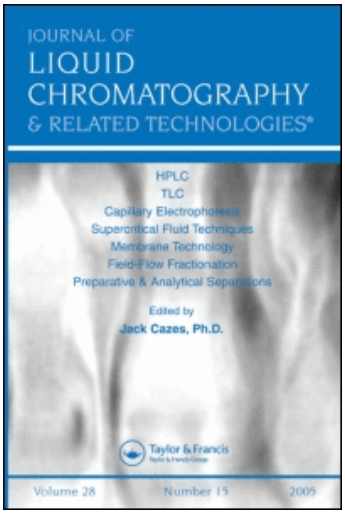

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Effect of Eluent pH on the Ionic and Molecular Forms of the Non-Steroidal Anti-Inflammatory Agents in Reversed-Phase High-Performance Liquid Chromatography

E. Nivaud-Guernet[a]; M. Guernet[a]; D. Ivanovć[b]; M. Medenica[b]
[a] Laboratoire de Chimie Analytique et d'Électrochimie Organiques, Chatenay-Malabry, Cedex, France [b] Faculty of Pharmacy, Belgrade, Yugoslavia

To cite this Article Nivaud-Guernet, E. , Guernet, M. , Ivanovć, D. and Medenica, M.(1994) 'Effect of Eluent pH on the Ionic and Molecular Forms of the Non-Steroidal Anti-Inflammatory Agents in Reversed-Phase High-Performance Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 17: 11, 2343 — 2357
To link to this Article: DOI: 10.1080/10826079408013484
URL: http://dx.doi.org/10.1080/10826079408013484

JOURNAL OF LIQUID CHROMATOGRAPHY, 17(11), 2343-2357 (1994)

EFFECT OF ELUENT pH ON THE IONIC AND MOLECULAR FORMS OF THE NON-STEROIDAL ANTI-INFLAMMATORY AGENTS IN REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

E. NIVAUD-GUERNET[1], M. GUERNET[1],
D. IVANOVIĆ[2]*, AND M. MEDENICA[2]
[1]*Laboratoire de Chimie Analytique et d'Électrochimie Organiques*
rue J. B. Clément
92296 Chatenay-Malabry Cedex, France
[2]*Faculty of Pharmacy*
Vojvode Stepe 450
Belgrade, Yugoslavia

ABSTRACT

High-performance liquid chromatographic conditions for the best separation of some non-steroidal anti-inflammatory agents were described. The dependence of eluent pH on the ionic (protonated) and molecular (non-protonated) forms of analysed compounds have been investigated. This paper is the study of the retention behavior of some anti-inflammatory agents depending of the eluent pH.

Some derivatives of phenol (acetaminophen, aspirin, salicylamide, phenacetin and salicylic acid), pyrazolidinedione (sulfinpyrazone, oxyphenbutazone, phenylbutazone and ketazone),

* To whom correspondence should be addressed.

amino-2-nicotinic acid (nixylic and niflumic acid) and amino-2-benzoic acid (mefenamic and flufenamic acid) were analysed with the isocratic RP-HPLC separation.

The different variations of mobile phase methanol-water v/v, containing 1% acetic acid were performed in order to obtain the best conditions of the separation. For all analysed substances the pH, pKa and the fitted pKa values were calculated in different mixtures of methanol-water using the graphical method given by G.Charlot and B.Trémillon. The capacity factors (k') and the separation factors (α) were calculated for all of them.

INTRODUCTION

In this paper are described the compounds from the non-steroidal anti-inflammatory drugs (NSAIDs), agents which possess analgesic and anti-inflammatory properties and often antipyretic properties. They are used in the treatment of rheumatic deseases.

Lechat (1) classified analgesic-antipyretic agents in two groups, which possesses anti-inflammatory properties and which does not. Analgesic-antipyretics which possess the anti-inflammatory properties are:

- phenolic derivatives
- pyrazolidinedione derivatives
- propionic acid derivatives
- amino-2-nicotinic acid derivatives
- amino-2-benzoic acid derivatives

The numbers of paper describe the high-performance liquid chromatographic method for assaing the mentioned non-steroidal anti-inflammatory agents. Some phenolic derivatives (acetaminophen, aspirin, salicylamide, phenacetin and salicylic acid) were determined in different pharmaceutical preparations

(2, 3), in multicomponent analgesic tablets (4, 5), in elixir formulations (6) and in cough mixtures (7). A group of non-steroidal anti-inflammatory agents were analysed by thin-layer chromatography (8). Aspirin in analgesic tablets was determined by high-performance reversed-phase TLC (9) and using second derivative UV-spectrophotometry (10). The simultaneous spectrophotometric assay of the active consituents of phenolic derivatives in multicomponent analgesics, using Kalmanfiltering, (11) have been developed. The mixtures of certain phenolic and pyrazolidinedione derivatives were analysed by spectrophotometric method using the different coloured or complexometric reactions (12, 13).

