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ION-PAIR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC PROCEDURE FOR THE QUANTITATIVE ANALYSIS OF THEOPHYLLINE IN SERUM SAMPLES

JOHN J. LAUFF

Research Laboratories, Eastman Kodak Company, Rochester, NY 14650 (U.S.A.)

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SUMMARY

An ion-pair chromatographic system is described for the separation of theophylline and related xanthines from serum samples. The mobile phase consisted of 0.02 *M* tetrabutylammonium ion and 0.015 *M* Tris buffer in water–acetonitrile–methanol (93:3.5:3.5, v/v/v) at a precisely controlled pH (7.50 ± 0.02, adjusted with hydrochloric acid). The flow-rate was 1.2 ml/min through a 15 cm × 4.6 mm I.D., 5-μm reversed-phase column (Ultrasphere C_{18} ion pair). Xanthines were extracted from serum (100 μl) with 1 ml of acidified chloroform–isopropanol (95:5, v/v). After reconstitution in 200 μl of mobile phase, the extracted xanthines, including theophylline, caffeine, theobromine and 1,7-dimethylxanthine, were baseline-resolved in less than 15 min. The method correlates well with a common clinical immunoassay for theophylline (EMIT Syva, $r^2 = 0.999$) and yields excellent recovery and precision (98–101% and better than 2% at therapeutic levels, respectively). In addition, the use of the ion-pair chromatography mode eliminates many of the interferences noted in the published literature for the common reversed-phase separations of theophylline.

INTRODUCTION

Numerous liquid chromatographic methods for the analysis of theophylline in serum samples have been proposed [1–27]. Although the analyte is easily detected when present in therapeutic concentrations (10–20 mg/l), many exogenous and endogenous substances in serum have been reported to interfere in subsequent reversed-phase chromatographic separations [28–35]. Although normal-phase separations are reported to give excellent separation of theophylline and its metabolites [6,9,21], this mode of chromatography is not routinely used because of the difficulty of maintaining a stable separation. Likewise, gradient elution can resolve many interferences but, in general, it is more time-consuming than isocratic methods [20, 26, 36]. Extraction of the drug from the serum matrix eliminates many interferences; however, a major metabolite of caffeine, 1,7-

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dimethylxanthine, is not completely resolved in most reversed-phase systems. In order to resolve as many interferences as possible, we have developed an ion-pair chromatographic system [37] that retains the ease and stability of isocratic, reversed-phase systems while adding another dimension, the ion-exchange properties of theophylline, to the selectivity of the separation.

In order to obtain a relatively clean extract for the subsequent high-performance liquid chromatographic (HPLC) separation which would then give a stable separation and relatively long column life, the common technique of extraction with organic solvents, as proposed by the AACC committee on theophylline [38], was adopted. To overcome the inadequate selectivity of the reversed-phase modes described in the literature, ion-pair or ion-interaction chromatography was employed [37]. By buffering the mobile phase at pH 7.5, ca. 10% of the theophylline present at equilibrium is in the anionic form. Since the xanthines (caffeine and 1,7-dimethylxanthine) are not ionized appreciably at this pH, the eluent composition can be tailored to maximize the separation of these potential interferents from theophylline. In addition, many of the non-xanthine interferences either are not eluted in this system or are retained much longer than theophylline.

EXPERIMENTAL

Instrumentation

The chromatographic system consisted of a WISP 710B sample processor (Waters Assoc., Milford, MA, U.S.A.) to automatically inject samples, Waters Model 720, a system controller, a Waters Model 6000A reciprocating pump to deliver mobile phase, and a Hewlett-Packard Model 1040A rapid-scan UV–Vis spectrometer to detect absorbance at 280 nm. The column was a reversed-phase 5-μm Ultrasphere ion-pair column, 15 cm$\times$4.6 mm I.D. (Altex), preceded by a 3-cm precolumn packed with the same material. Peak areas were integrated and the area ratios of analyte peaks to internal standard peak were automatically calculated by a Hewlett-Packard Model 1000 laboratory automation system.

