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The determination of metoclopramide in plasma by reversed-phase ion-pair high-performance liquid chromatography

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Abstract: Methodology based on reversed-phase ion-pair high-performance liquid chromatography is described for the determination of metoclopramide in plasma. The chromatography was optimized in terms of the peak shape for the drug and its resolution from endogenous plasma components by investigating the effects of quaternary ammonium (competing) ions and alkylsulphate (pairing) ions in an acidic mobile phase containing acetonitrile (20%) and 20 mM acetic acid. Optimum chromatographic conditions were obtained with an ODS-Hypersil column and a mobile phase containing 20% acetonitrile, 20 mM acetic acid, 0.6 mM sodium octylsulphate and 0.5 mM tetrabutylammonium chloride. A simplified method of sample preparation is described in which only 1 ml of plasma is required. The limit of detection (at 310 nm) was 7 ng/ml and no interference from endogenous plasma components or from any drugs commonly used in the treatment of cancer was observed. Consequently the methodology should be applicable to pharmacokinetic studies on metoclopramide, when used clinically to control the gastro-intestinal side-effects of chemotherapy.

Keywords: *Metoclopramide; ion-pair HPLC; plasma; chemotherapy.*

Introduction

Antiemetic drugs are frequently used to counteract the severe gastrointestinal disturbances produced as a result of cancer chemotherapy [1–3]. Cisplatin [4] is undoubtedly the antineoplastic agent which causes the most severe nausea and vomiting and drugs such as the phenothiazines [2] and synthetic cannabinoids [2, 3] are of limited value in controlling its side-effects. Recent studies, however, in dogs [5] and man [6, 7] have indicated that high doses of metoclopramide (3–10 mg/kg) can significantly reduce the gastrointestinal side-effects of cisplatin.

Metoclopramide (Fig. 1), a derivative of procainamide, is believed to exert its antiemetic effects in a manner similar to the phenothiazines by blocking dopamine receptors in the chemoreceptor trigger zone located near the fourth ventricle in the area postrema [8]. High intravenous doses of metoclopramide up to 2 mg/kg (given every 2 h up to a total of five doses) have been associated with only a slightly increased incidence

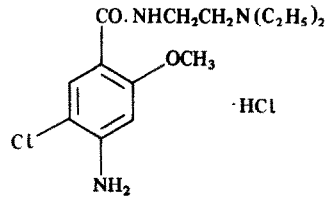

Figure 1
Metoclopramide hydrochloride.

of side-effects [7]. In the studies of Gralla *et al.* [7] 76% of the patients receiving metoclopramide experienced mild sedation. Extrapyramidal reactions appeared to be more common when metoclopramide was given orally in high doses to children and young adults under thirty (R. J. Gralla, personal communication). The increased incidence of side-effects with higher doses of metoclopramide and the suggestion of Lewin *et al.* [9] that there may be a correlation between anti-emetic effect and plasma levels indicates the need for close monitoring of plasma levels and has stimulated the present study.

The earliest methods [10–12] for the determination of metoclopramide in biological fluids involved separation by thin-layer chromatography followed by colorimetry. These methods are relatively tedious and require large volumes of sample to achieve the necessary sensitivity. Bateman *et al.* [13, 14] and Ross-Lee *et al.* [15] have described gas chromatography procedures which employ detection by mass spectrometry [13, 14] or electron capture [15], respectively. Several HPLC procedures [16–19] have been described for the determination for metoclopramide in biological fluids. However, these methods display features which limit their application to the routine monitoring of plasma levels. The normal phase methods [16, 17] require extractions into organic solvents, whereas the methods of Teng *et al.* [16] and Block *et al.* [18, 19] require large volumes of biological fluids to achieve the desired sensitivity.

Two reversed-phase HPLC methods recently described [18–20] both require high concentrations of organic modifiers in the mobile phase to elute the solute from the column. Moreover, the method of Bishop-Freudling and Vergin [20] requires elevated temperatures (50°C). Presumably the high concentrations of organic modifiers in the mobile phase used by these workers [18–20] dictate the rigorous sample pretreatment necessary to remove the polar components of plasma which would otherwise precipitate upon injection.