Pyrazolidinedione derivatives were separated and determined in bulk drug by HPLC and potentiometry (14). Stability control and determination of degradation products were performed in injectable formulations (15) by HPLC. The mentioned substances were determined in pharmaceutical preparations using the caracteristic reactions of functional groups by spectrophotometric method (16-18). Coulometric titration, with potentiometric determination of the end point, was used for the determination of pyrazolidinedione derivatives (19). Oxyphenbutazone was determined in tablets and ophtalmic ointments using the biamperometric titration (20). Phenylbutazone in tablets was determined by nuclear magnetic resonance (21).

The purity assay of amino-2-nicotinic acid derivatives have been devolped using HPLC method (22).

Mefenamic acid from the amino-2-benzoic acid derivatives was analysed in different pharmaceutical mixtures by means of semimicro liguid chromatography (23), ion-pair partition chromatography (24) and spectrophotometry (25, 26). Colorimetric

determination of two fenamates in antirheumatic drugs have been developed (27-30).

EXPERIMENTAL

Chemicals

All chemicals and reagents used were of an analytical reagent grade. Methanol "Normapur" Prolabo was used as eluent. Acetic acid "Normapur" Prolabo was added to eluent. The solvents were degased and after mixing filtered through a millipore DA 0.65μm filter. Double distilled water was used. All investigated pharmaceutical substances were obtained from commercial sources. Their identity was checked by IR and NMR spectra and their purity by TLC.

Apparatus

The chromatographic system consisted of a Shimadzu LC-6A pump, a Shimadzu SPD-6A detector and Shimadzu C-R3A data handling device. Separations were performed on Lichrosorb RP 18 column 250 x 4.6mm, with particles of 7μm sizes (Hibar, Merck). Samples were introduced through a Rheodyne injector valve with a 10μl sample loop. A mixture of methanol-water containing 1% acetic acid was used as a mobile phase with fixed flow rate 1.0ml/min, at room temperature and with a detection wavelength of 230 and 254nm.

Chromatographic conditions

The standard substances were dissolved in such a way that 1ml of the mobile phase contained 0.05mg of analysed substance.

Eleven different mixtures of mobile phase methanol/water v/v such as: 30/70; 35/65; 40/60; 45/55; 50/50; 55/45; 60/40; 65/35; 70/30; 75/25 and 80/20 containing 1% acetic acid were prepared.

The methanol-water levels containing 1% acetic acid were changed to obtain the acceptable separations.

An isocratic elution was made at room temperature with a flow rate 1ml/min and 10μl portions of the preparations were injected. The detector wavelength was usualy set up at 230nm. Pyrazolidinedione derivatives were detected at 254nm.

RESULTS AND DISCUSSION

The analysed non-steroidal anti-inflammatory agents ionize and can exist in a mobile phase in molecular and ionic forms. The increase in the ionization of the component causes an increase in dissolution in the water phase and reduces the retention time. In that case the ionic form of the analysed substance passes through the column without retaining. For that reason, the chromatogram may show that the peak has been coused to split.

High-performance liquid chromatography analysis of hidrosoluble and ionizing substances requires the ionization of molecules to be avoided. Only in that case the RP-HPLC method can be used in qualitative and quantitative analyses.

In our experiments of HPLC analysis the anti-inflammatory agents which belongs to acidic substances, such as phenolic, pyrazolidinedione, amino-2-nicotinic acid and amino-2-benzoic acid derivatives, have been investigated There is no acidic functional group in molecul of phenacetin, but it was analysed in combination with substances from phenolic derivatives because of the structural similarity with aminophenazone. The ionization has been avoided by the addition of acetic acid. An acetic acid belongs to the HA/A^- type of acid/base pair.