A Corning Model 130 pH meter was used to measure the pH of the mobile phase.

Distilled water was passed through a Barnstead Ultrapore cartridge (Cat. No. 09-034-3, Fisher Scientific) to obtain deionized water.

A rotary mixer (Eppendorf Model 5432) and a centrifuge (Eppendorf Model 5414) were used to manipulate samples contained in 1.5-ml plastic centrifuge tubes (VWR).

Materials

Tetra-*n*-butylammonium ($TNBu^+$) hydroxide as 1.0 *M* aqueous solution was purchased from Southwestern Analytical Chemicals (Austin, TX, U.S.A.); Trizma base [tris(hydroxymethyl)aminomethane (Tris)], theophylline, theobromine, 1,7-dimethylxanthine and β-hydroxyethyltheophylline were purchased from Sigma (St. Louis, MO, U.S.A.). Chloroform and caffeine were obtained

from Kodak Laboratory Chemicals (Rochester, NY, U.S.A.). Isopropyl alcohol, acetonitrile and methanol were HPLC grade from J.T. Baker (Phillipsburg, NJ, U.S.A.).

Reagents

Mobile phase was prepared by mixing 20 ml of 1.0 *M* tetra-*n*-butylammonium hydroxide, 910 ml of distilled, deionized water, 35 ml each of methanol and acetonitrile, and 1.82 g of Trizma base. The pH of the solution was adjusted to 7.50 ± 0.03 by the addition of concentrated hydrochloric acid. The eluent was degassed by bubbling with helium before use.

The extraction solution containing internal standard was prepared by mixing 95 ml of chloroform and 5 ml of isopropanol to which 1.5 mg of β-hydroxyethyltheophylline and 25 μl of glacial acetic acid were added.

A standard solution was prepared by dissolving weighed amounts of theophylline, 1,7-dimethylxanthine, theobromine and caffeine in a volumetic flask with deionized water. A calibrator set down to 2 ppm theophylline and similar concentrations of the other three xanthines was then prepared by serial dilution of the high-level standard. For standard additions to serum, accurate volumes of the high-level standard were added to pooled normal serum.

Analytical procedure

A 100-μl volume of sample or standard solution was added to a 1.5-ml plastic centrifuge tube followed by the addition of 1.0 ml of extraction solution containing internal standard. As many as 24 sample tubes were vortex-mixed on a rotary mixer for 10 min. After mixing, if a large emulsion layer was present, the samples were centrifuged for 2–3 min. The upper aqueous phase was removed to waste by aspiration. The organic layer was transferred to a WISP vial, evaporated to dryness under a stream of nitrogen, dissolved in 200 μl of mobile phase and transferred to a low-volume sample vial insert for automatic injection into the chromatograph.

For the chromatographic separation, an isocratic flow-rate of 1.2 ml/min was used with automatic injection of 40 μl of sample. The separation was carried out at room temperature and required less than 15 min. Compounds were detected by absorbance at 280 nm.

For calibration, extracted aqueous standards were injected interspersed among the samples during the chromatographic run. Standardization was by least-squares fitting of the ratios of analyte peak area to internal standard peak area for all standards. From the slope and intercept value and the area ratio obtained from each sample, the concentration of analyte in each unknown was calculated.

RESULTS AND DISCUSSION

Chromatographic system

Initial efforts were directed at improving the reversed-phase separation for theophylline and related xanthines first described by Orcutt et al. [2] and later modified by Miksic and Hodes [3]. In the unmodified Orcutt procedure, overlap of theophylline with 1,7-dimethylxanthine was observed. Since the metabolite of

caffeine can lead to falsely elevated theophylline values, Miksic and Hodes modified the mobile phase to bring about a partial separation of these species; however, dyphylline, a diuretic and cardioactive drug, is eluted nearly identically to theophylline. In addition numerous other drugs, in particular antimicrobial agents, have been reported to interfere in the reversed-phase analysis for theophylline [28–35].

