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CONTROLLED-RELEASE PENICILLIN COMPLEXES

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND ASSAY

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SUMMARY

High-performance liquid chromatographic assays for the controlled-release penicillin complexes benzathine–cloxacillin, benzathine–penicillin V, procaine–penicillin G, benethamine–penicillin G and benzathine–penicillin G are described. Reversed-phase ion-paired chromatography using related aqueous acetonitrile mobile phases enables both components of the complex to be determined simultaneously. The utility of the methods is demonstrated by application to various formulations including fortified injections, which contain two complexes, and a suspension which also contains four preservatives.

INTRODUCTION

Complexes of penicillin with strongly basic amines have been developed to provide depot-therapy and the controlled release of the penicillin over a period of time. Such complexes are considerably less soluble than penicillins at physiological pH. This causes a controlled availability of the penicillin and also enables liquid oral suspensions of adequate stability to be formulated[1–3]. Complexes which are presently available include benzathine–cloxacillin[4], benzathine–penicillin V[5], benzathine–penicillin G[6], benethamine–penicillin G[7] and procaine–penicillin G[8]. The official compendia maintain assay procedures for quality control. The monograph for fortified benethamine penicillin injection[9] requires that the content of benzylpenicillin sodium (penicillin G), procaine penicillin, benethamine penicillin and total penicillins be established. These are determined by iodine–thiosulphate back-titration after separation of the less soluble complexes, UV absorbance assay, extraction and sulphuric

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acid–borax back-titration and an iodine–thiosulphate back-titration on the whole sample. Such methods are complex, time-consuming, non-specific and precision is difficult to maintain. Although chromatographic methods are readily available for the assay of penicillins[10–21] until recently few methods were available for complexes[22–25], and these required derivatisation[12], detected only one component[22,23,25] or involved relatively long separation times[24]. Renewed interest in penicillin complexes has resulted in the appearance of methods which allow rapid detection and quantification of both components[26–28]. Our interest in the physicochemical aspects of release and interaction of these complexes leads us to present high-performance liquid chromatographic assays for a range of penicillin complexes which allow simultaneous quantification of penicillin and amine in simple and fortified formulations.

EXPERIMENTAL

Apparatus and conditions

Analyses were performed using a high-performance liquid chromatography (HPLC) interface constructed from an Altex 100A dual-reciprocating, constant-flow solvent-metering pump, a Rheodyne 7120 injection valve fitted with a 20-μl loop and a Pye LC3 variable-wavelength ultraviolet monitor, equipped with an 8-μl flow cell and operated at a wavelength of 258 nm with a variable sensitivity of 0.02–0.04 a.u.f.s. (benzathine), 0.08–0.16 a.u.f.s. (penicillin G), 0.16 a.u.f.s. (penicillin V and cloxacillin), 0.32 a.u.f.s. (procaine) and 0.02 a.u.f.s. (benethamine). Chromatography was performed using a Shandon 10 cm × 4.6 mm I.D. stainless-steel column packed with ODS-Hypersil 5 μm reversed-phase material. Pre-filtered mobile phase (0.45 μm) was delivered at a rate of 1 ml/min and the column was thermostatted at 34°C to enhance column efficiency. Mobile-phase compositions were:

(1) Benzathine–cloxacillin: acetonitrile (33%) in phosphate buffer (4.54 g/l potassium dihydrogen phosphate; 6.3 g/l sodium heptane-1-sulphonate) adjusted to an apparent pH of 3.40 with phosphoric acid.

(2) Benzathine–penicillin V: as for benzathine–cloxacillin but with final adjustment to pH 3.50.

(3) Procaine–penicillin G and benzathine–penicillin G: acetonitrile (28.5%) in phosphate buffer (4.32 g/l potassium dihydrogen phosphate; 6.00 g/l sodium heptane-1-sulphonate) adjusted to pH 3.17 with phosphoric acid.

(4) Benethamine–penicillin G fortified: acetonitrile (28.5%) in phosphate buffer (4.54 g/l potassium dihydrogen phosphate; 6.30 g/l sodium heptane-1-sulphonate) adjusted to pH 3.17 with phosphoric acid.