Eleven mixtures of methanol and water in various ratios containing 1% acetic acid were analysed. The Charlot-Trémillon graphical method (31) was used to determine the pKa values of acetic acid in various proportions of methanol and water, which are

TABLE I. pH mobile phase

MOBILE PHASE * (v/v)	pKa (corrected)	Δ pKa ($pKa_{cor.}$ - pKa)	pH
30/70	5.20	0.45	2.98
35/65	5.30	0.55	3.02
40/60	5.40	0.65	3.08
45/55	5.50	0.75	3.12
50/50	5.55	0.80	3.15
55/45	5.65	0.90	3.20
60/40	5.85	1.10	3.30
65/35	6.00	1.25	3.37
70/30	6.25	1.50	3.50
75/25	6.60	1.85	3.68
80/20	6.80	2.05	3.78

* mobile phase containing 1% acetic acid

$pKa_{acetic\ acid}$ = 4.75 (in water)

necessary for the calculation of pH eluants (Figure 1 and Table I). This graphical method gives the relation of pKa in the function of $1/\varepsilon$ (ε - dielectric constant of mobile phase). The values of pKa of acetic acid in pure methanol and water are 9.7 and 4.75 respectively. That makes possible the determination of the pKa of acetic acid in the mobile phases (pKa corrected). For example, for the mobile phase 60/40 v/v, pKa corrected for acetic acid is 5.85 (ΔpKa = 5.85 - 4.75 = 1.10). For all variations of mobile phases, it have been calculated the ΔpKa values and those value factors were used for the calculations of pH (Table I) from the equation: pH = 1/2 pKa - 1/2 log c.

For all the analysed substances, the fitted pKa (pKa corrected) in mobile phases were calculated respectively. For