In the interim many reversed-phase separation methods for the analysis of theophylline in serum have been published [4–27]. With the most common reversed-phase column packing, C_8 or C_{18} bonded to silica, the separation of the 1,7-dimethylxanthine metabolite of caffeine from theophylline is marginal [5,7,11,13,15,16] or not addressed [4,8,10,12,14,19], unless gradient elution is used [10,26,36]. With a less common, reversed-phase packing (phenyl bonded to silica) good separations of the caffeine metabolite from theophylline have been reported using isocratic elution [20,22].

With C_8 or C_{18} reversed-phase materials the addition of $TNBu^+$ [23–25,34,36] and other amine modifiers [27] to the mobile phase has been demonstrated to improve the separation of 1,7-dimethylxanthine from theophylline. However, at the pH values reported for use in the mobile phase (4–6), theophylline is not appreciably ionized, as indicated in Fig. 1. Thus ion pairing or dynamic ion exchange cannot be important in the separation mechanism. Most likely, the additive is modifying the surface properties of the column [27]. In any case, while baseline-separated, the 1,7-dimethylxanthine metabolite is still eluted just before theophylline as in the C_8 or C_{18} reversed-phase separations without $TNBu^+$ addition. Also, it has been our experience that dyphylline is eluted in this region and may constitute a positive interferent in the theophylline assay.

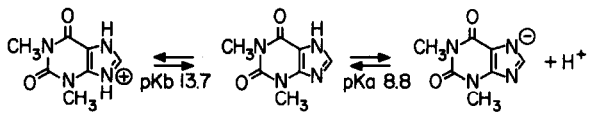
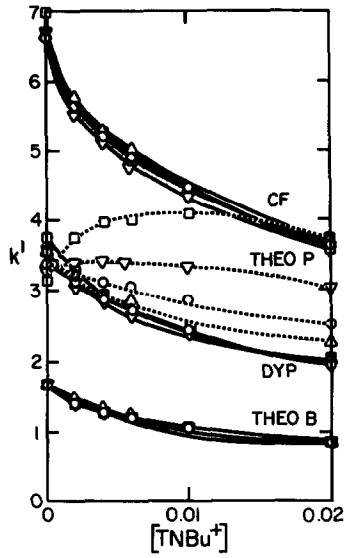


Fig. 1. Acid–base behavior of theophylline.

While earlier methods utilizing $TNBu^+$ as a mobile phase additive do not take full advantage of the potential benefits of ion pairing or dynamic ion exchange, as indicated in Fig. 1, by judicious choice of pH and $TNBu^+$ concentration in the eluent, theophylline retention relative to the other xanthines can be shifted dramatically by a dynamic ion-exchange effect. The effect is graphically illustrated in Fig. 2, which plots k' for theophylline and related xanthines as a function of $TNBu^+$ concentration at increasing pH values. At any constant pH, as $TNBu^+$ concentration increases, k' for all xanthines decreases, presumably because of competition for surface sites between the analytes and $TNBu^+$. Yet, even at pH 6.5 where less than 1% of theophylline should be ionized, theophylline retention decreases more slowly than the non-ionized xanthines because of ion-exchange retention. As the pH increases, theophylline retention increases relative to the other xanthines until, at pH 7.8 and 0.02 *M* $TNBu^+$ concentration, theophylline can be retained longer than caffeine. These observations are consistent with an



