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Ion interaction reagent reversed-phase high-performance liquid chromatography determination of anti-tuberculosis drugs and metabolites in biological fluids

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Abstract

New methods of ion interaction reagent (IIR) RP-HPLC are presented for the determination of anti-tuberculosis drugs and their metabolites, singly or in multi-component mixtures, in biological fluids. The following analytes are considered: isoniazid, ethionamide, pyrazinamide, morphazinamide, *p*-aminosalicylic acid, nicotinic and isonicotinic acids. Aqueous solutions of three different ion interaction reagents are alternatively or comparatively used as the mobile phases, namely: (A) 5.00 m*M* octylamine at pH 3.00 for *o*-phosphoric acid, (B) 5.00 m*M* octylamine at pH 8.00 for *o*-phosphoric acid, and (C) 5.00 m*M* 1,6 diaminohexane at pH 6.00 for *o*-phosphoric acid. The response linearity between peak area and analyte concentration is verified for all the analytes in the concentration range within the determination limits and 2.00 mg/l. Detection limits are always lower than 82 μg/l for standard solutions; in the analysis of samples of rat serum, rat plasma and human serum, the matrix effect is negligible, the detection limits are always lower than 94 μg/l and the average recovery yield is always greater than 96%.

Keywords: Isoniazid; Ethionamide; Pyrazinamide; Morphazinamide; *p*-Aminosalicylic acid

1. Introduction

Tuberculosis infection still causes the death worldwide of ~3 000 000 people a year [1] and the number is increasing, due to the recent diffusion of the acquired immuno-deficiency syndrome (AIDS), that potentially increases the frequency and the progression of pulmonary tuberculosis. The drugs used in the treatment of tuberculosis are generally assigned to two major categories. The ''first –line'' agents, that combine good efficiency with acceptable levels of toxic side-effects, include isoniazid and pyrazinamide and are the most widely used, often in combined formulations. In the presence of microbial resistance or HIV infection, ''second–line'' drugs, such as ethionamide and *p*-aminosalicylic acid, must also be used. Interest in morphazinamide, developed for its potential therapeutic superiority over pyrazinamide, has decreased since both its activity and toxicity have been ascertained to correspond to those of pyrazinamide, since morphazinamide is easily converted to pyrazinamide in vivo. Iso-

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nicotinic acid is of particular interest due to its inhibitory effect of isoniazid on mycobacteria.

Of the drugs employed against tuberculosis, isoniazid has in the past been the most used. However, due to recently recognised toxic side-effects (such as hepatic disorders) and the potential emergence of resistant strains of *Mycobacterium tuberculosis*, its use is now forbidden [2,3] in human and also in veterinary medicine because of its potential diffusion to milk and the consequent potential damage to human health.

Suitable analytical methods are therefore required for the identification and determination of isoniazid in plasma, serum and urine of patients, in order to carry out suitable individual pharmacokinetic studies of transport and localisation and to develop personalised therapies. Monitoring the drug and its metabolites in plasma in a systematic way together with anti-TB therapy is very advantageous, especially when drug resistance is likely. Interest in the development of reliable methods also concerns other anti-TB drugs often used as alternatives or combined with isoniazid.

A number of analytical methods have been described in the literature, essentially based on reversed-phase HPLC [2–7], ion-pair HPLC [8–11], and liquid capillary gas-chromatography with MS spectrometry detection [12]. Pretreatments of derivatisation with cinnamaldehyde [4,5,13,14], L-L or L-S extraction are employed [15–18]. Methods are reported for the determination of isoniazid in milk [2,3], in alveolar cells after bronchoalveolar lavage [19], in plasma of HIV-positive patients [19], in plasma [6], in human serum [4,12,15], in urine [7,14], in the presence of isonicotinic acid and acetylisoniazid in biological fluids [14,20], in rat hepatocytes [9] or cerebrospinal fluids [8], and in pharmaceutical formulations [21–23]. Pyrazinamide is mainly determined by GC and HPLC methods [24,25], and ethionamide and *p*-aminosalicylic acid in biological fluids by HPLC [26,27].

As can be seen, these methods are mainly devoted to the separation of single analytes and the required sensitivity is achieved through pretreatment steps based on extraction, preconcentration and derivatization.

In this work a liquid chromatographic ion interaction reagent (IIR) method is presented, that offers new advantages: the pretreatment steps are minimised and the simultaneous determination of isoniazid, pyrazinamide, ethionamide and *p*-aminosalicylic acid, as well as the separation of iso- and nicotinic acid can be obtained. The molecular structures of the analytes are reported in Fig. 1.

The multi-component method developed is easily applied to the analysis of rat serum, rat plasma and human serum.