Materials

The preparations analysed were: benzathine–cloxacillin B.P.C. (unformulated), benzathine–penicillin V (unformulated), procaine–penicillin injection B.P. (containing procaine–penicillin B.P.), benethamine–penicillin injection fortified B.P.C. (containing benethamine–penicillin B.P.C., procaine–penicillin B.P. and benzylpenicillin sodium B.P.), benzathine–penicillin injection (containing benzathine–penicillin B.P.), benzathine–penicillin injection fortified B.P.C. (containing benzathine–penicillin B.P., procaine–penicillin B.P. and benzylpenicillin potassium B.P.) and benzathine–penicillin suspension (containing benzathine–penicillin B.P.). 1-Heptane sulphonic acid

was supplied by Fluka, cloxacillin sodium, benzathine–cloxacillin and benzathine diacetate came from Beecham Pharmaceuticals Research Division Labs., Worthing, U.K. Benethamine diacetate came from Glaxo Operations U.K., Ulverston, Cumbria, U.K. Procaine–penicillin injection B.P., benethamine–penicillin injection fortified, benzathine–penicillin injection, benzathine–penicillin injection fortified B.P.C. and benzathine–penicillin suspension were obtained from a retail pharmacy. Solvents were HPLC grade. Water was double-distilled from glass vessels. All other materials were standard laboratory reagents.

Sample preparation

Benzathine–cloxacillin. A series of calibration solutions was prepared by dissolving cloxacillin sodium (75 mg) and benzathine diacetate (30 mg) in the same solvent as the benzathine–cloxacillin samples and adjusting to 100 ml. Dilutions were made to yield 20, 40, 60, 80 and 100% solutions.

Benzathine–penicillin V. A series of calibration solutions were prepared by dissolving penicillin V sodium (24 mg) and benzathine diacetate (12 mg) in the same solvent as the benzathine–penicillin V samples and adjusting to 100 ml. Dilutions were made to yield 20–100% solutions.

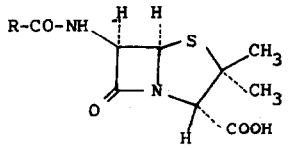
Procaine–penicillin injection B.P. The dry powder was reconstituted for injection by adding 8 ml of water and shaking. One ml of this suspension was diluted to 250 ml with water. All of the suspended material dissolved. Formulation details of the injection were not known, and it was not possible to prepare standards of procaine and penicillin G in identical solvents to the samples, however, dilution of the injection was high (1:250), thus the solvent composition was taken to approximate to water. Standards were therefore prepared in unbuffered water. One sample of procaine–penicillin injection B.P. [1 ml containing 300 mg procaine–penicillin G and equivalent to procaine hydrochloride (139.0 mg) and penicillin G sodium (181.6 mg)] was diluted 1:250 and aliquots were further diluted with water to produce samples of 80, 60, 40 and 20%. A standard containing 55.6 mg of procaine hydrochloride and 72.6 mg of penicillin G sodium in 100 ml was similarly diluted. Penicillin G exhibited a linear peak height *vs.* concentration relationship. Ion-paired procaine, in a similar way to benzathine (Fig. 6) produced a line with some deviation from linearity.

Benethamine–penicillin injection fortified B.P.C. The contents of one ampoule [containing benethamine-penicillin G (475 mg), procaine-penicillin G (250 mg) and penicillin G sodium (300 mg), equivalent to penicillin G sodium (762 mg), benethamine diacetate (184 mg) and procaine hydrochloride (116 mg)] were reconstituted with 1.3 ml of water. The resulting suspension was dissolved in 100 ml of methanol and then diluted to 500 ml with water. A series of standard solutions was prepared by dissolving procaine hydrochloride (25 mg) and penicillin G sodium (150 mg) in methanol–water (20:80) (100 ml) and diluting to give 20–100% solutions. A linear response between concentration and response was found for benethamine and penicillin G. Procaine produced a slightly curved response.

Benzathine–penicillin G. Due to its low solubility in common solvents, benzathine–penicillin G was dissolved in formamide before dilution with water. Samples were prepared by dissolving the injection [1 ml; containing benzathine–penicillin G (229 mg), equivalent to penicillin G sodium (179.6 mg) and benzathine diacetate

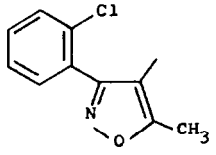
(90.8 mg)] in formamide (50 ml) and diluting to 250 ml with water. One ml of injection was dissolved in 50 ml of formamide and diluted to 250 ml with water. A series of standard solutions were prepared by dissolving benzathine diacetate (40 mg) and penicillin G sodium (75 mg) in formamide–water (20–80) (100 ml) and diluting to give 20–100% solutions. Penicillin G produced a straight line relationship between concentration and response, but benzathine gave a slightly curved calibration line.