The method described in the present work employs a reversed-phase HPLC system which can be operated at ambient temperatures with a low concentration of organic modifier (20% acetonitrile) in the mobile phase. Minimal sample pretreatment is necessary and only 1 ml of plasma is required.

Experimental

Materials

Metoclopramide hydrochloride was obtained from A. H. Robins (Richmond, VA, USA) and used as received. Deionized water was used throughout. The glacial acetic acid, tetrabutylammonium chloride (TBAC), hexadecyltrimethylammonium chloride (HTAB), sodium hexylsulphate (SHS) and sodium octylsulphate (SOS) were all reagent grade and were obtained from Fisher Scientific Company (Fairlawn, NJ, USA). The sodium acetate, methanol and acetonitrile were HPLC grade and were also obtained from Fisher.

Chromatography

A modular liquid chromatograph was constructed from a Constametric II pump, a Rheodyne 7125 injector fitted with a 20-μl loop and a Spectromonitor D variable wavelength UV detector (all from LDC/Milton Roy, Riviera Beach, FL, USA). The detector output (0–10 mV) was monitored by a Fisher 'Recordall' D-5000 chart recorder. A flow-rate of 2.0 ml/min was used throughout.

The ODS-Hypersil column (5 μm, 150 × 4.6 mm, i.d.) was slurry packed using the method previously described [21]. The mobile phases were prepared by diluting mixtures of the organic modifiers and stock solutions of the reagents to volume with deionized water. The mobile phases were filtered through Nylon 66 membranes (0.45 μm, Ranin Instrument Company, Woburn, MA, USA) and degassed *in vacuo*. Capacity ratios ($k' = t_r - t_0)/t_0$) were determined at least in duplicate using an injection of D_2O for the measurement of t_0. The peak asymmetry factors, A_s, were determined at 10% of the peak height [22]. For the determination of metoclopramide in plasma the following mobile phase was used: acetonitrile–water (20:80, v/v), containing 20 mM acetic acid, 0.6 mM SOS and 0.5 mM TBAC.

Plasma preparation

A 1-ml aliquot of plasma was transferred to a 15-ml Corex No. 8060A centrifuge tube (Fisher), and mixed with acetonitrile (4 ml) using a vortex mixer. After allowing to stand for 10 min, the precipitated plasma proteins were separated by centrifugation (2500 *g* for 10 min) and the clear supernatant was transferred to a second centrifuge tube. The solvent was evaporated to dryness at 50°C under a stream of nitrogen and the resultant residue reconstituted in mobile phase. The volume of mobile phase used to reconstitute the residue varied from 100 μl to 1 ml depending on the anticipated concentration of metoclopramide in each sample. A 20-μl aliquot of the reconstituted residue was then injected into the liquid chromatograph.

Results and Discussion

Previous attempts [18, 19] to develop a reversed-phase HPLC method for metoclopramide have employed mobile phases containing high concentrations of organic modifier. It is reasonable to assume that under these conditions [18, 19] a significant contribution to retention arises from interactions between the polar moieties of the solute and the residual silanol groups on the surface of the stationary phase [23–25]. It was found that this contribution to retention could be eliminated by the use of an ODS-Hypersil column and the addition of a competing quaternary ammonium ion (HTAB or TBAC) to the acidic mobile phase [23]. This substantially reduced the concentration of organic modifier required to elute metoclopramide from the column. Moreover, elution of the solute could be achieved from the column at ambient temperature.

Initial studies were performed with 0.1 mM HTAB in the mobile phase, when it was found that metoclopramide was poorly retained (k' 0.50) with mobile phases containing 0.02 M acetic acid and greater than 20% acetonitrile. Although decreasing the acetonitrile concentration below 20% produced adequate retention of the solute, the peaks tailed badly (A_s>3.0). Eluents based on maintaining the mobile phase concentration of acetonitrile at 15% and replacing the acetic acid with an acetate buffer (0.2 M, pH 2.8–5.4) also proved unsuccessful. The capacity ratio of metoclopramide increased from 0.6 at pH 2.8 to 2.2 at pH 5.4. However, this increase in retention was paralleled by

increases in band broadening and peak asymmetry. The increased retention with increasing pH may be attributed to the decreasing protonation of the aniline group, which in turn decreases the affinity of the solute for the mobile phase [26]. Increasing the pH of the mobile phase would also be expected to increase the activity of acetate ions (pK_a 4.76, [27]) available for ion-pair formation with the remaining cationic groups on the solute. Dissociation of the metoclopramide-acetate ion-pairs in the stationary phase would account for the poor peak shape observed at higher concentrations of acetate in the mobile phase.

