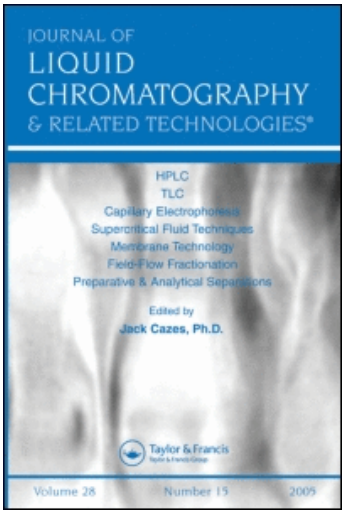

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Specific High-Performance Liquid Chromatographic Determinatioin of Ampicillin in Bulks, Injectables, Capsules, and Oral Suspensions by Reverse-Phase Ion-Pair Chromatography

Ronald G. Lauback[a]; James J. Rice[a]; B. Bleiberg[a]; N. Muhammad[a]; S. A. Hanna[a]
[a] Analytical Research & Development Department Bristol Laboratories, Division of Bristol-Myers Company, Syracuse, New York

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SPECIFIC HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF AMPICILLIN IN BULKS, INJECTABLES, CAPSULES & ORAL SUSPENSIONS BY REVERSE-PHASE ION-PAIR CHROMATOGRAPHY

Ronald G. Lauback, James J. Rice[X], B. Bleiberg, N. Muhammad, & S. A. Hanna

Analytical Research & Development Department
Bristol Laboratories, Division of Bristol-Myers Company
Syracuse, New York 13201

ABSTRACT

A rapid, specific, stability-indicating high-performance liquid chromatographic (HPLC) method has been developed for the assay of Ampicillin in Ampicillin Trihydrate bulk, capsules and oral suspensions and Sodium Ampicillin bulk and injectables. The assay is specific for Ampicillin in the presence of possible contaminants; Penicillin V, Phenylglycine, and 6-Aminopenicillanic Acid (6-APA); the degradation product, Penicilloic Acid of Ampicillin; and all excipients present in the formulations assayed. Ampicillin, Ampicillin formulations, and formulation excipient blends were force-degraded to further demonstrate specificity.

The assay is precise, accurate, linear over the range 50% to 125% of expected Ampicillin sample level, and stability-indicating toward the described thermal, acid, base, aqueous, and light degradations.

The procedure employs an ion-pairing eluent with UV detection at 254 nm. Ampicillin Trihydrate and Sodium Ampicillin bulk are stable in assay diluent for six hours allowing the use of automatic HPLC injectors for unattended analysis. One set of HPLC parameters can assay bulks and formulations.

INTRODUCTION

D(-)-α-aminobenzylpenicillin, Ampicillin, is a semi-synthetic penicillin with activity against both gram-positive and gram-negative

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bacteria. It is available in injectable, capsule, and oral suspension forms.

Although the iodometric titration assay (1) will differentiate between intact penicillin nucleus and degradation products containing open β-lactam structure, it is still subject to interference from penicillin precursors or polymers of ampicillin (2-3). Other methods of analysis include colorimetry, microbiology (4), and non-aqueous titration. These methods also lack specificity. A higher degree of specificity can be achieved using high-pressure liquid chromatography. Anion-exchange columns have been used to assay ampicillin in nitrofurantoin (5) and in pharmaceutical preparations (6). With the advent of microparticulate HPLC columns, greater efficiency and resolution can be achieved. The literature contains references to reverse-phase HPLC systems for chromatographing ampicillin (7), investigating impurities of ampicillin (8), analyzing ampicillin in body fluids (9-10), separation of ampicillin from epicillin (11) and polymers of ampicillin (12), and quantitative analysis of ampicillin (13).

Ampicillin possesses both carboxylic acid and primary amine functionalities. Therefore, reverse-phase ion-pairing techniques can be used to chromatograph ampicillin. Heptane sulfonic acid has been used to ion-pair the primary amine and analyze ampicillin in human urine (14) and to investigate ampicillin degradation products (15). Tetrabutylammonium hydroxide has been used to ion-pair the carboxylic acid and separate ampicillin from its penicilloic acid (16).

Our purpose was to develop a specific HPLC system based on the reverse-phase ion-pairing technique using dodecyl sodium sulfate to analyze ampicillin in commercial drug forms.