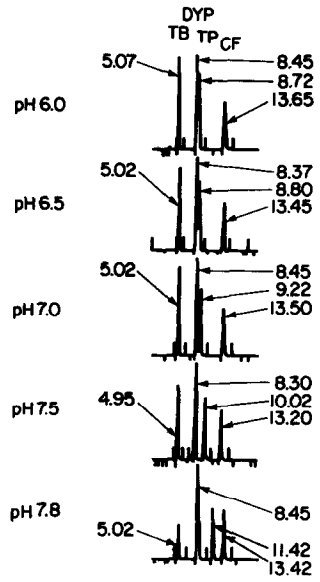


Fig. 2. Variation of k' for xanthines as a function of quaternary amine concentration and pH in the mobile phase (0.002 *M* phosphate buffer, chloride counter-ion). Key: △ = pH 6.5; ○ = pH 7.0; ▽ = pH 7.5; □ = pH 7.8; CF = caffeine; THEO P = theophylline; DYP = dyphylline; THEO B = theobromine.

Fig. 3. Chromatographic effect on the separation of xanthines by varying pH (buffer concentration = 0.002 *M* phosphate; tetrabutylammonium chloride concentration = 0.006 *M*). TB = theobromine. DYP = dyphylline; TP = theophylline; CF = caffeine.

added ion-exchange retention mechanism for theophylline, which does not affect the retention of the other xanthines. These effects are illustrated by the chromatograms in Fig. 3.

In addition to pH and $TNBu^+$ concentration, the relative retention of the xanthines in the ion-pair or ion-exchange mode can also be affected by the type of buffer and its concentration, as well as by the choice of anion and its concentration (counterion to $TNBu^+$). The influence of the concentration of Tris chloride on the separation of the xanthines is shown in Fig. 4. As the concentration of Tris increases, the retention of all components decreases because of competition for available surface sites; however, the retention of theophylline decreases faster relative to the non-ionized xanthines, because it also competes with Tris for ion-exchange sites. The effect of different anions on the separation using phosphate as buffer is shown in Fig. 5. The more polarizable anion competes more effectively for ion-exchange sites with theophylline so that its retention relative to the other xanthines decreases going from chloride to bromide to sulfate.

The final eluent composition chosen for theophylline analysis was determined after separating a number of serum extracts containing theophylline to optimize its separation from other xanthines, as well as other endogenous potential interferences in serum. The separation, nearly identical with that for 0.010 *M* Tris in Fig. 4, leads to complete separation from potential interferents (see Table IV).

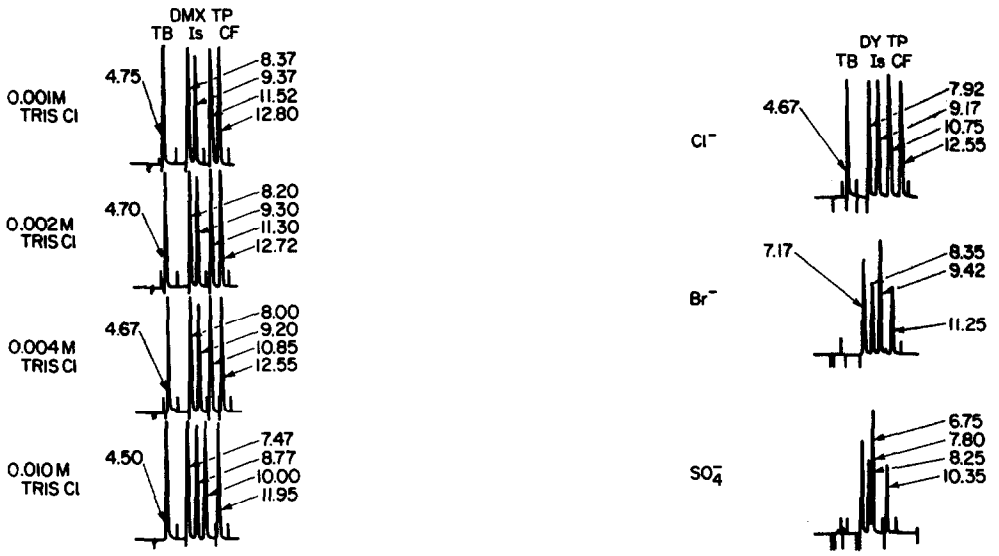


Fig. 4. Chromatographic effect of varying buffer concentration on the separation of the xanthines (pH 7.5, tetrabutylammonium chloride concentration = 0.01 *M*). TB = theobromine; DMX = 1,7-dimethylxanthine; Is = internal standard; TP = theophylline; CF = caffeine.