As is known [28], the IIR technique is based on the dynamic modification of a commercial reversed-phase column by a suitable ion interaction reagent that is contained in the mobile phase and is a salt containing a lipophilic cation (for instance an alkylammonium ion). Through adsorbtion and electrostatic forces, a double electric layer is assumed to form onto the surface of the RP stationary phase [28–31]. The properties of the stationary phase are thus modified and the new chromatographic system can simultaneously retain anionic and cationic species. The method is therefore very suitable for the simultaneous separation of amines and anionic species, if working at pH values at which the amines are present in their protonated form and the acids in their dissociated forms. A good selection of the variables involved, in particular the kind and

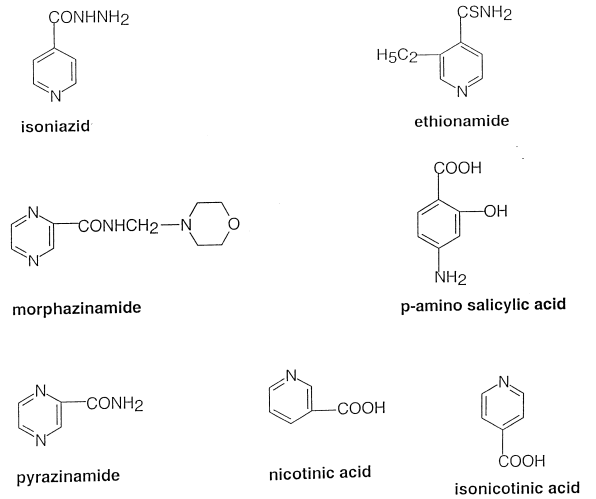

Fig. 1. Molecular structures of the analytes investigated.

concentration of IIR and the pH of the mobile phase, can modulate the retention and make it possible to separate the components even in a complex mixture.

2. Experimental

2.1. Apparatus

Analyses were carried out using a Merck-Hitachi Lichrograph L-6200 chromatograph, equipped with a two-channel Merck-Hitachi D-2500 chromato-integrator, interfaced with an L-4200 UV/VIS detector and an L-3720 conductivity detector with temperature control. The precolumn and column temperatures were held at 25.0±0.2°C.

A Metrohom 654 pH meter equipped with a combined glass-calomel electrode was employed for the pH measurements and a Hitachi Model 150-20 spectrophotometer for the absorbance measurements.

A ''endcapped'' 5-μm Merck-Lichrocart (250× 4.6 mm I.D.) Lichrospher 100 RP18 column was used; the analytical column was usually protected by a 5-μm Lichrospher RP 18 guard column. After use and between the use of different mobile phases, the stationary phase was washed with a mixture of water/acetonitrile 50/50, v/v (flow-rate 1.0 ml/min, 10 min) and 100% acetonitrile (flow-rate 1.0 ml/min, 5 min). With this washing treatment, no particular degradation of the stationary phase, when compared with use in conventional reversed-phase mode, was observed and good performances were maintained during this study (~3 months of intense work, and more than 500 injections).

2.2. Chemicals and reagents

Ultrapure water from Millipore Milli-Q system was used for the preparation of all solutions. Octylamine, *ortho*-phosphoric acid, isoniazid, nicotinic and isonicotinic acid, pyrazinamide, and 1,6 diaminohexane were analytical grade reagents from Fluka. *p*-Aminosalicylic acid and ethionamide were analytical grade reagents from I.C.N. Biomedicals and morphazinamide was analytical grade reagent from Bracco.

The standard solutions of isoniazid, pyrazinamide, morphazinamide, nicotinic acid, isonicotinic acid and *p*-aminosalicylic acid were prepared in dark flasks (1000 mg/l); the solutions were stable for at least 3 months. Standard solutions of ethionamide (500 mg/l) were prepared in methanol and stored at 4°C. The standard solutions were diluted, as required, with ultrapure water.

2.3. Sample preparation

All the samples of rat plasma and serum were prepared in the laboratories of the Dipartimento di Scienza e Tecnologia del Farmaco of the University of Torino, while the human plasma was prepared in hospital laboratories. The serum and plasma samples were stored at −18°C in polyvinyl containers and defrosted just before analysis. The only treatments required prior to HPLC analysis were filtration through Millex Fg 13 0.20-μm Millipore filter and dilution 1/20, v/v, with ultrapure water.

Two commercial pharmaceutical formulations, namely Nicizina® from Pharmacia and Upjohn, and Piraldina® from Bracco, were also analysed. A tablet of drug was dissolved in ultrapure water to obtain a solution of the expected concentration of 500 mg/l, diluted to 1.0 mg/l and analysed.

3. Results and discussion

3.1. Development of the methods

The ion interaction chromatographic technique is very versatile since retention depends on many experimental factors that must be optimised as a function of the separation process. In particular, the technique is very suitable for the separation of mixtures, whose components are characterised by different chemical structures and properties.

When using alkylammonium *o*-phosphate as the IIR, the factors that mainly affect retention are: the length of the alkyl chain, the concentration of the ion interaction reagent, the concentration of the organic modifier and the pH of the mobile phase.