EXPERIMENTAL

Instrumentation

The HPLC system consisted of a Waters 6000A pump operated at 3.0 ml/min., Model 440 fixed wavelength UV detector equipped to monitor 254 and 280 nm, WISP Model 710B autosampler programmed to inject 25-µl, and a 30 cm x 3.9 mm µ-Bondapak C-18 column (Waters Associates, Inc., Milford, Mass.). A Varichrom Variable Wavelength UV detector was used to monitor a third wavelength (Varian Associates, Palo Alto, CA.). The chromatograms were recorded on a Model 7100B dual-channel recorder and a Model 7127A single-channel recorder (Hewlett-Packard, Avondale, PA.). The assay wavelength was 254 nm. Integration was performed by a Model 3354 laboratory automation computer equipped with a 2:1 voltage divider (Hewlett-Packard, Avondale, PA.).

Reagents and Materials

Acetonitrile was UV grade, glass distilled (Burdick and Jackson, Lab., Inc., Muskegon, Mich.). Formic acid 88% w/w (Mallinckrodt Chem. Works, St. Louis, MO.), dodecyl sodium sulfate (Eastman Kodak, Rochester, N.Y.), and o-o´-biphenol (Aldrich Chem., Co., Inc., Milwaukee, Wisc.) were reagent grade. All aqueous reagents were prepared with water purified by reverse-osmosis (Millipore Corp., Bedford, Mass.). Ampicillin Trihydrate, Sodium Ampicillin, Penicillin V, Phenylglycine, 6-APA, and Penicilloic Acid of Ampicillin were high quality reference materials. Ampicillin bulks and formulations were of pharmaceutical quality.

Operating Parameters

The assay of Ampicillin was performed at ambient temperature. Detection was at 254 nm (0.1 AUFS). The flow rate was 3.0 ml/min.

Injection volume was 25-µl. The Ampicillin retention time was approximately 6.5 min.

Mobile Phase – 0.035M Dodecyl Sodium Sulfate/2.0M Formic Acid Stock Solution

10.1 g of Dodecyl Sodium Sulfate and 87 ml of formic acid were added to a 1-liter volumetric flask. The contents were dissolved in and diluted to volume with water.

Mobile Phase – 100 ml of 0.035M Dodecyl Sodium Sulfate/2.0M formic acid stock solution and 350 ml of acetonitrile were added to a 1-liter volumetric flask containing approximately 400 ml of water. The resulting solution was diluted to volume with water and mixed. The mobile phase was filtered through 0.45 micron filter paper. The proportion of acetonitrile may be modified in the range 30% to 38% to obtain the desired Ampicillin retention time. Increasing the proportion of acetonitrile will decrease retention time, decreasing the proportion of acetonitrile will increase retention time.

Standard and Sample Diluent – 35:65, acetonitrile:water.

Sample Diluent for Ampicillin/Probenecid Oral Suspensions – USP 1% pH 6.0 phosphate buffer.

System Suitability

A solution of Ampicillin Trihydrate reference material was prepared at 2.5 mg Ampicillin/ml. 25-µl was injected at 0.1 AUFS. The retention time of Ampicillin was between 4.5 and 8.5 minutes. If the Ampicillin retention time was not between 4.5 and 8.5 minutes, then new mobile phases would have been prepared, making the appropriate modifications in the acetonitrile concentration, until re-injection of the Ampicillin test solution produced an Ampicillin retention time between 4.5 and 8.5 minutes.

The column efficiency, based on the Ampicillin peak, was greater than 2100 plates per column as determined using the following formula:

$$\text{Efficiency (plates/column)} = 5.54 \left(\frac{\text{Retention Time}}{\text{Peak Width at Half Height}} \right)^2$$

where the retention time and peak width at half height were in the same units. If the efficiency was inadequate, then the column would have been replaced and the system suitability re-done.

Standard Preparation

Ampicillin Trihydrate in-house reference standard (assigned potency 862 mcg ampicillin activity/mg versus USP Ampicillin Trihydrate Standard Lot No. H-RD at 832 mcg/mg) was used 'as is'. Approximately 146 mg was accurately weighed into a 50-ml volumetric flask. The standard was dissolved in and diluted to volume with standard diluent. The solution was stable for six hours.

Internal Monitor Stock Solution

A solution of o-o'-biphenol was prepared in standard diluent at 0.8 mg/ml. An appropriate aliquot was pipetted into standards and samples prior to diluting to volume to produce a final concentration of 0.08 mg/ml.