The results obtained with acetate suggested that the retention of metoclopramide could be modified by the addition of a more hydrophobic pairing ion to the mobile phase [28]. Sodium hexylsulphate (SHS) and sodium octylsulphate (SOS) were investigated. However, they proved to be incompatible with HTAB, due to the precipitation of what was presumed to be hexadecyltrimethylammonium alkylsulphate. Consequently, subsequent studies (Fig. 2) were performed with 0.1 mM TBAC which was found to be compatible with the alkylsulphates and was equally effective in eliminating the interactions of the solute with the residual silanols. With a mobile phase of 20% acetonitrile, 0.02 M acetic acid and 0.1 mM TBAC, the capacity ratio for metoclopramide was 0.7.

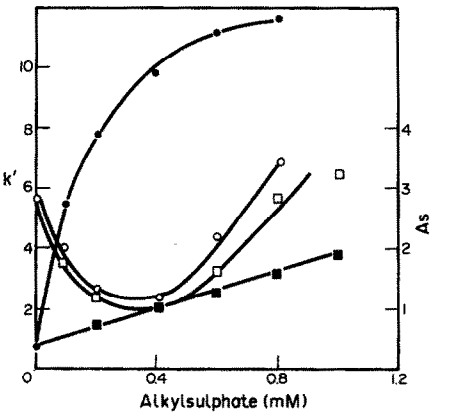

Figure 2
Effect of sodium octylsulphate (circles) and sodium hexylsulphate (squares) concentration on the capacity ratio, k', (closed symbols) and peak asymmetry, A_s (open symbol) of metoclopramide. Stationary phase; ODS-Hypersil (5 μm; 150 mm × 4.6 mm, i.d.). Mobile phase: 0–10 mM alkylsulphate in acetonitrile–water (20:80 v/v), containing 0.02 M acetic acid and 0.1 mM TBAC; temperature, ambient; flow-rate, 2.0 ml/min; injection volume, 20 μl; detection, 272 nm; solute concentration, 5 μg/ml.

Increasing the concentration of SHS and SOS in the mobile phase increased the retention of metoclopramide (Fig. 2), the effect being greatest for the more hydrophobic pairing ion (SOS). Figure 2 also shows the effect of alkylsulphate concentration on peak asymmetry and it can be seen that values of A_s approaching unity could be achieved at a concentration of 0.4 mM alkylsulphate. The combination of 0.1 mM TBAC and 0.4 mM SOS produced adequate retention and good peak shape for metoclopramide, although it resulted in incomplete resolution of the drug from the plasma components.

The separation was optimized in terms of peak shape and resolution from the interfering components of plasma (Fig. 3) by further adjustment to the concentrations of TBAC and SOS. Optimum conditions ($k' = 7.5$, $A_s = 1.15$) were obtained with a mobile phase of 0.02 M acetic acid, 0.6 mM SOS, 0.5 mM TBAC in acetonitrile–water (20:80, v/v). With a fixed concentration of SOS (0.6 mM), increasing the concentration of TBAC from 0.1 mM to 0.5 mM decreased the capacity ratio of metoclopramide from 11.1 to 7.5 and produced a marked improvement in peak shape ($A_s = 1.15$ compared with 2.30). Interestingly, if the concentration of the quaternary ammonium ion were increased from