In order to develop methods for the separation of the considered analytes, the effects of the different variables on retention in IIR mode were studied. The

results confirmed the dependencies already observed in previous papers [28–31]. Due to the more lipophilic properties assumed by the modified surface, the increasing length of the alkyl chain leads to increased retention of the anionic species (nicotinic and isonicotinic acids and *p*-aminosalicylic acid) and to decreased retention of amines (isoniazid, ethionamide, pyrazinamide and morphazinamide). A similar behaviour is observed with increased IIR concentration, since greater concentrations induce greater modification of the stationary phase surface. Anyway, this behaviour holds up to IIR concentrations that correspond to the capacity of the stationary phase surface. For greater concentration, a plateau behaviour is observed, if the ionic strength of the mobile phase is controlled. Otherwise, a retention decrease can be observed, due to competitive equilibria and electrostatic effects induced by the increased concentration in the mobile phase of the counter anions of IIR. In the present study, the concentration range of IIR was varied between 3.00 and 8.00 m*M*: in this range a retention increase with increasing concentration was observed and a concentration of 5.00 m*M* was shown to give good resolution in reasonable total analysis time. The effect on retention of the pH of the mobile phase is due not only to the analyte acid–base equilibria, but also to the modification of the stationary phase. For the analytes considered, the effect on the retention by the mobile phase pH was studied in the range between pH 3.00 and 8.00 (the pH limits allowed by a silica-based column). The increased concentration of the organic modifier in the mobile phase leads to a decrease in the retention times of both anionic and cationic species; the effect is due to two contributions, one from the increased eluotropic strength of the mobile phase and the other from the decreased concentration of the ion interaction reagent adsorbed onto the stationary phase surface.

UV detection at 264 nm was chosen to obtain the maximum average sensitivity of the components of the mixture.

1,6 Diaminohexane *o*-phosphate was here used for the first time as IIR to investigate the effect on the modification of the surface of the stationary phase by an IIR that contains in its molecule two lipophilic protonated groups, that can constitute the first positive layer of the electrical double layer. With respect to the data obtained with mono-polar alkylammonium chains, it was observed that when using this IIR, amines are generally retained better while carboxylic acids show generally lower retention times, with the final result of a generally improved resolution of analytes containing different functionalities.

The IIR methods developed utilise three different aqueous mobile phases, which can be used alternatively or, when possible, for comparison, and are: (A) 5.00 m*M* octylammonium *o*-phosphate at pH 3.00, (B) 5.00 m*M* octylammonium *o*-phosphate at

Table 1
Analyte detection limits in standard solutions and in biological fluids for the different mobile phases investigated[a]

Analyte	LOD (μg/l) in standard solution	LOD (μg/l) in biological fluids	Mobile phase
Isoniazid	82	91	(C): 5.00 m*M* 1,6 diaminohexane *o*-phosphate, pH 6.00, flow-rate 1.5 ml/min
Ethionamide	65	71	(A): 5.00 m*M* octylammonium *o*-phosphate, pH 3.00, flow-rate 1.5 ml/min
Pyrazinamide	24	63	(A): 5.00 m*M* octylammonium *o*-phosphate, pH 3.00, flow-rate 1.5 ml/min
Morphazinamide	81	94	(B): 5.00 m*M* octylammonium *o*-phosphate, pH 8.00, flow-rate 1.5 ml/min
p-Aminosalicylic acid	39	68	5.00 m*M* octylammonium *o*-phosphate, pH 8.00, flow-rate 1.5 ml/min

[a] Stationary phase: Phase Separation ODS-2 Spherisorb 5 μm, endcapped.

pH 8.00, and (C) 5.00 m*M* 1,6 diaminohexane *o*-phosphate at pH 6.00

3.1.1. Calibration plots and detection limits

In a total working time of ~3 months, the repeatibility of the measurements was, for all the analytes and the three mobile phases used, always within 3% for the same mobile phase preparation and within 6% for different mobile phase preparations.

By employing the optimal conditions of mobile phase for each analyte, 5-point calibration plots were built in order to evaluate: (i) the linearity between peak area and concentration; and (ii) the method sensitivity. All the plots indicate a good linearity with correlation coefficients R^2 always greater than 0.988. From sensitivity (peak area for 1.00-mg/l concentration) and for a signal-to-noise ratio=3, the detection limits reported in Table 1 were evaluated.

The same separations of interest are presented below.

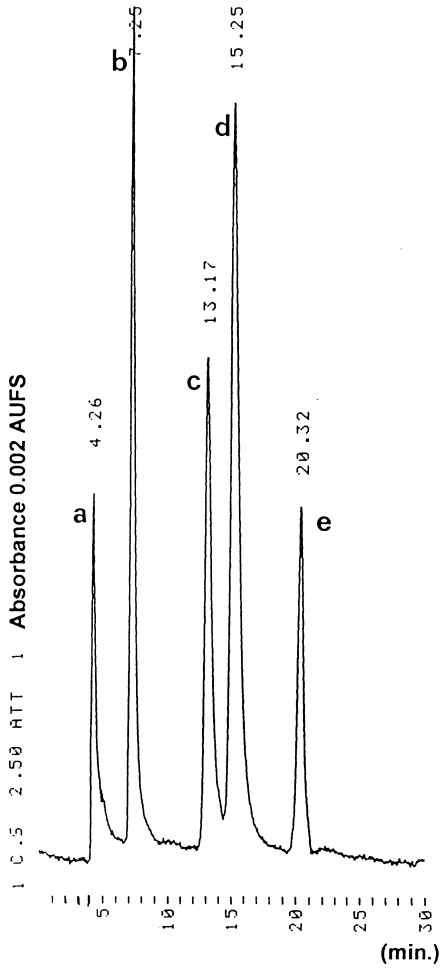

Fig. 2. Separation of a mixture of: (a) isoniazid (1.00 mg/l), (b) isonicotinic acid (0.50 mg/l), (c) nicotinic acid (0.50 mg/l), (d) pyrazinamide (1.00 mg/l), and (e) ethionamide (1.00 mg/l). Stationary phase: Phase Separation ODS-2 Spherisorb 5 μm, endcapped. Mobile phase: 5.00 m*M* octylammonium *o*-phosphate, pH 3.00. Spectrophotometric detection at 264 nm; flow-rate 1.0 ml/min.

