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**Note**

# High-performance liquid chromatographic determination of amoxycillin in pharmaceutical dosage forms

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Recently, several chemical methods for the determination of amoxycillin in biological fluids have been reported. Miyazaki *et al*[1]. described the determination of amoxycillin based on the formation of a fluorescent derivative. The high-performance liquid chromatographic (HPLC) determination of amoxycillin and ampicillin in biological fluids was reported by Vree *et al.*[2]. Subsequently, Lee *et al.*[3], and Carlqvist and Westerlund[4] described more sensitive HPLC methods involving post-column derivatization.

To date, HPLC methods have not been directed to the analysis of amoxycillin in pharmaceutical dosage forms. The present official assay method of the British Pharmacopoeia[5] involves a colourimetric determination based on the copper(II) ion catalysed formation of the penicillenic acid. This method was recommended previously for ampicillin but has been replaced by the method of Bundgaard[6] involving colourimetric determination of penicillins by reaction in imidazole–mercury solution. The iodometric method is official in the Code of Federal Regulations[7].

Canadian drug quality assessment programs require reliable and time-saving methods for the analysis of large numbers of samples. Due to the lack of specificity of the official methods, a reversed-phase HPLC method for the determination of amoxycillin in bulk drug substance and oral preparations was developed.

## EXPERIMENTAL

An SP 8000 liquid chromatograph (Spectra-Physics, Santa Clara, CA, U.S.A.), equipped with a SP 8300 fixed-wavelength detector (254 nm) and with a data system, was employed during the study. A reversed-phase column (RP-8, 10 $\mu$m, 25 cm $\times$ 4.6 mm I.D., Brownlee Labs., Santa Clara, CA, U.S.A.) was employed at 30°C with a flow-rate of 1.0 ml/min. Injections of 10 $\mu$l were used for all solutions to be analysed.

### *Mobile phase*

The mobile phase consisted of 6% (v/v) methanol in 0.05 *M* phosphate buffer (pH 5.0).

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*Solutions*

*Internal standard stock solution.* Phenoxyacetic acid was dissolved in water, 4 g/l.

*Bulk drug substance and capsule solutions.* To an accurately weighed amount of bulk drug substance or homogeneous capsule contents equivalent to 20 mg of amoxycillin, was added 2.0 ml of internal standard solution and the volume made to 10.0 ml with water. A small stirring bar was then added and the contents of the flask stirred until dissolution was complete (0.5–2 h). The amoxycillin standard (USP Amoxycillin Reference) solution was prepared in a similar manner.

## RESULTS AND DISCUSSION

A typical chromatogram of an amoxycillin capsule formulation is shown in Fig. 1. Excipients from capsule formulations did not interfere.

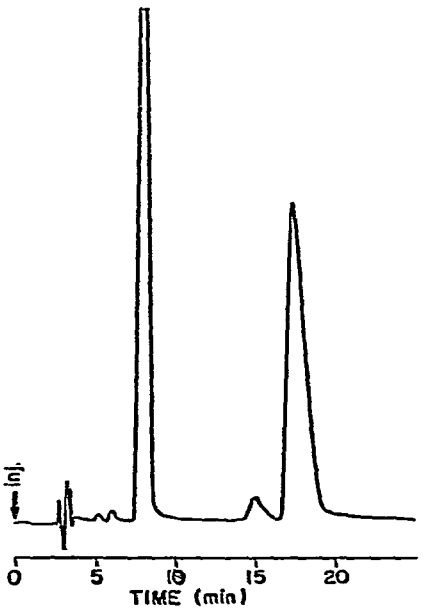


Fig. 1. Chromatogram of amoxycillin capsule contents on a reversed-phase column (RP-8) with a mobile phase of 6% methanol in 0.05 *M* phosphate (pH 5.0).

Table I shows the relative retention times of amoxycillin and other related compounds.

The linearity of the chromatography system was verified by injection of six solutions containing amoxycillin from 0.5 to 2.5 mg/ml and 0.8 mg/ml internal standard. A straight line, coefficient of correlation = 0.9998 ($y = 0.877x - 0.0197$), was obtained when the ratios of the area counts of the amoxycillin divided by the area counts of the internal standard were plotted *versus* concentration of amoxycillin.