Fig. 5. Chromatographic effect of varying anion type (pH 7.5, 0.004 *M* Tris, 0.01 *M* tetrabutylammonium ion). TB = theobromine; DY = dyphylline; Is = internal standard; TP = theophylline CF = caffeine.

Prechromatographic treatment

The separation of theophylline from serum proteins is necessary to maintain a stable column and separation system [18,27]. In our experience, removal of proteins by precipitation with acetonitrile as used by Orcutt et al. [2] and others led to build-up of species on the column that were eluted in succeeding chromatograms as very broad peaks. In addition, even with this simple pretreatment, it was necessary to evaporate the acetonitrile and reconstitute the sample with mobile phase to obtain good peak shapes. In contrast, the extraction with acidified chloroform–isopropanol gives a purer extract from which no spurious, late-eluting peaks have been observed. Also, other potential interferents, in particular charged species that might also be extracted with acetonitrile, are eliminated by this procedure. This extractant solution is similar to that proposed by others for the analysis of theophylline in serum [4,6–8,16,22,24,34,36] but was modified by the inclusion of a small amount of acid. The addition of acetic acid increased the recovery of theophylline in the extraction without requiring the use of a large volume of extractant. The use of a relatively small volume minimizes the time for evaporation to almost the same time required for evaporation of acetonitrile when it is used to precipitate proteins.

With a precolumn before the analytical column, more than 300 samples have been analyzed on a single column. At first, with a fresh column, retention times tend to decrease slowly and then stabilize with no evident column deterioration. Only very infrequent replacement of the first few millimetres of column packing

in the precolumn has been needed to maintain baseline resolution of the xanthines.

Calibration

Calibrations were obtained by treating aliquots of a seven-level aqueous calibration set, each of which contained known concentrations of the xanthines, theobromine, 1,7-dimethylxanthine, theophylline and caffeine, as sera. The reconstituted standard extracts were injected into the column interspersed with serum extracts. From the area ratio of each analyte peak to that of the added internal standard, β-hydroxyethyltheophylline, a linear least-squares fit of the data was made. For a typical analysis the data are given in Table I.

TABLE I

XANTHINE CALIBRATION DATA

Analyte	n	r^2	Slope*	Intercept
Theobromine	11	0.99958	0.00684	−0.00012
1,7-Dimethylxanthine	11	0.99942	0.00497	−0.00147
Theophylline	11	0.99976	0.00683	−0.00186
Caffeine	11	0.99971	0.00695	−0.00168

*Arbitrary area units per mg/ml analyte.

Recovery and precision

Recovery and precision were assessed by analyzing repeatedly, over a six-week period, four serum pools to which had been added known amounts of the four xanthines at four different levels. In addition, blanks were analyzed daily. The recovery data are summarized in Table II. Precision data were assessed for theophylline and caffeine and are shown in Table III. For the other analytes, theobromine and 1,7-dimethylxanthine, for which the separation was not optimized, the precision was not rigorously studied; however, in most cases it was in the range 2–4%.

Interferences

Various drugs and endogenous biological compounds considered from a review of the literature to be potential interferences were evaluated. These are listed in Table IV. Of the drugs tested, only acetaminophen interfered with the assay and then only at the highest concentration tested because it was eluted as a badly skewed peak and was only partially resolved from the internal standard. At a more moderate concentration (50 ppm) complete resolution was observed.