3.1.2. Separation of isoniazid, pyrazinamide, ethionamide, isonicotinic and nicotinic acid

Fig. 2 presents the separation of a mixture of: isoniazid, pyrazinamide, ethionamide (each at a concentration of 1.00 mg/l), isonicotinic acid and nicotinic acid (each at a concentration of 0.50 mg/l) obtained with 5.00 m*M* octylammonium *o*-phosphate at pH 3.00 as IIR (mobile phase A). The separation gives good resolution in a total analysis time of less than 25 min. As also reported in the literature, the peaks that correspond to the aminic species are somewhat larger and less symmetric than the peaks of the anionic species. This behaviour is explained by interactions of amines with sylanol groups that are always present on silica-based stationary phases, even following end-capping.

Table 2
Kinetics of the transformation from morphazinamide to pyrazinamide: mmoles evaluated from peak areas as a function of time[a]

Sampling time (h)	Morphazinamide (mmol)	Pyrazinamide (mmol)	Total (mmol)
0	5.795	–	5.795
1	5.670	0.300	5.970
2	5.046	0.308	5.354
3	4.905	0.512	5.417
4	4.761	0.674	5.435
24	2.191	2.995	5.186

[a] Stationary phase: Phase Separation ODS-2 Spherisorb 5 μm, endcapped. Mobile phase B: 5.00 m*M* octylammonium *o*-phosphate at pH 8.00. Flow-rate: 0.8 ml/min.

3.1.3. Kinetic of degradation of morphazinamide to pirazinamide

As mentioned, morphazinamide (*N*-morpholinomethylamide of pyrazinoic acid) is an active prodrug of pyrazinamide, to which it is converted in vivo. A solution of 1.00 mg/l (5.795 mmol) of morphazinamide was prepared and its natural transformation to pyrazinamide has been followed over 24 h by leaving the solution in a capped flask at room temperature (~20°C) and collecting samples every hour for the first 4 h. The samples have been analysed with the IIR-RP-HPLC method which utilises mobile phase B (5.00 m*M* octylammonium *o*-phosphate at pH 8.00). Through the calibration plots, the concentrations of both analytes have been evaluated and are reported in Table 2. The data show that the mass balance is nearly maintained: the sum of mmoles of morphazinamide and pyrazinamide is always within 15% with respect to the expected value, indicating that the transformation of morphazinamide to pyrazinamide can be considered as quantitative. Fig. 3 shows examples of the kinetics of