Standardization & Injection Sequence

A standard was injected at the beginning of the assay, after every six samples, and at the end of the assay. Each sample was injected once. Each sample was calculated using the average response factor of the standard injections that 'bracket' it.

Sample Preparation

Ampicillin Trihydrate Bulk Powder — approximately 146 mg of bulk powder was accurately weighed into a 50-ml volumetric flask. The sample was dissolved in and diluted to volume with sample diluent.

TABLE 1

Preparation of Sodium Ampicillin Injectables

Activity/Vial From Label (mg)	Appropriate Vol. Flask (ml)	Further Vol. Dilution
125	50	None
250	100	None
500	200	None
1000	100	25.0/100.0
2000	200	25.0/100.0
10000	200	10.0/200.0

Sodium Ampicillin Bulk Powder

Approximately 130 mg of bulk powder was accurately weighed into a 50-ml volumetric flask. The sample was dissolved in and diluted to volume with sample diluent.

Sodium Ampicillin Injectables

Using small portions of sample diluent, the contents of one vial were completely transferred to an appropriate volumetric flask. The sample was dissolved in and diluted to volume with sample diluent. Using the same sample diluent, further volumetric dilutions were made to achieve an acceptable final concentration as indicated in Table 1.

Ampicillin Trihydrate Capsules

The contents of ten capsules were combined. A portion of the combined Ampicillin Trihydrate capsule contents, equivalent to 125 mg of Ampicillin activity, was accurately weighed into a 50-ml volumetric flask. The sample was diluted to the neck of the flask with sample diluent. With occasional swirling, the sample was sonicated for five minutes until all particles were finely suspended. The sample was diluted to volume with sample diluent and mixed. The sample was filtered through 0.6 micron filter paper placed in a disc filter holder.

TABLE 2

Preparation of Multiple Dose Ampicillin Trihydrate Oral Suspensions (5 ml dose)

Activity from Label (mg/5 ml)	Appropriate Volumetric Flask (ml)
125	50
250	100
500	200

Multiple Dose Ampicillin Trihydrate Oral Suspensions (5 ml dose)

125 mg/5 ml, 250/5 ml, 500 mg/5 ml Ampicillin Activity — using a burette, the contents of the bottle were reconstituted with water as per label directions. The bottle was mechanically shaken in a horizontal position for 25 minutes. The bottle was inverted several times just prior to sampling to evenly resuspend all particles. Using a 5.0-ml glass syringe fitted with a 13-gauge needle, sufficient suspension was withdrawn to wet the syringe. The suspension was expelled back into the bottle. Slightly more than a 5.0-ml dose was withdrawn from the bottle. The syringe was inverted and brought to 5.0-ml expelling all air bubbles. The 5.0-ml dose was ejected into the appropriate volumetric flask (as indicated in Table 2) already one-half full of sample diluent. The sample was diluted to volume with sample diluent and mixed. The sample was sonicated five minutes and filtered through 0.6 micron filter paper placed in a disc filter holder.

Single Dose Ampicillin Trihydrate Oral Suspensions

125 mg/bottle, 250 mg/bottle, 500 mg/bottle Ampicillin Activity — using a glass syringe, the contents of the bottle were reconstituted with water as per label directions. The bottle was mechanically shaken in a horizontal position for 25 minutes. The bottle was inverted several

TABLE 3

Preparation of Single Dose Ampicillin Trihydrate Oral Suspensions

Activity/Bottle From Label	Appropriate Volumetric Flask (ml)
125	50
250	100
500	200

times just prior to sampling to evenly re-suspend all particles. The entire contents were drained into the appropriate volumetric flask (as indicated in Table 3) for 30 seconds. The sample was diluted to volume with sample diluent and mixed. The sample was sonicated five minutes and filtered through 0.6 micron filter paper placed in a disc filter holder.

Single Dose Ampicillin Trihydrate/Probenecid Oral Suspension

3.5 g/bottle Ampicillin Activity. Using a burette, the contents of the bottle were reconstituted as per label directions. The bottle was mechanically shaken in a horizontal position for 25 minutes. The bottle was inverted several times just prior to sampling to evenly re-suspend all particles. The entire contents were drained into a 1-liter volumetric flask for 30 seconds. The sample was diluted to volume with 1% pH 6.0 phosphate buffer and mixed. A 75.00-ml aliquot was transferred to a 100-ml volumetric flask and diluted to volume with 1% pH 6.0 phosphate buffer. The sample was filtered through filter paper discarding the first 10 ml of filtrate.