Comparison with a common immunoassay

An experiment was conducted to assess the correlation obtained for theophylline values obtained by HPLC with those obtained by a commercial immunoassay technique (EMIT, Syva). Twenty-eight serum samples from patients receiving theophylline therapy and eleven blank sera (to which had been added various

TABLE II

ANALYTICAL ACCURACY AND RECOVERY STUDY

Compound	Concentration added (mg/l)	*n*	Recovery (%)	Coefficient of variation (%)
Toxic level				
Theobromine	11.00	37	99.3	2.1
Dimethylxanthine	25.70	43	99.6	2.2
Theophylline	30.40	43	100.1	0.9
Caffeine	29.98	43	98.7	1.3
High therapeutic				
Theobromine	6.60	38	98.8	2.4
Dimethylxanthine	15.42	43	98.0	2.3
Theophylline	18.24	43	99.1	1.0
Caffeine	17.99	43	97.7	1.7
Low therapeutic				
Theobromine	4.40	36	100.0	4.1
Dimethylxanthine	10.28	42	98.7	3.2
Theophylline	12.16	43	101.2	1.5
Caffeine	11.99	42	98.4	1.9
Sub-therapeutic				
Theobromine	0.88	36	98.5	11.0
Dimethylxanthine	2.06	41	93.2	9.7
Theophylline	2.43	40	111.5	7.2
Caffeine	2.40	42	97.1	4.6

TABLE III

PRECISION STUDY FOR CAFFEINE AND THEOPHYLLINE

Level	Precision type	Coefficient of variation (%)	
		Theophylline	Caffeine
Toxic	Within-day	0.6	0.7
	Between-day	0.7	1.2
	Total	0.9	1.3
High therapeutic	Within-day	0.6	1.3
	Between-day	0.9	1.1
	Total	1.0	1.7
Low therapeutic	Within-day	1.1	1.1
	Between-day	1.2	1.5
	Total	1.5	1.9
Sub-therapeutic	Within-day	3.2	5.5
	Between-day	6.1	3.8
	Total	7.5	4.6

TABLE IV

RETENTION INDICES FOR XANTHINES AND COMMON DRUGS

Compound	k'	Compound	k'
Acetaminophen	3.4	Lidocaine	>10
Acetazolamide	29.6	Methicillin	>20
Allopurinol	0.8	Methylurea	>10
Ampicillin	>20	3-Methyluric acid	>10
Caffeine	6.3	3-Methylxanthine	2.5
β-Hydroxyethyltheophylline	4.2	Oxypurinol	2.7
Cefazolin	>25	Procainamide	1.8
Cephalexin	>25	Quinidine	>10
Cephalothin	>40	Sulfadiazine	9.1
Cephapirin	>40	Sulfamerazine	>25
Chlorotheophylline	>50	Sulfamethazine	19.1
Dimethylurea	10.3	Theobromine	1.5
1,3-Dimethyluric acid	>10	Theophylline	5.4
1,7-Dimethylxanthine	3.4	1,3,7-Trimethyluric acid	>10
Dyphylline	3.5	Uric acid	2.7
Gentamicin	>20		

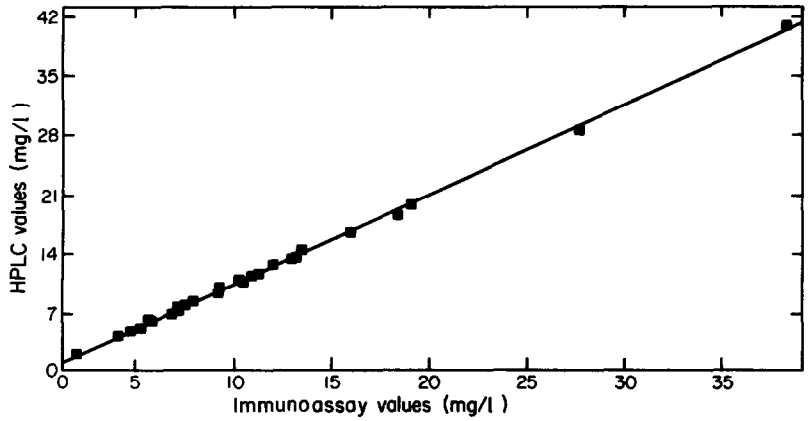


Fig. 6. Correlation for theophylline determination in 28 serum samples by HPLC and an immunoassay technique (EMIT by Syva): slope = 1.057; y-intercept = 0.0028; coefficient of determination = 0.999.

