spot^{-1}$) is less sensitive than that reported by Dhanesar[10] (0.24 to 16 ng $spot^{-1}$), our proposed TLC method can be used for analyzing pharmaceutical preparations (not pure ampicillin samples). After analyzing 15 batches of commercial ampicillin capsules produced by our Production Department using routine HPLC and this proposed TLC method (data are not shown), we did not find significant differences between the assay results using the pair t-test ($t_{calculated}$ = 1.436, p = 0.173 for n = 15).

Therefore, the proposed method is suitable for the routine analysis of products of similar composition in pharmaceutical industry quality control laboratories. Our experiences also showed that the TLC method is relatively cheaper, simpler, and faster, compared to our routine HPLC method.

ACKNOWLEDGMENTS

The authors are very grateful to Abdul Rahman (Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia) and Aloysius Indrayanto (ITS, Surabaya) for their technical assistance, and to Jan Masthoff (Camag,Muttenz, Switzerland) and Winy Tanudjojo (St. Margaret's College, Dunedin, New Zealand), for providing some of the newest references.

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Received August 23, 2000 Manuscript 5456
Accepted October 17, 2000