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Short communication

High-performance liquid chromatographic determination of 4-methylpyrazole in plasma and in dialysate

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Abstract

A rapid method for the determination of 4-methylpyrazole (4-MP) levels in plasma and in dialysate by isocratic reversed-phase high-performance liquid chromatography with UV detection is described. The internal standard was the 3-methylpyrazole (3-MP). Plasma sample preparation consisted of a protein precipitation. Dialysate samples were injected without preparation. The method was linear up to 30 mg l^{-1} in plasma and up to 5 mg l^{-1} in dialysate. The within-day precisions (C.V.) were less than 4% in plasma and were less than 2% in dialysate. The day-to-day precisions (C.V.) were less than 7% in plasma and were less than 3% in dialysate. This method is easy to perform and has practical interest for clinicians who need to monitor in emergency 4-MP levels in ethylene glycol and methanol poisonings. © 1997 Elsevier Science B.V.

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1. Introduction

The usual treatment of ethylene glycol and methanol poisonings consists in ethanol administration and hemodialysis [1–3]. The disadvantages of this treatment are the difficulty of maintaining ethanol blood levels in the range of 1 g l^{-1} [3] and also the fact that ethanol has adverse effects on the central nervous system [1,4].

4-Methylpyrazole (4-MP) is a competitive inhibitor of alcohol dehydrogenase, able to inhibit hepatic metabolism of ethylene glycol and methanol into their toxic metabolites [1,4] and has therapeutic advantages over ethanol: it does not exert CNS depressant activity and has a longer duration of action [5]. 4-MP has been used successfully without hemodialysis in severe ethylene glycol human intoxications before acute renal failure appeared [6–8].

The present work describes a simple and sufficiently rapid method for the determination in an emergency of 4-MP levels in plasma and in dialysate of patients intoxicated by ethylene glycol. The results obtained by this method have allowed us to propose a dosage regimen of 4-MP during hemodialysis [9] and to make more precise the data already available about dialysance of 4-MP [10].

2. Experimental

2.1. Reagents

3-MP and 4-MP were obtained from Aldrich (Steinheim, Germany) and were 97% and 99% pure

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respectively according to the manufacturer. Acetonitrile of HPLC-grade was purchased from Carlo Erba (Rodano, Italy). All other chemicals were of analytical range.

2.2. Sample preparation

Blood samples were collected in tubes containing lithium heparinate as anticoagulant and were centrifuged to obtain plasma. Plasma (200 μl) was introduced into 1.5-ml capped tubes and then 10 μl of internal standard solution (300 mg l^{-1} of 3-MP in distilled water) were added. Plasma proteins were precipitated by 100 μl of trichloroacetic acid solution (15%, v/v). The samples were centrifuged at 10 000 *g* for 4 min and 150 μl of the supernatant were mixed with 100 μl of a 0.5 *M* hydrogenphosphate disodium dihydrate solution in order to obtain a pH near 7.

Dialysate (200 μl) was injected into the column without any sample preparation and the external standard method was used for quantitation.

2.3. Chromatography

The HPLC system consisted of a Spectra-Physics P4000 liquid chromatographic pump, a Spectra-Physics AS3500 autosampler injector, a Spectra-Physics UV1000 variable wavelength detector, all controlled by PC1000 software. For the separation a 125×4-mm HPLC column Lichrospher 100 RP-18 (5 μm) with a 4×4 mm precolumn Lichrospher 60 RP-select B (5 μm) was used. The column temperature was 40°C.

The mobile phase consisted of 7.5% acetonitrile in 5 m*M* potassium phosphate buffer (pH 6), at a flow-rate of 1.5 ml min^{-1}. The wavelength used to monitor 3-MP and 4-MP was 220 nm. The injection volumes were 50 μl for plasma and 200 μl for dialysate. The concentrations of 4-MP were calculated from the 4-MP/3-MP peak area ratios for plasma samples and by external calibration for dialysate samples.