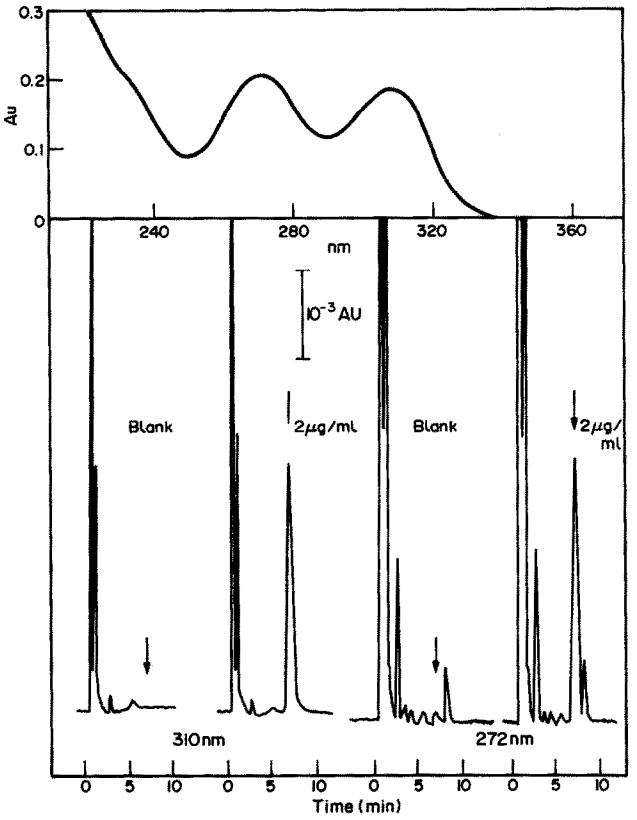

Figure 3
Chromatograms of blank plasma and plasma containing 2 μg/ml metoclopramide hydrochloride, showing the effects of detection at 310 and 272 nm. The UV spectrum shown above the chromatograms was obtained with a solution of metoclopramide hydrochloride dissolved in mobile phase using a Cary 219 UV–visible scanning spectrophotometer. The chromatographic conditions were: Stationary phase, ODS-Hypersil (5 μm; 150 × 4.6 mm, i.d.). Mobile phase: 0.5 mM TBAC, 0.6 mM SOS, 0.02 M acetic acid and acetonitrile–water (20:80 v/v); temperature, ambient; flow-rate, 2.0 ml/min; injection volume, 20 μl. The spectroscopic conditions were: slit-width, 1 nm; scan speed, 2 nm/s; range, 0–0.5 a.u.f.s.; chart speed, 20 nm/cm; pathlength, 1 cm. Solute concentration, 1.65×10^{-5} M metoclopramide hydrochloride in mobile phase.

0.1 mM to 0.5 mM, this had little effect on the retention and peak shape of metoclopramide when using acetate as a pairing ion.

Assay validation

Having achieved the optimum chromatographic conditions, the procedure for the analysis of plasma samples was validated in terms of selectivity, sensitivity, linearity of response and recovery from plasma.

The selectivity of the technique for the determination of metoclopramide in plasma arises from the specificity of the chromatographic separation and the appropriate choice of the wavelength of detection. Metoclopramide exhibits two UV absorption bands (Fig. 3) with maxima at 272 nm (ε = 12 700) and 307 nm (ε = 11 200). Consequently, maximum sensitivity was observed by detection at 272 nm. However, interference from a minor plasma component co-eluting with metoclopramide limited the usefulness of detection at this wavelength (Fig. 3). Detection at 310 nm completely eliminated the

interference by the plasma background (Figs 3 and 4) and was accompanied by only 8% reduction in sensitivity, compared with detection at 272 nm (Fig. 3). The peak-height ratio, P_{310}/P_{272}, was 0.903 and compared well with the absorbance ratio, A_{310}/A_{272}, of 0.886 obtained by spectroscopy (Fig. 3).