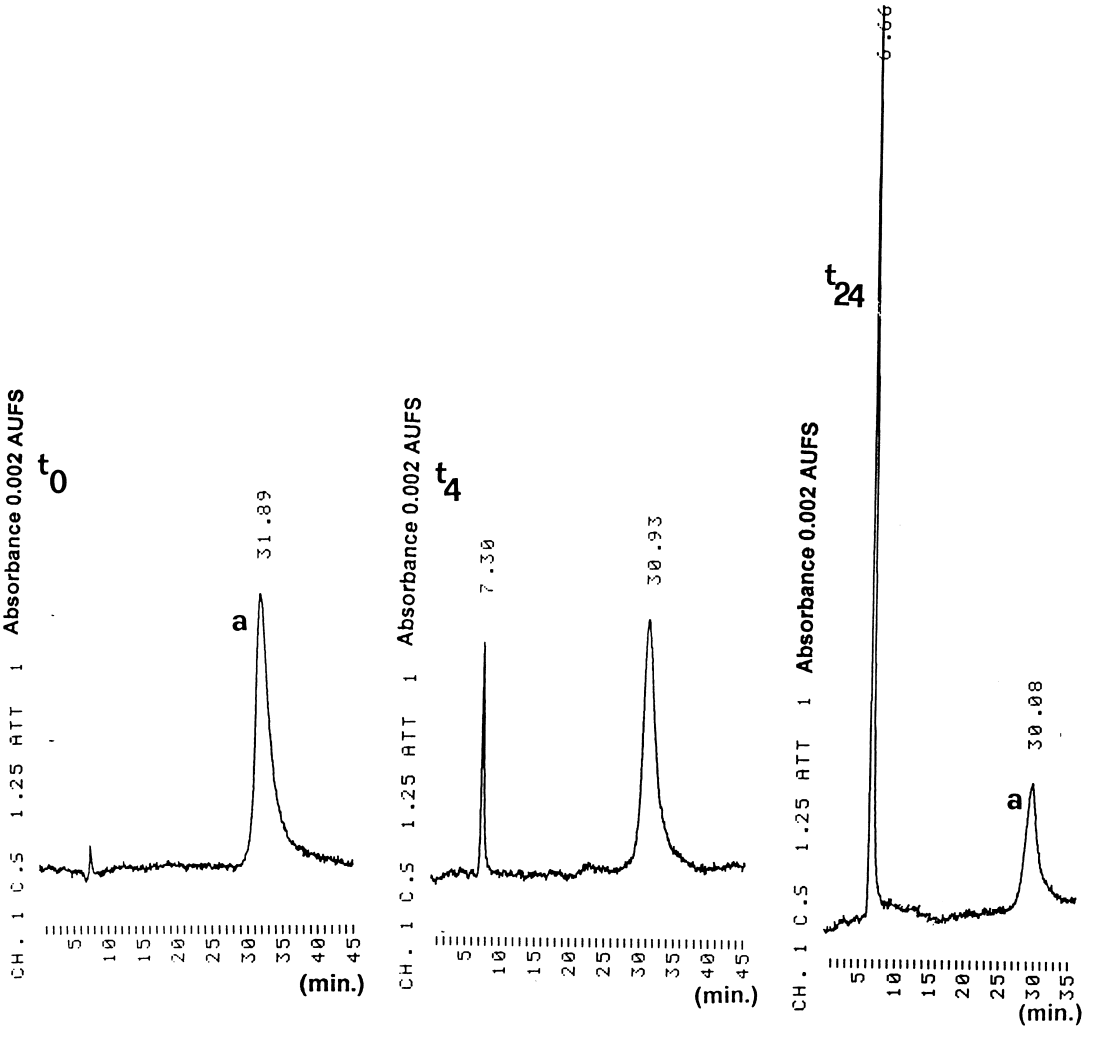

Fig. 3. Kinetics of the transformation from morphazinamide (a) 1.00 mg/l to pyrazinamide (b). (A) $t=0$ h; (B) $t=4$ h; (C) $t=24$ h. Stationary phase: Phase Separation ODS-2 Spherisorb 5 μm, endcapped. Mobile phase: 5.00 m*M* octylammonium *o*-phosphate, pH 8.00. Spectrophotometric detection at 264 nm; flow-rate 0.8 ml/min.

degradation of morphazinamide (1.00 mg/l) to pyrazinamide at times (A) $t=0$ min, (B) $t=4$ h and (C) $t=24$ h.

3.2. Application to real samples

The methods developed were then applied to the analysis of real samples, namely to pharmaceutical formulations, rat serum, rat plasma and human serum. For each analyte and each mobile phase the recovery yield is evaluated by spiking the real samples with the correct concentrations and by quantitative determination performed by 5-point standard addition method.

3.2.1. Determination of isoniazid and pyrazinamide in pharmaceutical formulations

A Piraldina® tablet containing pyrazinamide, and a Nicizina® tablet containing isoniazid, were analysed by using mobile phase A (5.00 m*M* octylammonium ortho-phosphate at pH 3.00). On the basis of the declared amounts, the tablets were dissolved