3. Results

The chromatograms obtained from plasma and dialysate are shown in Fig. 1. The retention times for 3-MP and 4-MP were 4.1 and 4.9 min, respectively. The equations of 4-MP calibration curves were $y = 6.77 \cdot 10^{-2} \times (\text{area ratio 4-MP/3-MP}) + 1.8 \cdot 10^{-2}$ and $y = 281\,075 \times (\text{4-MP area}) - 1566$ in plasma and in dialysate, respectively. The assays were linear between 1.25 and 30 mg l^{-1} in plasma and between 0.30 and 5 mg l^{-1} in dialysate. The regression coefficients were 0.9991 and 0.9999 in plasma and in dialysate, respectively. The limits of quantitation were 0.3 mg l^{-1} in plasma and 0.05 mg l^{-1} in dialysate. The within-day precisions and the day-to-day precisions (C.V.) were respectively less than 4% and 7% both for plasma and dialysate assays as shown in Tables 1 and 2. The recoveries of 4-MP from spiked plasma were 100%±6.7%, 99%±1.6% and 102%±0.5% (means±S.D., $n=3$) for plasma concentrations of 3, 11 and 24 mg l^{-1}, respectively. The recoveries of 4-MP from spiked dialysate were 101%±1.7%, 98.4%±0.5% and 101%±0.4% (means±S.D., $n=3$) for dialysate concentrations of 0.70, 1.85 and 4.5 mg l^{-1}, respectively as illustrated in Table 3.

4. Discussion

To our knowledge, this is the simplest HPLC method described for the determination of 4-MP levels in plasma and in dialysate: both sample preparation and chromatographic analysis need less than 1 h. Other described methods need either liquid extraction of 4-MP with ethyl ether [11] or a solid-phase extraction [12]. The disadvantages of these methods are the critical stage of evaporation with ethyl ether due to the volatility of 4-MP or the time consuming step for solid-phase extraction through Bond Elut SCX column.

The limit of quantitation of 0.3 mg l^{-1} in plasma is sufficient since therapeutic 4-MP plasma concentrations described in a case report were about 15 mg l^{-1} [6]. The range of linearity in plasma up to 30 mg l^{-1} means that samples need not be diluted since a loading dose as high as 20 mg kg^{-1} in humans has given mean peak plasma levels below 30 mg l^{-1} [13].

Recently we have used this method to measure 4-MP levels in two anuric patients severely intoxicated with ethylene glycol and treated with