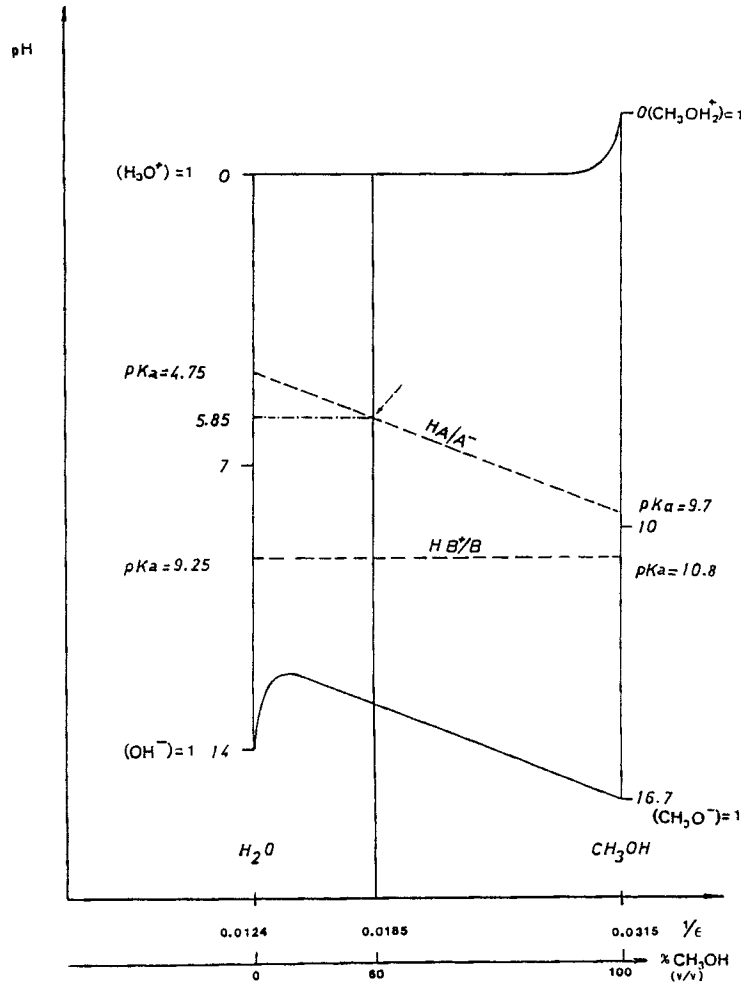

Figure 1. Graphical method Charlot-Trémillon

TABLE II. Values of pKa in mobile phase

	pKa (in water)	30/70	35/65	40/60	45/55	50/50	55/45	60/40	65/35	70/30	75/25	80/20
		pKa (corrected)										
A	3.5	3.95	4.05	4.15	4.25	4.30	4.40	4.60	4.75	5.00	5.35	5.55
B	3.0	3.45	3.55	3.65	3.75	3.80	3.90	4.10	4.25	4.50	4.85	5.05
C	8.2	8.65	8.75	8.85	8.95	9.00	9.10	9.30	9.45	9.70	10.05	10.25
D	9.5	9.95	10.05	10.15	10.25	10.30	10.40	10.60	10.75	11.00	11.35	11.55
E	3.7	4.15	4.25	4.35	4.45	4.50	4.60	4.80	4.95	5.20	5.55	5.75
F	4.7	5.15	5.25	5.35	5.45	5.50	5.60	5.80	5.95	6.20	6.55	6.75
G	2.8	3.25	3.35	3.45	3.55	3.60	3.70	3.90	4.05	4.30	4.65	4.85
H	4.4	4.85	4.95	5.05	5.15	5.20	5.30	5.50	5.65	5.90	6.25	6.45
I	5.0	5.45	5.55	5.65	5.75	5.80	5.90	6.10	6.25	6.50	6.85	7.05
J	5.0	5.45	5.55	5.65	5.75	5.80	5.90	6.10	6.25	6.50	6.85	7.05
K	3.9	4.35	4.45	4.55	4.65	4.70	4.80	5.00	5.15	5.40	5.75	5.95
L	4.2	4.65	4.75	4.85	4.95	5.00	5.10	5.30	5.45	5.70	6.05	6.25

A - Acetylsalicylic acid
B - Salicylic acid
C - Salicylamide
D - Acetaminophen
E - Ketazone
F - Oxyphenbutazone
G - Sulfinpyrazone
H - Phenylbutazone
I - Niflumic acid
J - Nixylic acid
K - Flufenamic acid
L - Mefenamic acid

Table III. Eluent pH dependence on molecular form percent

	MOBILE PHASE (METHANOL/WATER)										
%	30/70	35/65	40/60	45/55	50/50	55/45	60/40	65/35	70/30	75/25	80/20
pH	2.98	3.02	3.08	3.12	3.15	3.20	3.30	3.37	3.50	3.68	3.78
SUBSTANCE	MOLECULAR FORM IN PERCENT										
A	90	91	92	93	93	94	95	96	97	98	98
B	74	77	79	81	82	83	86	88	91	94	95
C	100	100	100	100	100	100	100	100	100	100	100
D	100	100	100	100	100	100	100	100	100	100	100
E	94	94	95	95	96	96	97	97	98	98	99
F	99	99	100	100	100	100	100	100	100	100	100
G	65	68	70	72	75	77	80	82	86	91	92
H	99	99	100	100	100	100	100	100	100	100	100
I	100	100	100	100	100	100	100	100	100	100	100
J	100	100	100	100	100	100	100	100	100	100	100
K	96	96	97	97	98	98	99	99	100	100	100
L	98	98	98	98	99	99	99	99	99	100	100

example: pKa for acetylsalicylic acid in water is 3.5; in the mobile phase 60/40 pKa fitted is 3.5 + 1.1 = 4.6 (Table II). Acetaminophen belongs to HB^+ type of acids and it does not necessery to correct the pKa value.

Using the values of pKa fitted and the pH calculated, it was possible to calculate the ratio of the molecular (non-protonated) and ionic (protonated) percent of the substances (Table III) from the equations:

$$\% \text{ ionization} = \frac{100}{1 + 10^{(pK_a - pH)}} \qquad \text{for HA acids}$$

$$\% \text{ ionization} = \frac{100}{1 + 10^{(pH - pK_a)}} \qquad \text{for } HB^+ \text{ acids}$$

TABLE IV. HPLC separation factors

SAMPLE	λ (nm)	$E^{1\%}_{1cm}$	k'	α
Acetaminophen	230	486	1.65	1.94
Salicylamide	230	464	3.20	1.28
Acetylsalicylic acid	230	474	4.09	1.45
Phenacetin	230	473	5.94	1.12
Salicylic acid	230	486	6.63	
Ketazone	254	472	3.17	1.51
Oxyphenbutazone	254	492	4.79	1.66
Sulfinpyrazone	254	467	7.94	1.83
Phenylbutazone	254	471	14.57	
Niflumic acid	230	485	4.30	2.09
Nixylic acid	230	491	9.00	
Flufenamic acid	230	486	7.46	1.12
Mefenamic acid	230	488	8.36	

k' - capacity factor
α - separation factor
mobile phase: methanol/water v/v containing 1% acetic acid
flow rate: 1ml/min

Table IV presents the values of the capacity factor k' and separation factor α for all the substances in the mobile phase which consists methanol-water in various proportions containing 1% acetic acid. For all of the applied mixtures of methanol-water in the mobile phase, salicylamide, acetaminophen, oxyphenbutazone, phenylbutazone, niflumic acid, nixylic acid and mefenamic acid, which pKa > 4.0, were in 98-100% molecular form. These experimental conditions enable a quantitative HPLC separation.