Benzathine–penicillin injection fortified B.P.C. The contents of one ampoule [containing benzathine–penicillin G (458 mg), procaine–penicillin G (300 mg) and penicillin G potassium (190 mg) equivalent to penicillin G sodium (722 mg), procaine hydrochloride (139 mg) and benzathine diacetate (182 mg)] were reconstituted with 1.5 ml of water, dissolved in 100 ml of formamide and diluted with water to 500 ml. A series of calibration solutions were prepared by dissolving penicillin G sodium (140 mg), procaine hydrochloride (30 mg) and benzathine diacetate (36 mg) in formamide (20 ml) and diluting to 100 ml with water then further diluting to give 20–100%

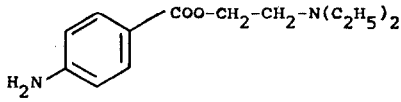


R = $C_6H_5-CH_2-$, Penicillin G, Benzylpenicillin

R = $C_6H_5-O-CH_2-$, Penicillin V, Phenoxymethylpenicillin

R = 

, Cloxacillin



Procaine

$C_6H_5-CH_2-NH-CH_2-CH_2-NH-CH_2-C_6H_5$

Benzathine

$C_6H_5-CH_2-NH-CH_2-CH_2-C_6H_5$

Benethamine

Fig. 1. Component structures of controlled-release penicillin complexes. Complexes with benzathine contain two molecules of penicillin to one of base, other complexes are 1:1.

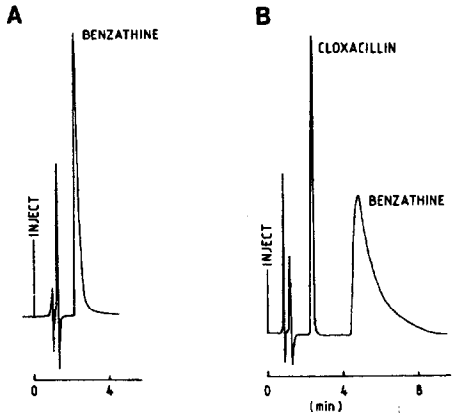


Fig. 2. Chromatography of benzathine–cloxacillin. Mobile phase: (A) acetonitrile–water (15:85), pH 3.12; (B) acetonitrile–water (30:70), pH 6.0.

solutions. Penicillin G exhibited linearity of response, but benzathine and procaine produced slightly curved calibration lines.

Benzathine–penicillin suspension. Two ml of suspension [containing benzathine–penicillin G (91.6 mg) equivalent to penicillin G (71.8 mg) and benzathine diacetate (36.3 mg)] were dissolved in 20 ml of formamide and diluted to 100 ml with water. A series of standard solutions was prepared from penicillin G sodium (75 mg) and benzathine diacetate (38 mg) dissolved in formamide (20 ml) diluted to 100 ml with water and further diluted to give 20–100% solutions. Penicillin G gave a linear response, but benzathine produced a slightly curved relationship.

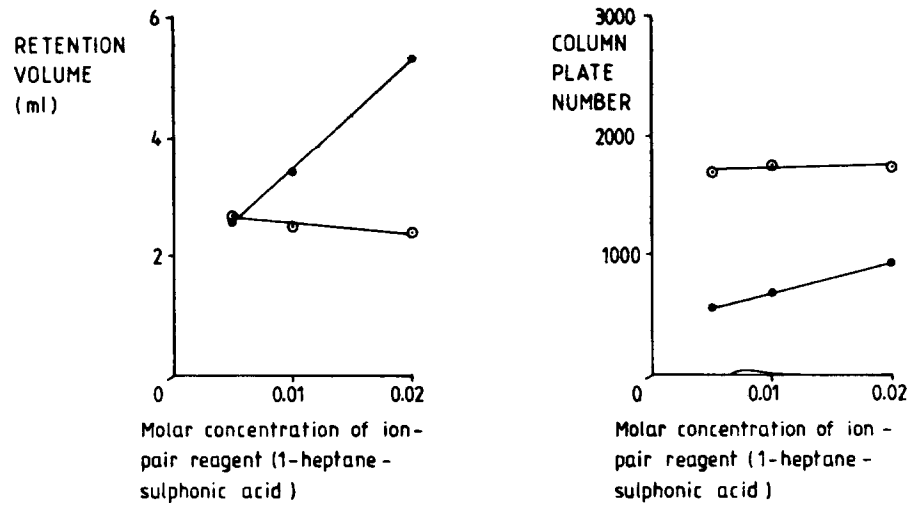


Fig. 3. Effect of ion-pair agent concentration on the chromatography of benzathine (●) and cloxacillin (⊙). Mobile phase: acetonitrile–water (30:70) containing 9.08 g/l KH_2PO_4 adjusted to pH 4.5.