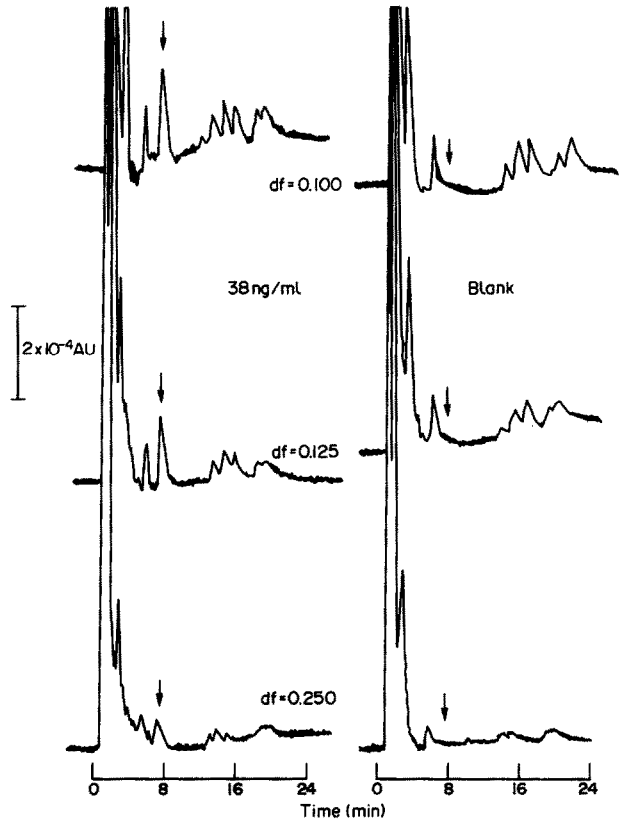

Figure 4
Chromatograms of blank plasma and plasma containing 38 ng/ml metoclopramide hydrochloride showing the effect of dilution factor, DF. Chromatographic conditions as in Fig. 3; detection at 310 nm.

The capacity ratios of drugs commonly used in cancer chemotherapy were determined in order to investigate the possibility that they might interfere with metoclopramide. None of the compounds investigated were found to interfere with metoclopramide (Table 1), since they were either well separated or undetected.

Using an injection volume of 20 μl, the relationship between peak height and concentration was shown to be linear for metoclopramide hydrochloride concentrations from 1–5 μg/ml (equation 1) and 100–500 ng/ml (equation 2).

$$P_{310\ nm} = 22.7\ (0.58)\ [MCP] - 0.60\ (1.83); \quad (1)$$

(correlation coefficient, $r = 0.999$; $n = 5$; detector sensitivity = 0.01 a.u.f.s.)

$$P_{310\ nm} = 115.7\ (1.51)\ [MCP] + 0.15\ (0.47); \quad (2)$$

($r = 0.999$; $n = 5$; detector sensitivity = 0.002 a.u.f.s.).

Table 1
Interference study for the determination of metoclopramide in plasma

Drug*	k'†
Antineoplastics	
Cisplatin (CDDP)	0.10
Methotrexate	1.27
Melphalan (L-PAM)	14.8
Vindesine	49.9
Dacarbazine (DTIC)	0.56
Mitoxanthrone	26.8
Vincristine	‡
Doxorubicin (Adriamycin)	‡
Teniposide (VM-26)	‡
Cyclophosphamide	‡
Actinomycin D	‡
Cytosine arabinoside (Ara C)	‡
Antiemetics	
Chlorpromazine	‡
Metoclopramide	7.45
Anti-Parkinsonian	
Diphenhydramine	‡

* *ca.* 100 μg/ml.
† Capacity ratios with chromatographic conditions as in Fig. 3.
‡ Not detected after 60 min with a flow-rate of 2.0 ml/min.

For equations (1) and (2), the numbers in parentheses are the standard errors of the regression coefficients; [MCP] refers to the concentration of metoclopramide hydrochloride in μg/ml; and $P_{310\ nm}$ is the peak height (in millimeters) at 310 nm.