Calculations — Ampicillin Response Factor

$$F = \frac{W_{STD} \times P_{STD}}{A_{STD} \times 50 \text{ ml} \times 1000 \text{ mcg/mg}}$$

TABLE 4

DF Values For Injectables

Ampicillin Potency (mg/vial)	DF
125	50
250	100
500	200
1000	400
2000	800
10000	4000

Ampicillin Trihydrate & Sodium Ampicillin Bulks

The potency in mcg/mg was calculated using the following formula:

$$P_{SAMP} = \frac{F \times A_{SAMP} \times 50 \text{ ml} \times 1000 \text{ mcg/mg}}{W_{SAMP}}$$

Sodium Ampicillin Injectables

The potency in mg/vial was calculated using the following formula:

$$V_{SAMP} = F \times A_{SAMP} \times DF$$

Ampicillin Trihydrate Capsules

The potency in mg/average capsule weight was calculated using the following formula:

$$C_{SAMP} = F \times A_{SAMP} \times \frac{ACW}{W_{SAMP}} \times 50 \text{ ml}$$

Ampicillin Trihydrate Oral Suspensions

The potency in mg/5 ml dose or mg/bottle was calculated using the following formula:

$$OS_{SAMP} = F \times A_{SAMP} \times DF$$

TABLE 5

DF Values For Ampicillin Trihydrate Oral Suspensions

Ampicillin Potency (mg/5 ml dose) or (mg/bottle)	DF
125	50
250	100
500	200
3500	1333

Where: F = Ampicillin standard response factor

P_{SAMP} = Ampicillin bulk potency, mcg/mg

P_{STD} = Ampicillin standard potency, mcg/mg

A_{SAMP} = Ampicillin peak area of sample

A_{STD} = Ampicillin peak area of standard

W_{STD} = Standard weight, mg

W_{SAMP} = Sample weight, mg

V_{SAMP} = Ampicillin potency, mg/vial

C_{SAMP} = Ampicillin potency, mg/average capsule weight

ACW = Average capsule weight

OS_{SAMP} = Ampicillin potency, mg/5 ml dose or mg/bottle

DF = Dilution Factor

Specificity

The specificity of the method was determined by injecting precursors of Ampicillin, a degradation product (Penicilloic Acid of Ampicillin), and all Ampicillin Trihydrate capsule and oral suspension excipients. The specificity of the assay was further demonstrated by force degrading both Ampicillin Trihydrate and Sodium Ampicillin Bulk under heat, acidic, basic, aqueous, and UV light conditions. Actual Ampicillin injectable, capsule, and oral suspension formulations as well as excipient placebo blends were

force degraded under heat, aqueous, and UV light conditions. These degraded samples were chromatographed to check for visible interferences. Three detection wavelengths (254, 280, and either 227, 233, or 241 nm) were monitored for these degradation samples. Ampicillin peak height ratios, among the three wavelengths, for these degradation samples were compared to the peak height ratios of an undegraded sample of Ampicillin. On a given day and instrument, the peak ratios of undegraded and degraded Ampicillin should be different if interfering degradation products with different absorptivities than Ampicillin are present. The absorptivity of Ampicillin at 280 nm is very poor. Monitoring 280 nm was a method of determining whether any interfering degradation products were produced.

Recovery Studies

Ampicillin Trihydrate was spiked into two different capsule excipient blends at the 50%, 100%, and 150% levels. Ampicillin Trihydrate was spiked into two different oral suspension excipient blends at the 50%, 100%, and 150% levels.

The precursors of Ampicillin; Penicillin V, Phenylglycine, and 6-APA and the degradation product, Penicilloic Acid of Ampicillin were completely resolved from Ampicillin (Fig. 1). None of the capsule excipients or oral suspension excipients interfered with Ampicillin (Fig. 2).

Ampicillin Trihydrate and Sodium Ampicillin Bulk were degraded thermally, in 0.01N HCl, in 0.01N KOH, in water, and by accelerated light (3 x 10^{16} photons/sec/cm^2 at 254 nm). Elevated temperatures were used for the acid and aqueous degradations to facilitate otherwise slow degradation.