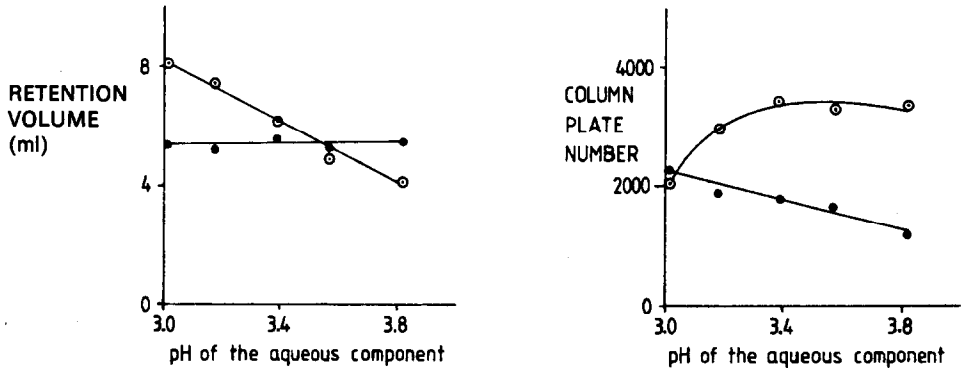


Fig. 4. Effect of pH on the chromatography of benzathine (●) and cloxacillin (⊙). Mobile phase: acetonitrile–aqueous buffer (30:70) containing 9.08 g/l KH_2PO_4 and 6.3 g/l sodium heptane-1-sulphonate.

RESULTS AND DISCUSSION

The controlled-release penicillin preparations (Fig. 1) are composed of an acidic penicillin complexed with a mono- or di-basic amine presented in aqueous vehicles. Reversed-phase chromatography with pH control for the ionisable functions was thus indicated. Fig. 2 shows that both cloxacillin (pK_a 2.7)[29] and benzathine (pK_a 9.2, 6.2) may be eluted as sharp peaks but variation of the pH alone does not allow simultaneous assay. Fig. 3 illustrates the effect of incorporating an ion-pairing agent (sodium heptane-1-sulphonate) into the mobile phase. Benzathine is almost fully protonated at low pH values and the ion-pairing agent interacts strongly with this component retarding its elution. The cloxacillin retention is but little affected and column efficiency is increased. In contrast, pH adjustment in the acid region affects the retention of cloxacillin alone (Fig. 4). At pH 3.0 cloxacillin is eluted later

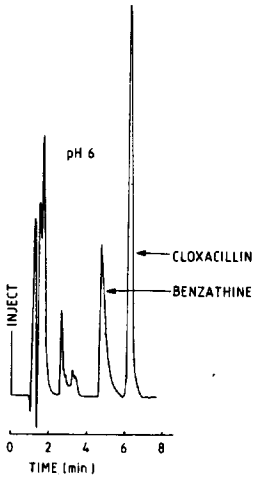


Fig. 5. Separation of benzathine–cloxacillin from cloxacillin degradation products at pH 6.0. Mobile phase: acetonitrile–aquous buffer (33:67) containing 4.54 g/l KH_2PO_4 and 6.3 g/l sodium heptane-1-sulphonate, adjusted to pH 3.0.