The absolute limit of detection with an injection volume of 20 μl was 80 ng/ml metoclopramide hydrochloride (71 ng/ml as the free base), equivalent to 1.6 ng MCP injected on-column for a signal/peak-to-peak noise ratio of 2:1. The limit of detection in plasma could be reduced to 8 ng/ml (7.1 ng/ml free base) by reducing the volume of mobile phase used to reconstitute the residue (equivalent to 0.16 ng injected on-column). This introduced a dilution factor, *DF*, into the calculations, as follows:

$$DF = \frac{\text{Volume of mobile phase used to reconstitute}}{\text{Volume of original plasma sample}} \quad (3)$$

Figure 4 confirms that the peak height of metoclopramide (38 ng/ml as the hydrochloride) in plasma was, as expected, inversely proportional to the dilution factor. The limit of detection of 7.1 ng/ml metoclopramide (0.142 ng injected on-column) obtained in this study compares with values of 5 ng/ml and 8 ng/ml reported previously using GC–EC [15] and reversed phase HPLC [19], respectively.

The concentration of metoclopramide, as the free base, (*C*) in plasma was determined by comparing the peak heights of the drug in the plasma extracts (*P*) with the peak

heights (P_s) of an external analytical standard of metoclopramide hydrochloride dissolved in mobile phase, according to equation (4).

$$C = \frac{P \times C_s \times DF \times 0.892}{P_s} \quad (4)$$

where C_s is the concentration of metoclopramide hydrochloride in the analytical standard. The factor 0.892 represents the ratio of the molecular weights of metoclopramide free base to that of the hydrochloride salt.

Depending on the concentration of the drug in the extracts, analytical standards containing either 2 μg/ml or 400 ng/ml metoclopramide hydrochloride were used. The analytical standards were injected after every fourth plasma sample and the mean of the peak heights of the analytical standards bracketting the samples was used for calculation (equation 4). The relative standard deviation ($n = 8$) at 2 μg/ml and at 400 ng/ml for the analytical standards was 0.62% and 2.11%, respectively. By using this method of quantitation, it was not found to be necessary to use an internal standard or to generate daily calibration curves.

The recovery of metoclopramide from plasma was determined by analysing spiked samples containing 2 μg/ml, 200 ng/ml and 40 ng/ml metoclopramide hydrochloride respectively, as shown in Table 2. The recovery from plasma was found to be essentially complete (~100%) and independent of concentration. The recovery experiments (Table 2) also indicated that there was a negligible contribution from the plasma background at 310 nm. This observation was confirmed independently (Figs 3 and 4) by extracting blank plasma samples.

Table 2
Recovery of metoclopramide from plasma

Concentration added (ng/ml)	Concentration found (mg/ml)	Percentage recovery*	Relative S.D. (%)
1680	1704	101.4	1.46
168.0	168.4	100.3	3.76
33.6	33.7	100.1	1.62

$n = 4$ at each concentration studied.

The methodology described here is being applied in this laboratory to pharmacokinetic studies designed to optimize high dose regimens of metoclopramide in children who are also receiving chemotherapy [7]. The results of these studies will be published elsewhere.

Recently the methodology was transferred to a second laboratory necessitating the preparation of additional ODS-Hypersil columns [21]. It was found that although the retention of metoclopramide on the new columns was identical to that seen on the original column, the peaks exhibited greater asymmetry. However, the peak shape could be reproduced using the new columns after they had been conditioned with 50 ml of a solution containing 60% acetonitrile, 0.02 M acetic acid and 10^{-4} M hexadecyltrimethylammonium bromide, followed by 50 ml of a solution containing 20% acetonitrile, 0.02 M acetic acid and 10^{-4} M hexadecyltrimethylammonium bromide. It was found that the columns apparently required no further reconditioning and could be washed periodically with methanol, without affecting the chromatographic behaviour of metoclopramide.

These results indicate that there are two types of residual silanols on the surface of the stationary phase (ODS Hypersil). The first site may only be accessible to hexadecyltrimethylammonium bromide which apparently binds to it irreversibly. The second site may be masked less specifically by either hexadecyltrimethylammonium bromide or by the more bulky tetrabutylammonium bromide.