Acetylsalicylic acid, ketazone and flufenamic acid, which 3.5 < pKa < 4.0, exist in the applied mobile phase in 90-100% molecular form.

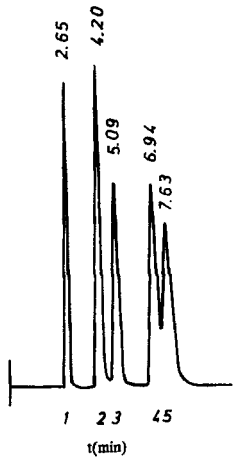

Figure 2. Chrtomatogram of Acetaminophen (1), Salicylamide (2), Acetylsalicylic acid (3), Phenacetin (4) and Salicylic acid (5)
mobile phase: methanol/water 45/55 v/v containing 1% acetic acid

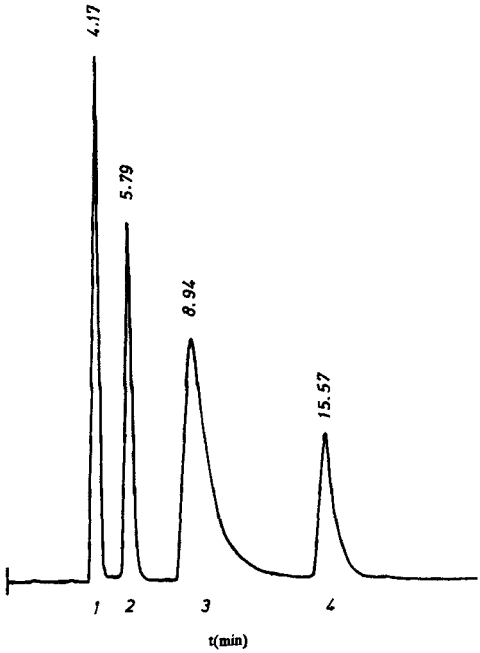

Figure 3. Chrtomatogram of Ketazone (1), Oxyphenbutazone (2), Sulfinpyrazone (3) and Phenylbutazone (4)
mobile phase: methanol/water 50/50 v/v containing 1% acetic acid

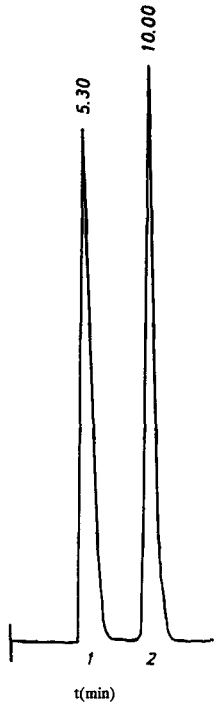

Figure 4. Chrtomatogram of Niflumic acid (1) and Nixylic acid (2) mobile phase: methanol/water 70/30 v/v containing 1% acetic acid

For the salicylic acid and sulfinpyrazone, which pKa < 3.5, it is necessery to increase the acetic acid percent (> 1%) to get the acceptable percent of molecular form.

The capacity factors, k' were calculated using the equation: $k' = (t_R - t_o) / t_o$, where t_R is the retention time of the analyte and t_o is the retention time of the non-retained peak (taken as a first deviation of the baseline following the injection of 100 μl of methanol).

Separation factors, α were calculated using the equation: $\alpha = k'_2 / k'_1$.

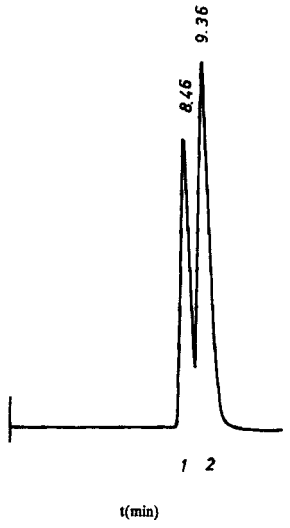

Figure 5. Chromatogram of Flufenamic acid (1) and Mefenamic acid (2)
mobile phase: methanol/water 75/25 v/v containing 1% acetic acid

Representative chromatograms of a mixed standard solution are shown in Fig. 2-5. UV detections have been done at 230 and 254nm. The eluent consists of methanol-water in various proportions containing 1% acetic acid with a flow rate 1ml/min at room temperature.