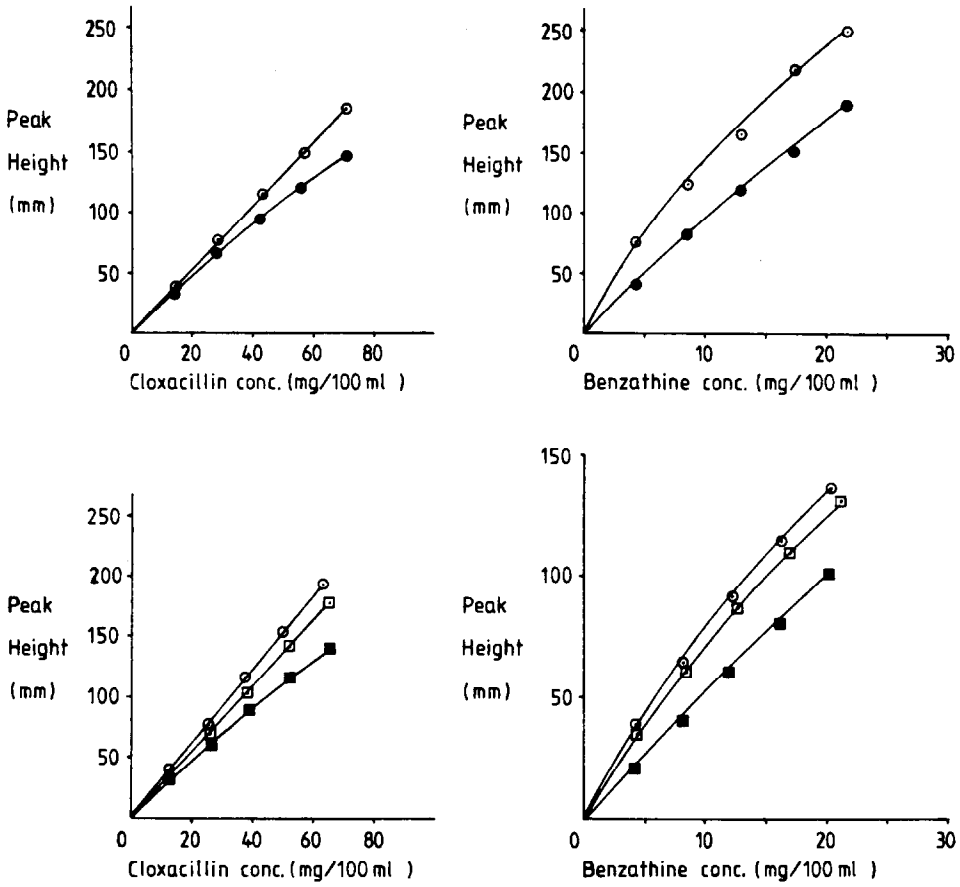


Fig. 6. Effect of sample-solvent on peak heights of benzathine and cloxacillin in water (⊙), buffer at pH 6.0 (●), buffer at pH 9.0 (■), and buffer at pH 9.0 diluted 1 to 6 (⊡).

than benzathine whereas at pH 3.8 it appears at an earlier retention time. This behaviour is in accordance with the expectation that the greater the ionisation of cloxacillin (at higher pH) the shorter is the retention time. Column efficiency is also influenced by the pH effect and a value above pH 3.40, where ionisation is largely complete, appears optimum. An increase in the concentration of buffer salts decreases the retention time of both components. Variation in pH and sodium heptane-1-sulphonate concentration allows total control of the retention times of the individual components of benzathine–cloxacillin. Fig. 5 shows the separation under optimum conditions to allow resolution from possible degradation products of cloxacillin. Under alkaline conditions (pH 9.0) cloxacillin and benzathine undergo reaction together. This product has a very long retention time under the conditions described here[1].

Quantification is best achieved in HPLC by dissolving samples in the mobile phase. If this is not desirable or possible, calibration and test samples should be prepared in the same solvent system. Failure to ensure sample–solvent standardisation may have a profound effect upon accuracy[30,31]. In particular, when the sample

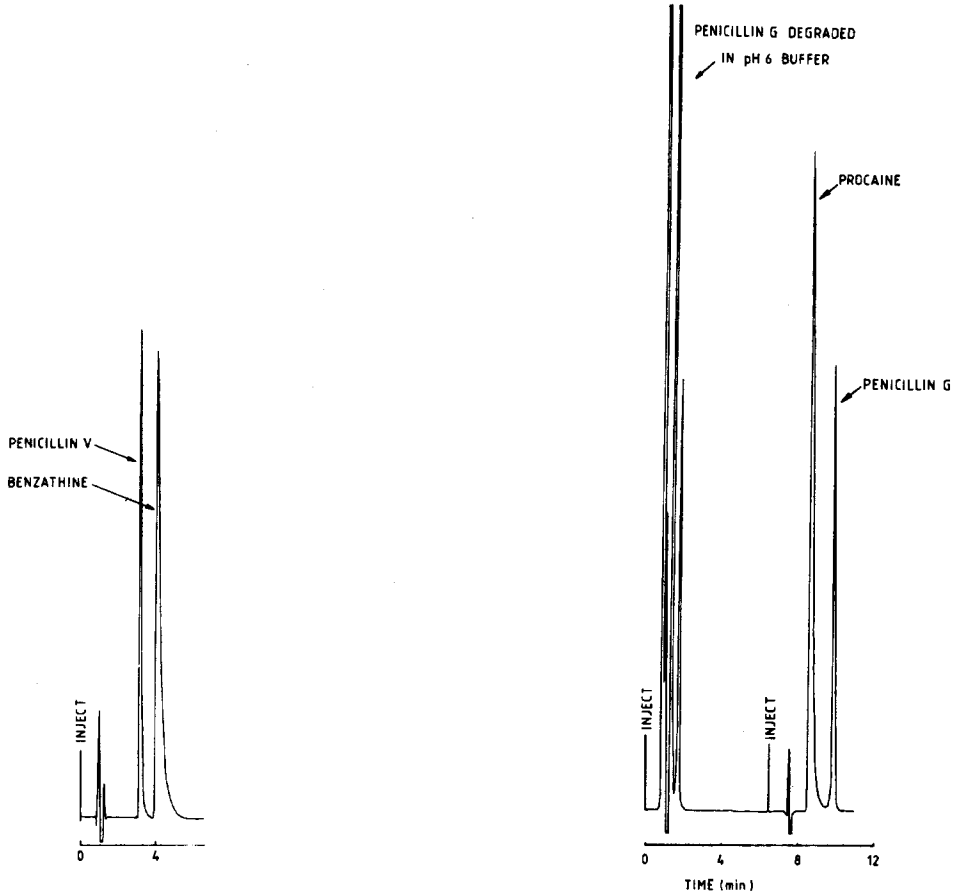


Fig. 7. HPLC separation of benzathine–penicillin V. Mobile phase: acetonitrile–aqueous buffer (33:67) containing 4.54 g/l KH_2PO_4 and 6.3 g/l sodium heptane-1-sulphonate, adjusted to pH 3.50.