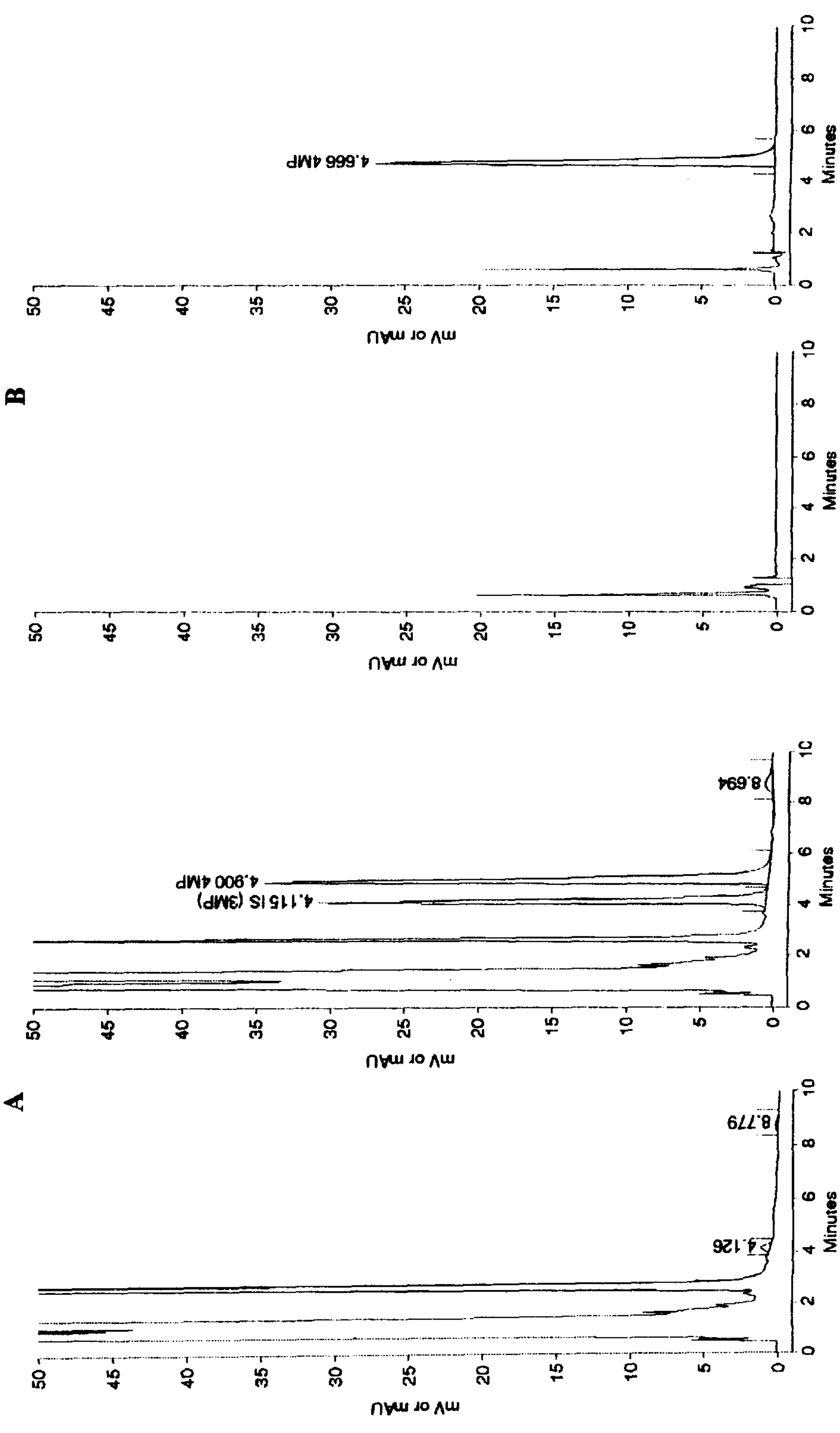

Fig. 1. Chromatograms of (A) plasma sample (blank without internal standard and spiked 4-MP: 20 mg l^{-1}) and (B) dialysate sample (blank and spiked 4-MP: 1.25 mg l^{-1}).

Table 1
Within-day precisions in plasma and in dialysate

Concentration added (mg l^{-1})	*n*	Concentration found (mean±S.D.) (mg l^{-1})	C.V. (%)
Plasma			
5	10	4.98±0.153	3.1
20	10	20.33±0.731	3.6
Dialysate			
0.625	10	0.60±0.013	1.7
2.5	10	2.61±0.027	0.5

Table 2
Day-to-day precisions in plasma and in dialysate

Concentration added (mg l^{-1})	*n*	Concentration found (mean±S.D.) (mg l^{-1})	C.V. (%)
Plasma			
5	9	4.61±0.293	6.4
20	11	19.95±0.889	4.5
Dialysate			
0.625	10	0.56±0.020	2.7
2.5	10	2.12±0.089	2.1

hemodialysis and we have shown that a large amount of 4-MP was eliminated in the dialysate. The mean hemodialysis clearances of 4-MP were 80 and 52 ml min^{-1}. The results obtained allowed us to propose the following administration of 4-MP: first a loading dose of 10–20 mg kg^{-1} before dialysis and then continuous infusion of 1–1.5 mg kg^{-1} h^{-1} during dialysis [9] in cases of ethylene glycol (EG) poisoning with renal failure and EG plasma concentrations higher than 0.1 g l^{-1}.

Table 3
Recoveries of 4-methylpyrazole from plasma and dialysate

Added 4-MP (mg l^{-1})	*n*	Recovered 4-MP (mean±S.D.) (mg l^{-1})	Recovery (%)
Plasma			
3	3	3.0±0.20	100
11	3	10.9±0.17	99
24	3	24.4±0.11	102
Dialysate			
0.70	3	0.71±0.012	101
1.85	3	1.82±0.010	98
4.50	3	4.56±0.020	101

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