REFERENCES

1. P.Lechat, *Abrégé de Pharmacologie Médical*, Masson, Paris, 226, (1981).
2. Y.Matsushima, Y.Nagata, K.Takakusagi, M.Niyomura, *J.Chromatogr.*, 332, 265, (1985).
3. V.D.Gupta, A.R.Heble, *J.Pharm.Sci.*, 73, 1553, (1984).
4. D.J.Krieger, *J.Assoc.Off.Anal.Chem.*, 87, 339 (1984).

5. A.Verstraeten, E.Roets, J.Hoogmartens, *J.Chromatogr.*, 388, 201, (1987).

6. W.R.Sisco, C.T.Rittenhouse, L.A.Everhart, A.M.McLaghlin, *J.Chromatogr.*, 354, 355, (1986).

7. L.Carnevale, *J.Pharm.Sci.*, 72, 196, (1983).

8. P.R.Kamath, N.M.Sanghavi, *Indian Drugs*, 22(6), 336, (1985).

9. J.Sherma, S.Stellmacher, T.J.White, *J.Liq.Chromatogr.*, 8(16), 2961, (1985).

10. K.Kitamura, M.Mabuchi, T.Fukui, K.Hozumi, *Chem.Pharm.Bull.*, 35(9), 3914, (1987).

11. A.Van Loosbroek, H.J.G.Debets, P.M.J.Coenegracht, *Anal.Lett.*, 17(B9), 779, (1984).

12. A.A.El Kheir, S.Belal, M.El Sadek, A.El Shanwani, *Analyst*, 111(3), 319, (1986).

13. A.A.El Kheir, S.Belal, A.El Shanwani, *Pharmazie*, 40(1), 62, (1985).

14. C.Herrenknecht, D.Ivanović, E.Guernet-Nivaud, M.Guernet, *J. Pharm. Biomed. Anal.*, 8, 1071, (1990).

15. H.Fabre, N.Hussam-Eddine, B.Mandrou, *J.Pharm.Sci.*, 73(12), 1706, (1984).

16. E.M.El-Kommos, *Analyst*, 108(1290), 1144, (1983).

17. S.Belal, A.A.El Kheir, A.Afaf, A.El Shanwani, *Analyst*, 110(2), 205, (1985).

18. A.S.Issa, Y.A.Beltagi, M.G.Kassem, H.S.Daabees, *Talanta*, 32(8A), 657, (1985).

19. C.Herrenknecht, E.Guernet-Nivaud, M.Guernet, C.Guetin, *Ann.Pharm. Fr.*, 43, 557, (1985).

20. D.Sebkova, L.Safarik, *J.Assoc.Off.Anal.Chem.*, 69(2), 191, (1986).

21. R.Stromberg, *J.Pharm.Sci.*, 73(11), 1653, (1984).

22. C.Guechot, P.Nicolle, *J.Chromatogr.*, 303, 440, (1984).

23. Y.Matsushima, Y.Nagata, M.Niyomura, K.Takakusagi, *J.Chromatogr.*, 332, 269, (1985).

24. W.M.Adams, *J.Assoc.Off.Anal.Chem.*, 66(5), 1178, (1983).

25. C.S.P.Sastry, D.S.Mangala, K.Ekambareswara, *Indian J.Pharm.Sci.*, 47(3), 123, (1985).

26. S.Zommer-Urbanska, H.Bajorowicz, *J.Pharm.Biomed.Anal.*, 4(4), 475, (1986).

27. M.S.Mahrous, M.M.Abdel-Khalek, M.E.Abdel-Hamid, *Talanta*, 32(8A), 651, (1985).

28. S.T.Hassib, H.M.Safwat, R.I.El-Bagry, *Analyst*, 111(1), 45, (1986).

29. S.M.Hasan, S.A.M.Shaaban, *Pharmazie*, 39(10), 691, (1984).

30. A.S.Issa, Y.A.Beltagi, M.G.Kassem, H.S.Daabees, *Talanta*, 32(3), 209, (1985).

31. Charlot, G., Trémillon, B., Les réactions chimiques dans les solvants et les sels fondus, Masson, Paris, 78, (1963).

Received: October 24, 1993
Accepted: December 21, 1993