Sodium Ampicillin injectables were degraded thermally, by accelerated light, and upon reconstitution with water. Elevated temperature was

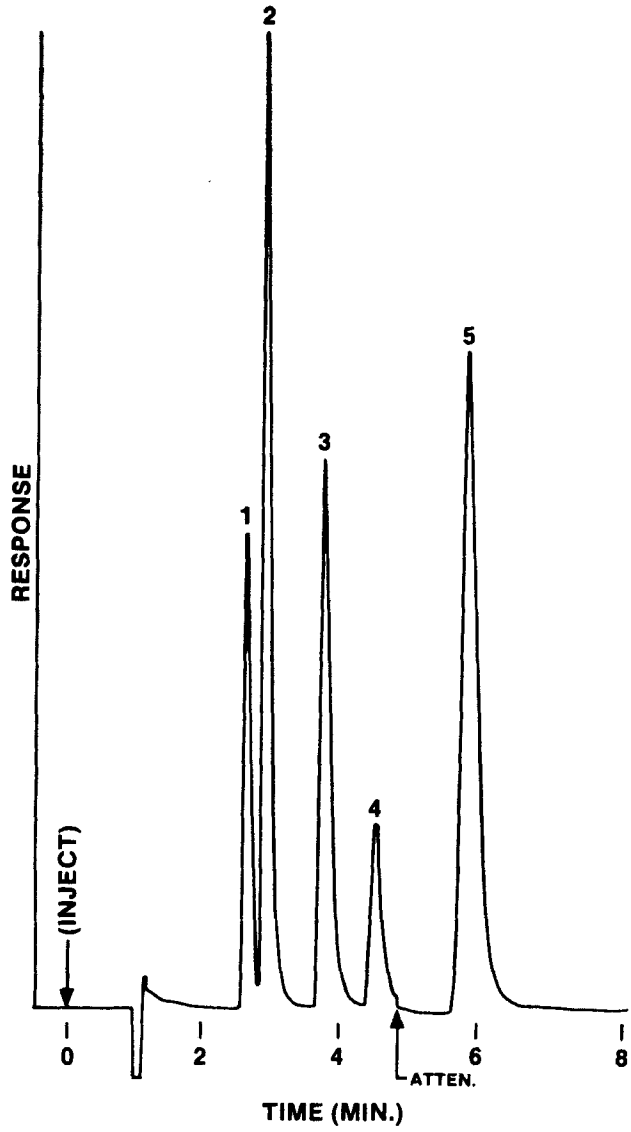

Figure 1 — Separation of Phenylglycine (1), 6-APA (2), Penicillin V (3), Penicilloic Acid of Ampicillin (4), and Ampicillin (5).

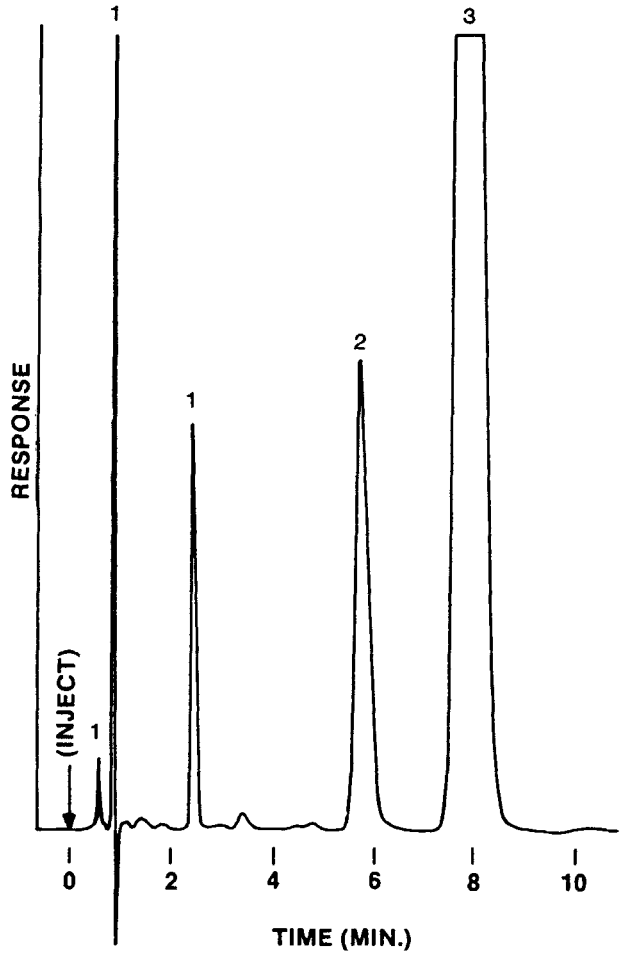

Figure 2 — Chromatogram of an authentic Ampicillin Oral Suspension.
Key: 1 = Excipients; 2 = Ampicillin; 3 = Probenecid.

used for the aqueous degradation. The degradations were performed in the injectable vial to allow any interferences from the container to form.