amounts of caffeine, theobromine, theophylline and 1,7-dimethylxanthine) were analyzed for theophylline by both methods. Figs. 6 and 7 plot each set of data with linear correlations for the equation $\delta(\text{HPLC}) = \text{slope } x \times (\text{EMIT}) + \text{intercept}$. In both sets of data the correlation coefficients are >0.99, but the slopes differ. The apparent greater response in the immunoassay for the sera to which the xanthines, in addition to theophylline, were added presumably indicates some cross-reactivity of these xanthines in that assay.

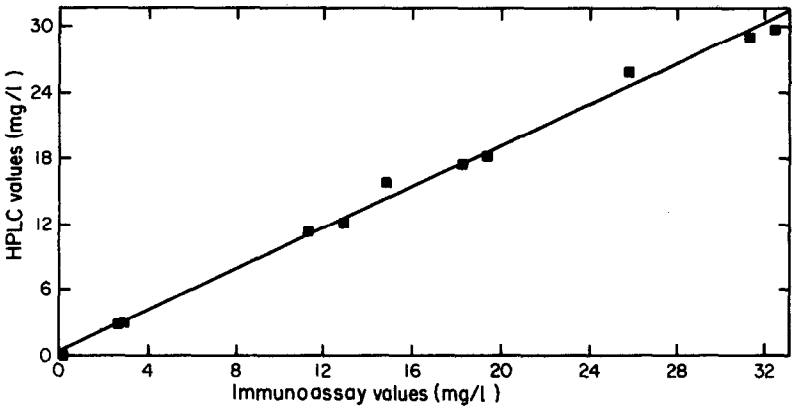


Fig. 7. Correlation for theophylline determination in eleven blank serum samples, to which four xanthines (caffeine, 1,7-dimethylxanthine, theophylline and theobromine) were added, by HPLC and an immunoassay technique (EMIT by Syva): slope = 0.942; y-intercept = 0.391; coefficient of determination = 0.996.

CONCLUSION

An ion-pair reversed-phase system has been developed for the separation of theophylline from related xanthines in serum samples. The ion-pair or dynamic ion-exchange mode affords additional variables including pH, buffer type and strength, ion-exchange agent type and strength, as well as counter-ion type, which can be manipulated to enhance selectivity. With this eluent many interferences noted in the literature for reversed-phase separations of theophylline from serum are eliminated. In addition, the use of a relatively small volume of organic solution to extract theophylline from serum leads to a relatively rapid analysis.

REFERENCES

1 M. Weinberger and C. Chidsey, Clin. Chem., 21 (1975) 599.
2 J.J. Orcutt, P.P. Kozak, Jr., S.A. Gillman and L.H. Cummins, Clin. Chem., 23 (1977) 599.
3 J.R. Miksic and B. Hodes, J. Pharm. Sci., 68 (1979) 1200.
4 S.J. Soldin and J.G. Hill, Clin. Biochem., 10 (1977) 74.
5 B. Kinberger and A. Holmén, J. Chromatogr., 229 (1982) 492.
6 P. Van Aerde, E. Moerman, R. Van Seeveren and P. Braeckman, J. Chromatogr., 222 (1981) 467.
7 R. Klassen and B. Stavric, J. Liq. Chromatogr., 6 (1983) 895.
8 L. Broussard, F.M. Stearns, R. Tulley and C.S. Frings, Clin. Chem., 27 (1981) 1931.
9 J.P. Sommadossi, C. Aubert, J.P. Cano, A. Durand and A. Viala, J. Liq. Chromatogr., 4 (1981) 97.
10 C. Ou and V.L. Frawley, Clin. Chem., 28 (1982) 2157.
11 P.M. Kabra and L.J. Marton, Clin. Chem., 28 (1982) 687.
12 K. Desiraju, E.T. Sugita and R.L. Mayock, J. Chromatogr. Sci., 15 (1977) 563.
13 C. Ou and V.L. Frawley, Clin. Chem., 29 (1983) 1934.
14 H. Imai, H. Yoshida, T. Masujima, I. Morita, K. Matsuura, A. Nakamaru, K. Katayama and H. Matsuo, Anal. Lett., 16 (1983) 1109.
15 J.L. Bock, S. Lam and A. Karmen, J. Chromatogr., 308 (1984) 354.