Fig. 8. HPLC of procaine–penicillin G from degradation products of penicillin G. Mobile phase: acetonitrile–aqueous buffer (28.5:71.5) containing 4.32 g/l KH_2PO_4 and 6.00 g/l sodium heptane-1-sulphonate, adjusted to pH 3.17.

solvent has more organic modifier than is present in the mobile phase significant peak spreading, with a concurrent loss of sensitivity and accuracy, is observed. This condition is not found in the system developed for benzathine–cloxacillin, because all of the samples were aqueous, but the complex chromatographic mechanisms (ion-pairing for benzathine and ionisation for cloxacillin) makes it susceptible to similar effects. For kinetic work it is necessary to analyse samples maintained at different pH values[1] and the effect of different sample solvents on the chromatography of benzathine and cloxacillin was therefore investigated by dissolving soluble salts of these compounds in water, pH 6 citrate buffer, pH 9 borate buffer, pH 9 borate buffer diluted 1:6 with water and pH 2 citrate buffer (Fig. 6). It was not possible to examine the effects of injecting cloxacillin in pH 2 citrate buffer due to its rapid rate of degradation in this solvent (approximately ten times more rapid than at pH 9). A considerable sample–solvent effect was found with this chromatographic system, and

TABLE I

HPLC ASSAY DATA FOR CONTROLLED-RELEASE PENICILLIN COMPLEXES

Preparation	*Component*	*Assay value (mg/ml)*	*Label claim (mg/ml)*	*Correlation coefficient for calibration line*
Benzathine–cloxacillin	Cloxacillin			0.9997
	Benzathine			0.9879
Benzathine–penicillin V	Penicillin V			0.9998
	Benzathine			0.9998
Procaine–penicillin injection	Penicillin G	174.8, 175.8	170.4	1.0000
	Procaine	132.5, 132.5	120.4	0.9997
Benethamine–penicillin injection fortified	Penicillin G	780 , 790*	736*	0.9980
	Procaine	110 , 114	108	0.9998
Benzathine–penicillin G injection	Penicillin G	173.5, 173.8	173.6	1.0000
	Benzathine	63.5, 63.5	60.5	0.9939
Benzathine–penicillin injection fortified	Penicillin G	745.0, 751.5*	677.8*	0.9999
	Procaine	126.0, 123.7	120.4	0.9999
	Benzathine	124.8, 127.5	121.0	0.9940
Benzathine–penicillin suspension	Penicillin G	177.7, 183.3	168.4	0.9993
	Benzathine	62.3, 63.0	60.5	0.9968

* Assay value in mg/ampoule.

although the precise cause of these effects is not known, it is probably associated with the buffering action of the sample solvent. The sample solvents used in this study were of a different pH to the mobile phase. The greater the buffering action of these sample solvents and the greater the difference in pH between the sample solvent and the mobile phase, the greater the effect they would have on the local pH of the mobile phase immediately after injection. This parameter has a marked effect on the

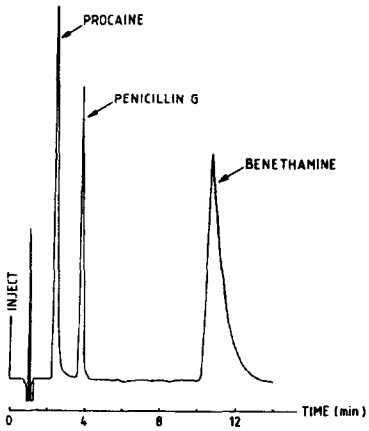
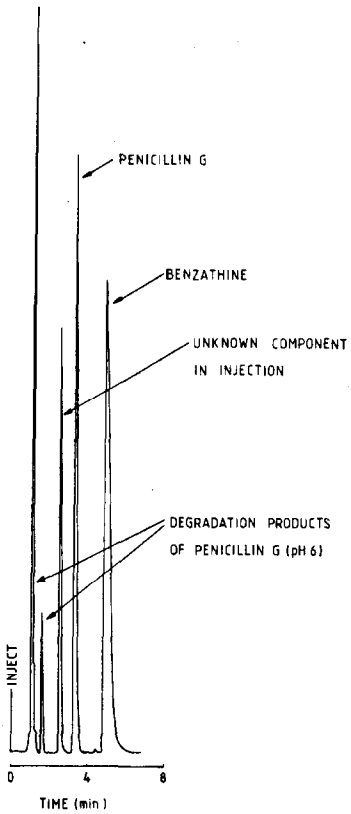