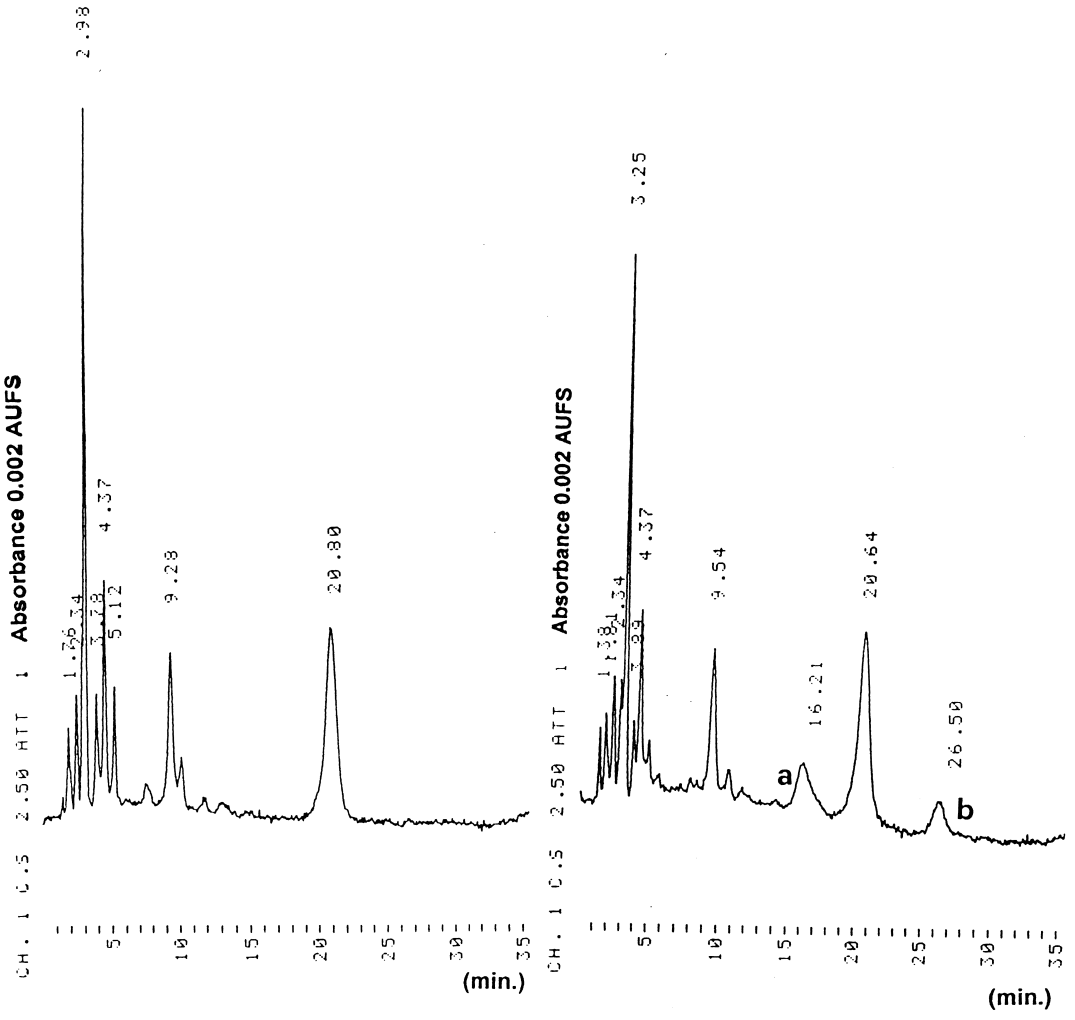

Fig. 4. (A) Chromatogram recorded for a typical rat plasma sample, diluted 1/20, v/v. (B) Chromatogram recorded for a typical rat plasma sample, diluted 1/20, v/v, and spiked with (a) morphazinamide (0.18 mg/l) and (b) *p*-aminosalicylic acid (0.05 mg/l). Stationary phase: Phase Separation ODS-2 Spherisorb 5 μm, endcapped. Mobile phase: 5.00 m*M* octylammonium *o*-phosphate, pH 8.00. Spectrophotometric detection at 264 nm; flow-rate 1.5 ml/min.

in ultrapure water and diluted to the expected concentration of 1.00 mg/l. The results showed a good correlation between the amount found and that declared: in Piraldina® tablet 478.7 mg were found with respect to the expected 500.0 mg and in Nicizina® tablet 207.6 mg with respect to the expected 200.0 mg were found.

3.2.2. Determination of isoniazid, pyrazinamide and ethionamide in rat serum and p-aminosalicylic acid and morphazinamide in rat plasma

On the basis of information based on the metabolic processes, the amounts generally found in real samples are: 0.19–1.13 mg/l of isoniazid, 0.10–2.74 mg/l of pyrazinamide, 0.10–1.12 mg/l of ethionamide, 0.05–3.84 mg/l of *p*-aminosalicylic acid and 0.18–3.50 mg/l of morphazinamide.

Samples of drug-free rat plasma were spiked with the proper amounts of the analytes. Due to the good sensitivity shown by the IIR method in this analysis, the biological samples could be diluted 1/20, v/v, so that also the matrix effect was lowered.

For the determination of pyrazinamide and ethionamide in rat serum, the best mobile phase was shown to be mobile phase A (5.00 m*M* octylammonium *o*-phosphate at pH 3.00). The same IIR but at pH 8.00 (mobile phase B) was shown to be the most suitable for the determination of mor-