16 R. Soto-Otero, E. Mendez-Alvarez and G. Sierra-Marcuño, J. Clin. Chem. Clin. Biochem., 23 (1985) 303.
17 M.V. St.-Pierre, A. Tesoro, M. Spiro and S.M. MacLeod, J. Liq. Chromatogr., 7 (1984) 1593.
18 G. Schumann, I. Isberner and M. Oellerich, Fresenius' Z. Anal. Chem., 317 (1984) 677.
19 H. Ong and S. Marleau, J. Liq. Chromatogr., 7 (1984) 779.
20 S.H.Y. Wong, N. Marzouk, S.L. McHugh and E. Cazes, J. Liq. Chromatogr., 8 (1985) 1797.
21 L. Ferron, J. Weber, J. Hébert and P. Bédard, Clin. Chem., 31 (1985) 1415.
22 Y.H. Park, C. Goshorn and O. Hinsvark, J. Chromatogr., 343 (1985) 359.
23 B.J. Starkey and G.P. Mould, Ther. Drug Monit., 6 (1984) 322.
24 T. Kikuta, A. Wakamatsu, M. Umetsu, H. Motoya, J. Sato, H. Nakata, H. Tada, E. Owada and K. Ito, Byoin Yakugaku, 10 (1984) 219.
25 M.B. Kester, C.L. Saccar, M.L. Rocci, Jr. and H.C. Mansmann, Jr., J. Chromatogr., 380 (1986) 99.
26 N.R. Scott, J. Chakraborty and V. Marks, J. Chromatogr., 375 (1986) 321.
27 N. Daoud, T. Arvidsson and K.-G. Wahlund, J. Pharm. Biomed. Anal., 4 (1986) 253.
28 C.A. Robinson, Jr. and J. Dobbs, Clin. Chem., 24 (1978) 2208.
29 R.C. Kelly, D.E. Prentice and G.M. Hearne, Clin. Chem., 24 (1978) 838.
30 J.R. Miksic and B. Hodes, Clin. Chem., 25 (1979) 1866.
31 D.R. Clark, Clin. Chem., 25 (1979) 1183.
32 C.A. Robinson, B. Mitchell, J. Vasiliades and A. Siegel, Clin. Chem., 24 (1978) 1847.
33 J.H.G. Jonkman, R.A. de Zeeuw and R. Schoenmaker, Clin. Chem., 28 (1982) 1987.
34 H.H. Farrish and W.A. Wargin, Clin. Chem., 26 (1980) 524.
35 L.W. Bond and D.L. Thornton, Clin. Chem., 25 (1979) 1186.
36 J.T. Muir, J.H.G. Jonkman, D.-S. Tang, M. Kunatani and S. Riegelman, J. Chromatogr., 221 (1980) 85.
37 B.A. Bidlingmeyer, S.N. Deming, W.P. Price, Jr., B. Sachok and M. Petrusek, J. Chromatogr., 186 (1979) 419.
38 H. Martin, Rhode Island Hospital, Providence, RI, personal communication.