Fig. 9. HPLC of procaine–penicillin G and benethamine–penicillin G. Mobile phase: acetonitrile–aqueous buffer (28.5:71.5) containing 4.54 g/l KH_2PO_4 and 6.30 g/l sodium heptane-1-sulphonate, adjusted to pH 3.17.



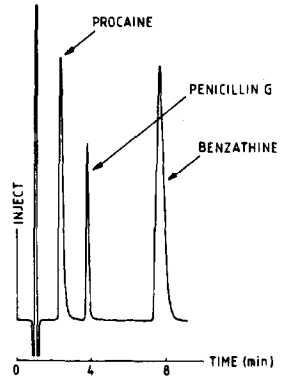


Fig. 10. HPLC of degraded benzathine–penicillin G. Mobile phase: acetonitrile–aqueous buffer (28.5:71.5) containing 4.32 g/l KH_2PO_4 and 6.30 g/l sodium heptane-1-sulphonate, adjusted to pH 3.17.

Fig. 11. HPLC of procaine–penicillin G and benzathine–penicillin G. Mobile phase: acetonitrile–aqueous buffer (28.5:71.5) containing 4.32 g/l KH_2PO_4 and 6.30 g/l sodium heptane-1-sulphonate, adjusted to pH 3.17.

peak-shape and separation of benzathine and may affect the immediate fate of the injected material allowing rapid spreading down the column before equilibrium is achieved.

The only practical way to overcome sample–solvent effects is to prepare standards in solvents of the same composition as the samples. When this is done good accuracy and precision are attained. The analytical conditions developed for benzathine–cloxacillin were modified for the analysis of benzathine–penicillin V by adjusting the mobile phase pH to 3.4. This enhanced the separation of the two components (Fig. 7) and allowed separation from penicillin degradation products. Although sample solvent composition had an effect upon chromatography, this was much smaller than that exposed with benzathine–cloxacillin. Procaine–penicillin G was best analysed with a reduced acetonitrile level (28.5%) at pH 3.17 (Fig. 8). Under these conditions the penicillin degradation product caused no interference. Typical assay results for the determination of procaine–penicillin injection B.P. are displayed in Table I.

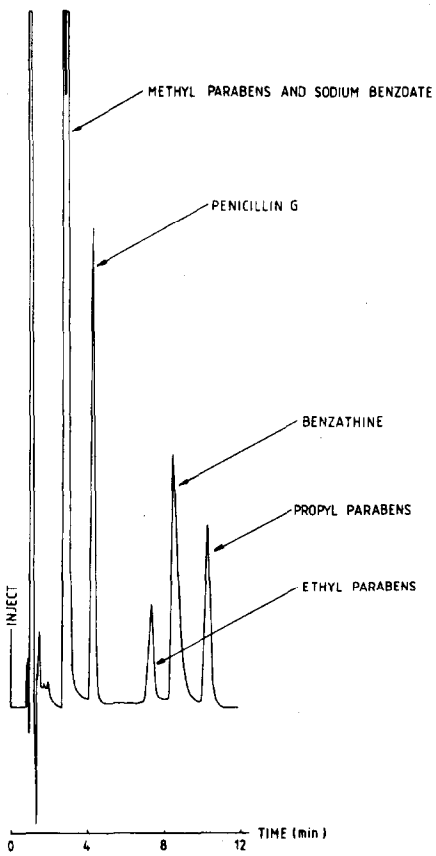


Fig. 12. HPLC of benzathine–penicillin G in an oral suspension. Mobile phase: acetonitrile–aqueous buffer (28.5:71.5) containing 4.54 g/l KH_2PO_4 and 6.30 g/l sodium heptane-1-sulphonate, adjusted to pH 3.17.

Benethamine–penicillin injection fortified B.P.C. contains benethamine–penicillin G, penicillin G and procaine–penicillin G. Solution was achieved in 20% aqueous methanol. The previous conditions were modified by increasing the buffer concentration and the level of ion-pairing agent to enable simultaneous estimation of all three components (Fig. 9). No conditions which improved the peak shape of benethamine whilst still enabling quantification of all three components were found. Penicillin degradation products caused no interference and assay results for this preparation are shown in Table I.