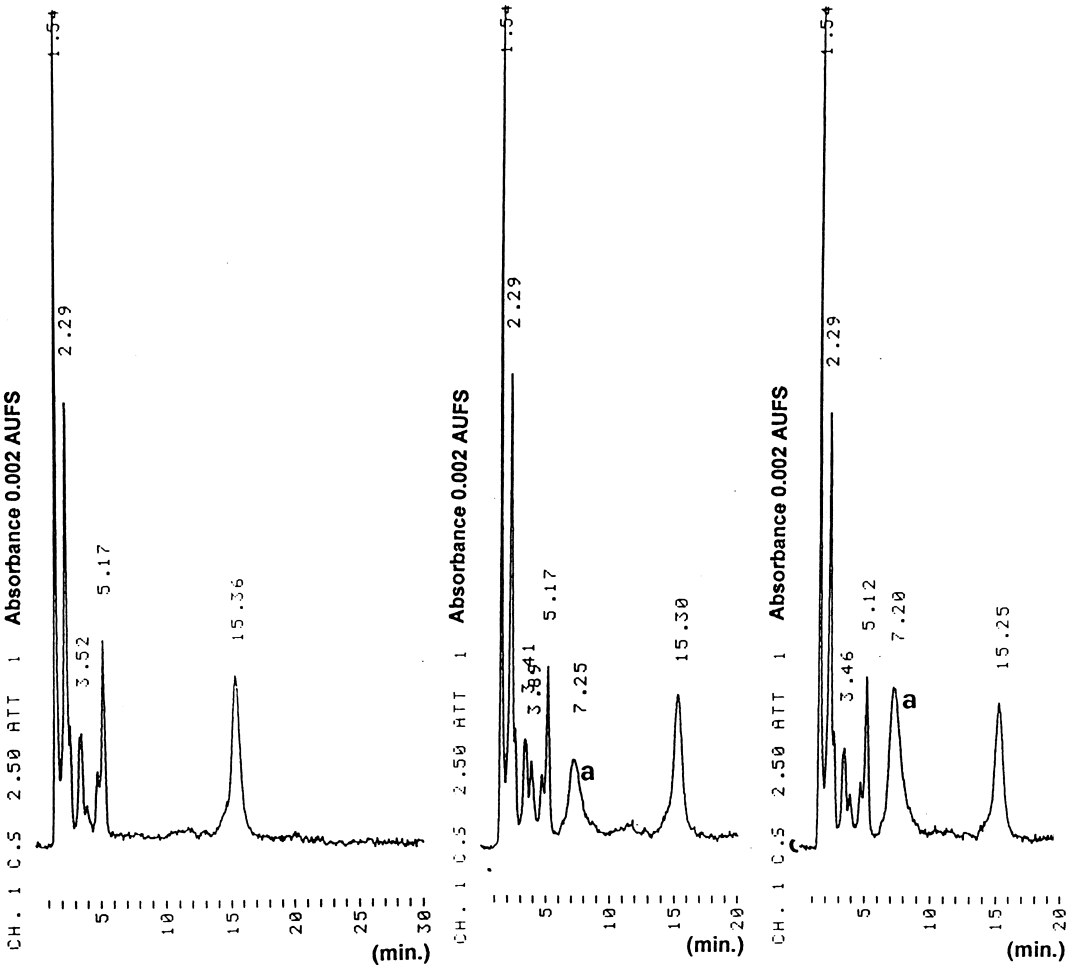

Fig. 5. (A) Chromatogram recorded for a typical rat serum sample, diluted 1/20, v/v, and spiked, respectively, with isoniazid at concentration of (B) 0.38 mg/l, and (C) 0.76 mg/l. Stationary phase: Phase Separation ODS-2 Spherisorb 5 μm, endcapped. Mobile phase: 5.00 m*M* 1,6 diaminohexane *o*-phosphate, pH 6.00. Spectrophotometric detection at 264 nm; flow-rate 1.5 ml/min.

phazinamide and of *p*-aminosalicylic acid in rat plasma. Fig. 4 presents the chromatogram recorded for this sample diluted 1/20, v/v, and, respectively, (A) not spiked, and (B) spiked with 0.18 mg/l of morphazinamide (peak a) and with 0.05 mg/l of *p*-aminosalicylic acid (peak b).

For the determination of isoniazid in rat serum, 5.00 m*M* 1,6 diaminohexane *o*-phosphate at pH 6.00 (mobile phase C) is the most suitable. Fig. 5 presents the chromatogram of rat serum diluted 1/20, v/v, and, respectively, (A) not spiked, (B) spiked with 0.38 mg/l, and (C) spiked with 0.76 mg/l of isoniazid (peak a).

For the determination of isoniazid in samples of spiked human serum, 5.00 m*M* 1,6 diaminohexane *o*-phosphate at pH 6.00 (mobile phase C) as IIR was shown to be suitable.

The recovery determination was performed by the standard addition method. The good linearity of the plots correlating peak areas and concentration (R^2 always greater than 0.975) and the slopes very similar to those obtained in the external calibration plot suggest that the matrix interference is negligible.

Table 3
% Recovery yields in spiked samples[a]

Analyte	Concentration range (mg/l)	Average % recovery
(I) Rat serum		
Ethionamide	0.10–1.12 (n=5)	102.7±11.2
Pyrazinamide	0.10–2.77 (n=5)	102.6±16.2
(II) Rat plasma		
p-Aminosalicylic acid	0.10–3.84 (n=6)	100.2±5.7
Morphazinamide	0.18–3.50 (n=5)	96.5±9.4
(III) Rat serum		
Isoniazid	0.19–1.13 (n=5)	97.6±3.6
(IV) Human serum		
Isoniazid	0.19–1.13 (n=5)	103.7±5.2

[a] The values are the mean of n experiments performed at different concentrations in the range indicated. Each experiment was replicated three times. Stationary phase: Phase Separation ODS-2 Spherisorb 5 μm, endcapped. (I) Ethionamide and pyrazinamide in rat serum (mobile phase A: 5.00 m*M* octylammonium *o*-phosphate, pH 3.00; flow-rate: 1.5 ml/min). (II) *p*-Aminosalicylic acid and morphazinamide in rat plasma (mobile phase B: 5.00 m*M* octylammonium *o*-phosphate, pH 8.00; flow-rate: 1.5 ml/min). (III) Isoniazid in rat serum (mobile phase C: 5.00 m*M* 1,6 diaminohexane *o*-phosphate, pH 6.00; flow-rate: 1.5 ml/min). (IV) Isoniazid in human serum (mobile phase C: 5.00 m*M* 1,6 diaminohexane *o*-phosphate, pH 6.00; flow-rate: 1.5 ml/min.