Ampicillin Trihydrate capsule contents, the intact capsule, and a placebo blend were degraded thermally, by accelerated light, and in water. Elevated temperature was used for the aqueous degradation.

Ampicillin Trihydrate oral suspension blend and the intact oral suspension bottle were degraded thermally and by accelerated light. The oral suspension blend was degraded in water with elevated temperature.

Approximately 15-50% degradation was targeted for, although in some cases more or less degradation was produced.

No visibly interfering degradation products were produced in any of the aforementioned forced degradations of drug substance or placebo blends. The stress conditions were purposely chosen to be more severe than any conditions to which the product may be subjected. Many unknown degradation peaks were produced.

The peak height ratios, among the three wavelengths monitored, for degraded Ampicillin compared to undegraded Ampicillin were very consistent. This is one confirmation of the stability-indicating nature of the assay. At 280 nm, where Ampicillin's absorptivity is very low, no interfering degradation peaks appeared.

Forced degradation studies produced an appreciable degradation of Ampicillin (15-50%), the possibility of finding an internal standard that will be absolutely free of potential interferences from the degradation products of Ampicillin or the excipients present in the drug forms is doubtful.

We used an internal monitor, a compound that elutes in a zone free from interference in undegraded drug forms (Fig. 3). The internal

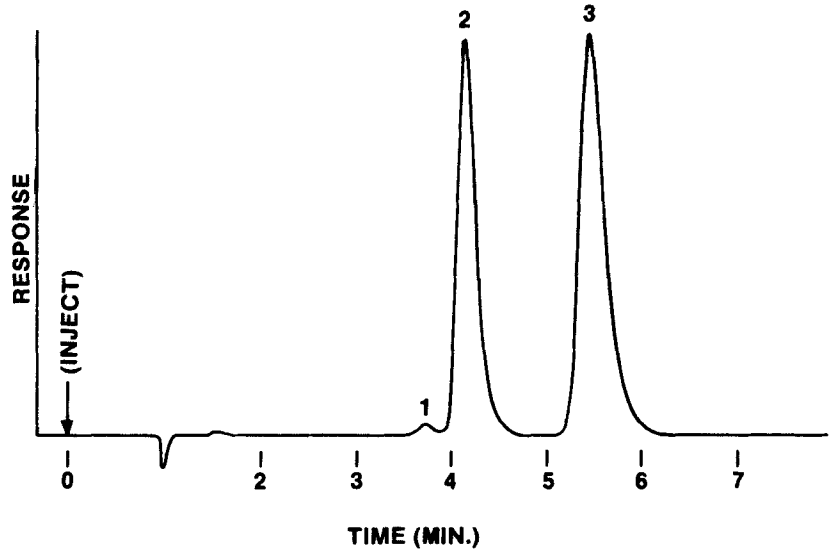

Figure 3 — Chromatogram of Sodium Ampicillin and internal monitor. Key: 1 = Penicilloic Acid of Ampicillin, 2 = o-o´-biphenol (internal monitor) 3 = Ampicillin.

monitor served to monitor the injection volume of the autoinjector. Due to its potential of having an interference in degraded drug forms, it was not and cannot be used as an internal standard to calculate assay results.

Six injections of one preparation of both Ampicillin Trihydrate and Sodium Ampicillin were made at the 2.5 mg/ml level. The reproducibility of injection of Ampicillin Trihydrate bulk (2 RSD = 1.2%) and Sodium Ampicillin bulk (2 RSD = 1.1%) was found to be excellent.

Both Ampicillin Trihydrate and Sodium Ampicillin were injected in triplicate at 1.25, 1.90, 2.50, and 3.10 mg/ml. The levels were chosen to correspond to 50%, 75%, 100%, and 125% of expected sample level.