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References

[1] S. Frytak and C. G. Moertel, *J. Am. Med. Assoc.* **245**, 393–396 (1981).
[2] S. Frytak, C. G. Moertel, J. R. O'Fallon, J. Rubin, E. T. Creagan, M. J. O'Connell, A. J. Schutt and N. W. Schartau, *Ann. Intern. Med.* **91**, 825–830 (1979).
[3] A. E. Chang, D. J. Shilling, R. C. Stillman, N. H. Goldberg, C. A. Seipp, I. Barofsky, R. M. Simon and S. A. Rosenberg, *Ann. Intern. Med.* **91**, 819–824 (1979).
[4] A. W. Prestayko, S. T. Cooke and S. K. Carter (eds), *Cisplatin Current Status and New Development*. Academic Press, New York (1980).
[5] J. A. Gylys, K. M. Doran and J. P. Buyiski, *Res. Comm. Chem. Path. Pharmacol.* **23**, 61–68 (1979).
[6] T. Kahn, E. G. Elias and G. R. Mason, *Cancer Treat. Rep.* **62**, 1106–1107 (1978).
[7] R. J. Gralla, L. M. Itri, S. E. Pisko, A. E. Squillante, D. P. Kelson, D. W. Braun, L. A. Bordin, T. J. Braun and C. W. Young, *New Eng. J. Med.* **305**, 905–909 (1981).
[8] R. M. Pinder, R. N. Brogden, P. R. Sawyer, T. M. Speight and G. S. Avery, *Drugs* **12**, 81–131 (1976).
[9] M. Lewin, B. Meyer, D. Drayer, M. Pasmantier and M. Reidenberg, *Proc. Am. Soc. Clin. Oncol.* **2**, 97 (1983).
[10] O. M. Bakke and J. Segura, *J. Pharm. Pharmacol.* **28**, 32–39 (1976).
[11] G. Berner, B. Emschermann, E. Gladtke, W. Haase, F. Leuschner, U. Vogtle-Junkert and H. H. Wagner, *Arzneim.-Forsch.* **32**, 169–173 (1982).
[12] D. Schuppan, I. Schmidt and M. Heller, *Arzneim.-Forsch* **29**, 151–154 (1979).
[13] D. N. Bateman, D. S. Davies, C. Kahn and K. Mashiter, *Br. J. Clin. Pharmacol.* **6**, 401–407 (1978).
[14] D. N. Bateman, C. Kahn, K. Mashiter and D. S. Davies, *Br. J. Clin. Pharmacol.* **4**, 650P (1977).
[15] L. M. Ross-Lee, M. J. Eadie, F. Bochner, W. D. Hooper and J. H. Tyrer, *J. Chromatogr.* **183**, 175–184 (1980).
[16] L. Teng, R. B. Bruce and L. K. Dunning, *J. Pharm. Sci.* **66**, 1615–1618 (1977).
[17] C. Graffner, P.-O. Lagerstrom and P. Lundborg, *Br. J. Clin. Pharmacol.* **8**, 469–474 (1979).
[18] W. Block and A. Pingould, *Z. Anal. Chem.* **301**, 109–110 (1980).
[19] W. Block, A. Pingould, M. Khan and P. Kjellerup, *Arzneim. Forsch.* **31**, 1041–1045 (1981).
[20] G. B. Bishop-Freudling and H. Vergin, *J. Chromatogr.* **273**, 453–457 (1983).
[21] P. A. Bristow, P. N. Brittain, C. M. Riley and B. F. Williamson, *J. Chromatogr.* **131**, 57–64 (1977).
[22] J. P. Foley and J. G. Dorsey, *Anal. Chem.* **55**, 730–737 (1983).
[23] K.-G. Wahlund and A. Sokolowski, *J. Chromatogr.* **151**, 299–310 (1978).
[24] A. Nahum and C. Horvath, *J. Chromatogr.* **203**, 53–63 (1981).
[25] K. E. Bij, C. Horvath, W. R. Melander and A. Nahum, *J. Chromatogr.* **203**, 65–84 (1981).
[26] C. Horvath, W. Melander and I. Molnar, *Anal. Chem.* **49**, 142–154 (1977).
[27] A. Albert and E. P. Serjeant, *The Determination of Ionization Constants. A Laboratory Manual*, p. 84. Chapman & Hall, London (1971).
[28] S. Eksborg, P.-O. Lagerstrom, R. Modin and G. Schill, *J. Chromatogr.* **83**, 99–110 (1973).

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