Table 1 reports the LODs evaluated for the different analytes in the real samples, in comparison with the detection limits evaluated for the standard solutions.

The per cent recovery yields, reported in Table 3, are always greater than 96.5%.

4. Conclusion

In conclusion, it can be said that the method presented here is suitable for the determination of anti-tuberculosis drugs and metabolites in different matrices. The method is based on the ion interaction reagent technique and the use of three different mobile phases is proposed, as a function of the specific separation.

The detection limits for the analytes investigated are always lower that 90 μg/l in biological fluids and the recovery yields in the different matrices are always greater than 96%. When possible, for identification purposes, two different mobile phases can be comparatively used.

It is also worth noting that the sensitivity offered by the method is good enough to permit the determination of the amounts of interest without pretreatments (like derivatisation) or preconcentration processes.

Also noticeable are: (i) the possibility of simultaneously separating more components of a mixture and (ii) the use of completely aqueous mobile phases: the absence of organic solvent in the mobile phase is an advantage both economically and ecologically.

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References

[1] J.P. Edmond, Biol. Prat. 83 (3) (1990) 1.
[2] A. Defilippi, G. Piancone, R. Costa Laia, G.P. Tibaldi, Chromatographia 40 (1995) 170.

[3] A. Defilippi, G. Piancone, R. Costa Laia, S. Balla, G.P. Tibaldi, J. Chromatogr. 656 (1994) 466.
[4] N. Sadeg, N. Pertat, H. Dutertre, M. Dumontet, J. Chromatogr. 675 (1996) 113.
[5] H.I. Seifart, P.B. Kruger, D.P. Parkin, P.P. van Jaarsveld, P.R. Donald, J. Chromatogr. 619 (1993) 285.
[6] E.B. Hansen Jr., K.L. Dooley, H.C. Thompson Jr., J. Chromatogr. 670 (1995) 259.
[7] A. Walubo, K. Chan, C.L. Wong, J. Chromatogr. 567 (1991) 261.
[8] H. Kubo, T. Kinoshita, K. Matsumoto, T. Nishikawa, Chromatographia 30 (1990) 69.
[9] Y. Ono, A. Noda, Y. Zaima, N. Jitsufuchi, S. Eto, H. Noda, J. Chromatogr. 677 (1996) 339.
[10] J.O. Svensson, A. Muchtar, O. Ericsson, J. Chromatogr. 341 (1985) 193.
[11] M.A. Moulin, F. Albessard, J. Lacotte, R. Camsonne, J. Chromatogr. 226 (1981) 250.
[12] G. Karlaganis, E. Peretti, B.H. Lauterburg, J. Chromatogr. 420 (1987) 171.
[13] C. Lacroix, G. Laine, J.P. Goulle, J. Chromatogr. 307 (1984) 137.
[14] H.I. Seifart, W.L. Gent, D.P. Parkin, P.P. van Jaarsveld, P.R. Donald, J. Chromatogr. 674 (1995) 269.
[15] M. Guillaumont, M. Leclercq, Y. Frobert, B. Guise, R. Harf, J. Chromatogr. 232 (1982) 369.
[16] A. Hutchings, R.D. Monie, B. Spragg, P.A. Routledge, J. Chromatogr. 277 (1983) 385.
[17] G. Miscoria, A. Leneveu, C. Walle, A. Roux, Ann. Biol. Clin. 46 (1988) 734.
[18] C.D. Gaitonde, P.V. Pathak, J. Chromatogr. 532 (1990) 418.
[19] T. Delahunty, B. Lee, J.E. Conte, J. Chromatogr. 705 (1998) 323.
[20] K. Kitamura, M. Hatta, S. Fukuyama, K. Hozumi, Anal. Chim. Acta 201 (1987) 357.
[21] S.C. Sharma, S. Das, S.K. Talwar, J. Assoc. Off. Anal. Chem. 70 (1987) 679.
[22] J. Manes, M.J. Gimeno, J.C. Moltò, G. Font, J. Pharm. Biomed. Anal. 6 (1988) 1023.
[23] A. Walubo, P. Smith, P.I. Folb, J. Chromatogr. 658 (1994) 391.
[24] J. Woo, C.L. Wong, R. Teoh, K. Chan, J. Chromatogr. 420 (1987) 73.
[25] P.J. Jenner, G.A. Ellard, J. Chromatogr. 225 (1981) 245.
[26] C.A. Peloquin, G.T. James, E. McCarthy, J. Chromatogr. 563 (1991) 472.
[27] I.L. Honigberg, J.T. Stewart, T.C. Clark, D.Y. Davis, J. Chromatogr. 181 (1980) 266.
[28] M.C. Gennaro, Adv. Chromatogr. 35 (1995) 343.
[29] S. Angelino, A. BiancoPrevot, M.C. Gennaro, E. Pramauro, J. Chromatogr. A 845 (1999) 257.
[30] E. Marengo, M.C. Gennaro, S. Angelino, J. Chromatogr. A 799 (1998) 47.
[31] M.C. Gennaro, C. Abrigo, E. Pobozy, E. Marengo, J. Liq. Chromatogr. 18 (1995) 311.