Linearity of response versus Ampicillin concentration for Ampicillin Trihydrate was found to be linear in the range tested

TABLE 6

Linearity of Ampicillin Trihydrate & Sodium Ampicillin

Compound	Concentration Range Tested mg/ml	Correlation Coefficient	Percent Deviation From Origin, %
Ampicillin Trihydrate	1.23-3.09	0.99986	−0.6
Sodium Ampicillin	1.28-3.09	0.9985	−0.7

TABLE 7

Calculated Biases For Ampicillin Trihydrate & Sodium Ampicillin Over The Range of 50% to 125% of Sample Level

Compound	Actual Conc. mg/ml	Calculated Conc. mg/ml	% Deviation
Ampicillin Trihydrate	3.089	3.101	0.4%
	1.870	1.877	0.4%
	1.232	1.230	−0.2%
Sodium Ampicillin	3.093	3.044	−1.6%
	1.908	1.907	<0.1%
	1.276	1.235	−3.2%

TABLE 8

Ampicillin Recovery From Capsule Blends

Blend No.	Spike Added mg/ml	Spike Recovered mg/ml	% Recovery
1	3.563	3.520	98.7
1	2.484	2.486	100.1
1	1.298	1.290	99.4
2	3.791	3.782	99.8
2	2.514	2.517	100.1
2	1.267	1.270	100.2

(correlation coefficient = 0.99986) and the percent deviation from the origin was extremely small (intercept x 100/response at standarization level). Linearity of response versus Ampicillin concentration for Sodium Ampicillin was linear (correlation coefficient = 0.9985) and the percent deviation from the origin was extremely small (Table 6).

Using single-point standardization at the 100% Ampicillin level, the biases calculated for Ampicillin Trihydrate at the 50, 75, and 125% levels was not greater than 1% (relative). The biases for Sodium Ampicillin were not more than 2% (relative) except for the 50% level which was −3.2%. This was not considered a serious bias especially at the 50% level. Refer to Table 7.

Based on the data in Tables 6 and 7, single-point standardization may be used.

Table 8 shows the accuracy of the procedure for two different capsule excipient blends. Recovery in all cases at three different Ampicillin levels was >98%.

Table 9 shows the accuracy of the procedure for two different oral suspension excipient blends. Recovery in all cases at three different Ampicillin levels was >98%.

TABLE 9

Ampicillin Recovery From Oral Suspension Blends

Blend No.	Spike Added mg/ml	Spike Recovered mg/ml	% Recovery
1	3.782	3.778	99.9
1	2.502	2.512	100.4
1	1.257	1.269	101.0
2	3.742	3.748	100.2
2	2.494	2.466	98.9
2	1.259	1.249	99.2

TABLE 10

Overall Procedural Variability

Sample No.	Ampicillin Trihydrate Bulk, mcg/mg	Sodium Ampicillin Bulk, mcg/mg	Injectable 250 mg/vial	Capsule 250 mg/cap	Capsule 500 mg/cap
1	830	887	247	240	473
2	832	883	250	236	472
3	828	880	242	237	472
4	826	889	237	233	473
5	826	883	244	234	473
6	825	889	245	235	473
Average	828	885	244	236	473
RSD (%)	0.3	0.4	1.8	1.0	0.1

Sample No.	Oral Suspension 125 mg/5 ml	Oral Suspension 250 mg/5 ml	Oral Suspension 500 mg/5 ml	Oral Suspension 3.50 g/bottle
1	118	244	496	3.48
2	120	239	496	3.54
3	120	242	487	3.71
4	118	231	482	3.55
5	117	237	491	3.53
6	120	237	475	3.54
Average	119	238	488	3.56
RSD (%)	1.1	1.9	1.7	2.2

Six different preparations of Ampicillin Trihydrate bulk, Sodium Ampicillin bulk, Sodium Ampicillin injectables, two lots of Ampicillin Trihydrate capsules, and four lots of Ampicillin Trihydrate oral suspensions were assayed by this procedure.

Table 10 shows the overall procedural variability of the method for all the dosage forms studied. As the sample handling became more involved, the RSD increased.