TABLE I

RELATIVE RETENTION TIMES OF AMOXYCILLIN AND OTHER RELATED COMPOUNDS

| *Compound* | *Relative retention time** |
|---|---|
| D(—) *p*-Hydroxyphenylglycine | 0.38 |
| Amoxycilloic acid | 0.76 |
| 6-Aminopenicillanic acid | 0.83 |
| Amoxycillin | 1.00 |
| Methyl paraben | 1.93 |
| Phenoxyacetic acid (internal standard) | 2.22 |
| Ethyl paraben | 3.97 |
| Propyl paraben | 7.65 |

* See text for chromatographic conditions.

Ten consecutive injections of a solution of amoxycillin (2 mg/ml) resulted in a relative standard deviation of the area ratio of the amoxycillin peak to that of the internal standard of 0.72%.

A number of samples of bulk drug substance were analysed for amyxocillin content by HPLC. These samples were also determined by the colourimetric method of Bundgaard[6] based on the reaction of penicillins with imidazole and mercuric chloride. The results are shown in Table II. Good correlation between the HPLC and chemical assay was obtained. Additionally the water content of each bulk was determined by the standard Karl Fischer techniques. Generally, the bulk drugs having a higher potency, *e.g.* USP and BP standards, also showed a higher water content.

TABLE II

HPLC AND CHEMICAL ASSAY OF AMOXYCILLIN BULK DRUG SUBSTANCE

| *Sample* | *Potency** (μg/mg)* | | *Water (%)* |
|---|---|---|---|
| | *HPLC* | *Chemical* | |
| USP standard | 850** | 850** | 13.97 |
| BP Standard | 853 | 855 | 14.49 |
| Manufacturer 1 A | 885 | 885 | 14.45 |
| B | 819 | 791 | 12.70 |
| Manufacturer 2 A | 806 | 799 | 11.98 |
| B | 806 | 824 | 12.41 |
| Manufacturer 3 A | 806 | 825 | 12.02 |
| B | 830 | 839 | 11.77 |
| Manufacturer 4 A | 852 | 850 | 12.20 |
| B | 842 | 848 | 11.60 |
| C | 819 | 816 | 12.30 |
| Manufacturer 5 | 833 | 836 | 12.34 |
| Manufacturer 6 | 815 | 829 | 12.11 |

* Average of duplicate determinations. All values based on USP Standard.
** Label claim.

Capsule formulations from four manufacturers were also assayed by both the HPLC and chemical assay methods. The results are presented in Table III. Again good correlation between the two methods was obtained. Ten replicate determinations of sample A from manufacturer number 3 gave a mean of 93.5% and relative standard deviation of 0.58%.

TABLE III

HPLC AND CHEMICAL ASSAY OF AMOXYCILLIN CAPSULE FORMULATIONS

| *Sample* | *Label claim (%)** | |
|---|---|---|
| | *HPLC* | *Chemical* |
| Manufacturer 1 A | 96.8 | 97.3 |
| B | 101.6 | 99.5 |
| Manufacturer 2 A | 95.6 | 95.9 |
| B | 97.3 | 96.1 |
| Manufacturer 3 A | 94.0 | — |
| B | 97.1 | 97.9 |
| Manufacturer 4 | 92.4 | 89.1 |

* Average of duplicate determinations. All results relative to USP Standard.

Most chromatograms of bulk drug samples and formulations contained a small peak eluting prior to amoxycillin, relative retention time, 0.76. Degradative studies showed this to be the penicilloic acid of amoxycillin. Relative to the amoxycillin peak and based on relative peak areas,there was *ca.* 0.2–0.6% of the penicilloic acid present in the sample solutions. 6-Aminopenicillanic acid is also eluted prior to amoxycillin; however, it is only partially resolved from the penicilloic acid of amoxycillin.

The HPLC system described is a rapid, precise and accurate method for the specific analysis of amoxycillin bulk drug substance and solid oral dosage forms.

REFERENCES

1 K. Miyazaki, O. Ogino, H. Sato, M. Nakano and T. Arita, *Chem. Pharm. Bull.*, 25 (1977) 253.
2 T. B. Vree, Y. A. Hekster, A. M. Baars and E. van der Kleijn, *J. Chromatogr.*, 145 (1978) 496.
3 T. L. Lee, L. D'Arconte and M. A. Brooks, *J. Pharm. Sci.*, 68 (1979) 454.
4 J. Carlqvist and D. Westerlund, *J. Chromatogr.*, 164 (1979) 373.
5 *British Pharmacopoeia, 1973*, Her Majesty's Stationery Office, London, 1973.
6 H. Bundgaard, *J. Pharm. Pharmacol.*, 26 (1974) 385.
7 *Code of Federal Regulations*, Title 21, Part. 440, 1979, U.S. Government Printing Office, Washington, DC, 1979.