Benzathine–penicillin G injection showed low solubility in common solvents and solution was effected in 20% formamide. The mobile phase suitable for procaine–penicillin was also satisfactory for this preparation (Fig. 10). Assay values are illustrated in Table I. The fortified injection also contains procaine–penicillin G. Formamide is necessary for solubilisation while separation is best achieved with the mobile phase suitable for procaine–penicillin (Fig. 11).

The final product, benzathine–penicillin G suspension, was a more complex chromatographic problem. Suspensions require adequate preservation from micro-

organisms and conditions must be chosen to minimise interference from these additives. Fig. 12 reveals the presence of methyl, ethyl and propyl paraben (*p*-hydroxybenzoate esters) and benzoate as the preservatives and illustrates that adequate separation between these components and benzathine and penicillin G has been achieved. Typical assay results are displayed in Table I.

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REFERENCES

1 W. J. Irwin, J. M. Hempenstall, A. Li Wan Po and A. H. Andrews, *Int. J. Pharm.*, (1984) in press.
2 J. L. Szabo, C. D. Edwards and W. F. Bruce, *Antibiot. Chemother.*, 1 (1951) 499.
3 R. L. Scott, S. F. Colalongo and N. O. Oldroyd, Jr., *Antibiot. Chemother.*, 4 (1954) 691.
4 *British Pharmacopoeia (Vet.)*, HMSO, London, 1977, p. 21.
5 *United States Pharmacopeia XX, National Formulary XV*, U.S. Pharmaceutical Convention, Mack, Rockville, MD, 1980, p. 595.
6 *British Pharmacopoeia*, HMSO, London, 1980, p. 49.
7 *The Pharmaceutical Codex*, Pharmaceutical Press, London, 1979, 11th ed., p. 82.
8 *British Pharmacopeia*, HMSO, London, 1980, p. 656.
9 *British Pharmaceutical Codex*, Pharmaceutical Press, London, 1973, p. 709.
10 I. J. McGilveray and R. D. Strickland, *J. Pharm. Sci.*, 56 (1967) 77.
11 J. Birner, *J. Pharm. Sci.*, 59 (1970) 757.
12 C. Hishta, D. L. Mays and M. Garafolo, *Anal. Chem.*, 43 (1971) 1530.
13 H.-L. Wu, M. Masada and T. Uno, *J. Chromatogr.*,137 (1977) 127.
14 E. R. White, M. A. Carrol, J. E. Zarembo and A. D. Bender, *J. Antibiot.*, 28 (1975) 205.
15 K. Tsugi, in K. Tsugi and W. Morozowich (Editors), *GLC and HPLC Determination of Therapeutic Agents, Part 2*, Marcel Dekker, New York, 1978, pp. 773–780.
16 J. M. Blaha, A. M. Knevel and S. L. Hem, *J. Pharm. Sci.*, 64 (1975) 1384.
17 W. A. Vadino, E. T. Sugita, R. L. Schnaare, H. Y. Ando and P. J. Niebergall, *J. Pharm. Sci.*, 68 (1979) 1316.
18 F. Nachtmann, *Chromatographia*, 12 (1979) 380.
19 F. Barbato, C. Grieco, C. Silipo and A. Vittoria, *Farmaco (Ed. Prat.)*, 34 (1979) 233.
20 W. J. Irwin, J. M. Hempenstall, A. Li Wan Po and A. H. Andrews, *J. Pharm. Sci.*, 72 (1983) 668–673.
21 W. J. Irwin, J. M. Hempenstall, A. Li Wan Po and A. H. Andrews, in preparation.
22 A. Holbrook, *J. Pharm. Pharmacol.*, 10 (1958) 762.
23 M. Le Belle, K. Graham and W. L. Wilson, *J. Pharm. Sci.*, 68 (1979) 555.
24 K. Tsuji, J. F. Goetz and W. Vanmeter, *J. Pharm. Sci.*, 68 (1979) 1075.
25 S. K. Wahba Khalil and W. H. Shelver, *J. Pharm. Sci.*, 65 (1976) 606.
26 F. Nachtmann and K. Gstreinn, *J. Chromatogr.*, 236 (1982) 461.
27 B. Stuber, *Pharm. Acta Helv.*, (1982) 293.
28 M. Puttemans, M. Lippens, L. Dryon and D. L. Massart, *J. Pharm. Biomed. Anal.*, 1 (1983) 99.
29 J. P. Hou and J. W. Poole, *J. Pharm. Sci.*, 60 (1971) 503.
30 K. J. Williams, A. Li Wan Po and W. J. Irwin, *J. Chromatogr.*, 194 (1980) 217.
31 W. J. Irwin, J. M. Hempenstall, A. Li Wan Po and A. H. Andrews, *Lab. Pract.*, (1984) in press.