TABLE 11

Ampicillin Stability in 1% pH 6.0 Phosphate Buffer

Time (hr.)	% Ampicillin Trihydrate Remaining	Time (hr.)	% Sodium Ampicillin Remaining
0	100.0	0	100.0
2.8	98.9	2.0	99.5
3.8	98.0	3.0	98.9
4.9	96.6	4.0	97.4
5.9	95.7	5.1	97.2
6.9	95.1	6.1	96.0
8.0	93.1	7.1	94.5
9.0	92.8	8.2	92.8
10.0	92.0	9.2	89.8

TABLE 12

Ampicillin Stability in 35:65, Acetonitrile:Water

Time (hr.)	% Ampicillin Trihydrate Remaining	% Sodium Ampicillin Remaining
0	100.0	100.0
0.4	100.7	100.3
1.0	100.2	99.7
1.9	100.0	99.7
2.3	99.6	99.6
2.9	99.5	99.6
3.3	99.4	99.9
3.8	99.6	99.0
4.2	99.1	99.6
4.8	99.3	99.3
5.2	99.2	99.7
5.8	99.1	99.4
6.1	98.8	98.8
6.7	99.9	98.9
7.1	99.3	98.2

TABLE 13

Automated, Unattended Assays

LABORATORY 1			LABORATORY 2		
Time (hr.)	Ampicillin Trihydrate Lot 1, mcg/mg	Sodium Ampicillin Lot 1, mcg/mg	Time (hr.)	Ampicillin Trihydrate Lot 2, mcg/mg	Sodium Ampicillin Lot 2, mcg/mg
0	855	902	0	821	873
0.4	861	905	0.4	821	874
0.9	858	901	0.8	821	872
1.3	856	902	1.4	821	876
1.9	857	901	1.8	822	873
2.3	854	900	2.2	819	876
2.9	854	901	2.8	821	875
3.2	853	904	3.1	819	873
3.8	852	894	3.5	823	871
4.2	847	898	4.1	822	874
4.8	846	894	4.5	824	872
5.2	845	896	4.9	822	872
5.7	852	902	5.4	821	879
6.0	849	896	5.8	828	881
6.6	866	904	6.2	818	890
7.0	860	898	6.8	797	882
			7.2	829	870
			7.6	817	870
Average	854	900		820	874
RSD (%)	0.6	0.4		0.8	0.5

The precision of the bulk, capsule and oral suspension results (125, 250, 500 mg/5 ml) also depends on uniformity of blend as well as the HPLC method. The precision of the injectable and oral suspension (3.50 g/bottle) results depend on the uniformity of blend and fill weight as well as the HPLC method.

Tables 11 and 12 show the stability of both Ampicillin Trihydrate and Sodium Ampicillin in two proposed assay diluents.

Ampicillin is stable in 35:65, acetonitrile:water for approximately six hours (<1% degradation). The degradation is very slow and an Ampicillin standard prepared at the same time as the samples

TABLE 14

Comparison of HPLC versus Iodometric Assay For
Ampicillin Trihydrate & Sodium Ampicillin

	Ampicillin Trihydrate mcg/mg Ampicillin		Sodium Ampicillin mcg/mg Ampicillin	
Lot	HPLC[a]	Iodometric[a]	HPLC[b]	Iodometric[c]
1	848	856	872	872
2	850	834	890	879
3	822	805	886	886
4	854	838	885	874
5	840	864	884	886
6	854	846	893	876
7	854	850	905	903
8	853	846	881	889
9	854	854	899	896
10	858	847	894	878
11	838	843	890	886
12	846	849	894	892
13	856	848	900	891
14	841	835	897	889
15	848	846	896	886
16	800	836	882	890
17	839	856	869	876
18	844	846	879	844
19	848	842	879	889
20	827	842	877	886
Average	844	844	887	883

a Average of duplicate preparations.

b Average of five preparations.

c Average of four preparations.

will exhibit the same degradation. Therefore, if samples are calculated versus standard injections that 'bracket' those samples, no apparent loss will be evident and automated, unattended assays may be performed for up to six hours. Table 13 shows the assay results of two different laboratories using the standard 'bracketing' technique.

Table 14 shows the comparison of the HPLC method to the iodometric method for twenty lots of Ampicillin Trihydrate and twenty lots of

Sodium Ampicillin. There was no significant difference between the means at the 95% confidence level as determined by the null hypothesis for material of this quality.

CONCLUSION

The described HPLC procedure has proven to be applicable to various Ampicillin formulations. One set of HPLC parameters successfully assayed all the formulations tested; separated precursors, degradation products, and formulation excipients; and showed good agreement with the iodometric assay. An assay diluent in which Ampicillin is stable was found. This will allow for the full benefits of the new autosampling equipment and be amenable to quality control operations.

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