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Short communication

Simple and efficient method for the detection of diethylenetriaminepentaacetic acid

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Abstract

Diethylenetriaminepentaacetic acid (DTPA) is a commonly used chelating agent. Its antiviral, antibacterial and immunomodulatory effects are well documented. DTPA forms a highly stable complex with lead (II) with an increased absorption coefficient and a bathochromic shift of the absorption maximum compared to pure DTPA. Based on this complex a high-performance liquid chromatographic method for the quantitative detection of DTPA in biological fluids was developed. A calibration curve was prepared and linearity was shown in the concentration range between 10 mg l^{-1} and 1000 mg l^{-1} DTPA. The recovery in water and in human plasma showed the method to be suitable for routine use.

Keywords: Diethylenetriaminepentaacetic acid

1. Introduction

Chelating agents are commonly used for decontamination after heavy metal poisoning [1,2] or exposition to radioactive metals, respectively [3] and are widely used in cases of iron-overload in some diseases like β-thalassemia [4].

In addition to their chelating properties they influence inflammatory diseases [5], reduce the side-effects of some chemotherapeutics in cancer therapy [6], and have antitumoral [7,8], antibacterial [9], antiviral [10,11], antiprotozoal [6] and immunomodulatory [12] effects. The activity against malaria was discovered to be independent of the iron status of the host [13]. An overview of these pharmacological effects is given by Voest et al. [6].

The most frequently investigated chelator is desferrioxamine (DFO) although diethylenetriaminepentaacetic acid (DTPA) offers advantages due to its lower toxicity [14].

For in vitro testing and clinical trials an efficient DTPA assay is necessary. Since a simple assay in biological media still is lacking, in the present study a simple and efficient high-performance liquid chro-

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matography (HPLC) method was developed. The applicability of the method was shown for the determination of DTPA in aqueous solutions and in human plasma.

2. Experimental

2.1. Reagents and chemicals

DTPA was purchased from Fluka (Buchs, Switzerland). Lead (II) nitrate, sodium acetate, potassium chloride and methanol (LiChrosolv) were obtained from Merck (Darmstadt, Germany). Glacial acetic acid was purchased from Caesar and Loretz (Hilden, Germany). All reagents were of analytical grade and used as received.

2.2. Instrumentation

For the determination of DTPA the chromatographic separation was performed on a Merck–Hitachi HPLC unit (L-6220 Intelligent Pump, Column Oven L-7350 and Autosampler L-7200 from Hitachi, Tokyo, Japan) equipped with spectralphotometric detection (Lamda-Max Model 481, Waters, Eschborn, Germany) and attached to a Kontron PC Integration Pack (Kontron, Milan, Italy). Separation was achieved using a silica-based ET 125/4 Nucleogen DEAE 60-7 anion-exchange column (Macherey–Nagel, Düren, Germany) at a temperature of 40°C. For ultrafiltration of the DTPA plasma samples Microcon 30 microconcentrators (Amicon, Witten, Germany) were used.

2.3. Sample preparation

For the preparation of the DTPA samples, aliquots (200.0 μl) of a 1 mg ml^{-1} lead (II) nitrate solution, containing 10 mg ml^{-1} glacial acetic acid, were transferred into each HPLC vial. Aqueous DTPA solutions with DTPA concentrations ranging between 20 mg l^{-1} and 2000 mg l^{-1} DTPA were diluted 1:1 with water. Aliquots of 200.0 μl of these dilutions were added to the HPLC vials. Fixed volumes (25.0 μl) of each sample were injected into the HPLC system.

2.4. Chromatographic separation

Two mobile phases containing different salt concentrations were used for the chromatographic separation of DTPA. Solvent A, the lower salt mobile phase, consisted of 0.02 *M* sodium acetate in 30% (v/v) methanol at a pH of 6.0. The higher salt mobile phase solvent B was composed of 0.02 *M* sodium acetate and 1 *M* potassium chloride in 30% (v/v) methanol at a pH of 6.0. For separation, the following gradient program was used: the gradient program started with 100% solvent A. During the next 10 min, solvent B was gradually increased to 100% and maintained at this level for 5 min. Over the following 5 min solvent B was decreased to 0%. The column was equilibrated with solvent A for further 45 min prior to the next injection. At a flow-rate of 1.0 ml per min and a detection wavelength of 260 nm, DTPA showed a retention time of approximately 5.3 min.

2.5. Calibration curve and recovery

A calibration curve for the HPLC method was prepared by using DTPA solutions containing 10 mg l^{-1}, 20 mg l^{-1}, 75 mg l^{-1}, 100 mg l^{-1}, 133 mg l^{-1}, 163 mg l^{-1}, 400 mg l^{-1}, 500 mg l^{-1}, 600 mg l^{-1}, 800 mg l^{-1} and 1000 mg l^{-1} DTPA after the 1:1 dilution with water, respectively. The calculated peak area was plotted against the used DTPA concentration.

Using the described HPLC method, the recovery of DTPA was determined by comparing the results of the actual DTPA concentration with the calculated values based on the calibration curve. The actual concentrations used were in the range of 40 to 60 mg l^{-1} for the low level, 200 to 300 mg l^{-1} for the medium level and 700 to 900 mg l^{-1} of DTPA for the high level. The samples were prepared and analyzed as described previously.

2.6. Calibration curve and recovery in human plasma

The samples for the recovery of DTPA in plasma were prepared as described for the calibration samples with only one exception: the aqueous DTPA solution was diluted 1:1 with plasma instead of

water. In order to separate the plasma proteins from the samples, the DTPA-plasma samples were ultrafiltrated using Microcon 30.000 microconcentrator units. Aliquots (200 μl) of the filtrate were added to the lead (II) nitrate solution as described previously for the calibration samples.

For the determination of the recovery of DTPA in human plasma a calibration curve was prepared using DTPA concentrations in the range of 20 to 40 mg l^{-1} for the low level, 100 mg l^{-1} to 200 mg l^{-1} for the medium level and 600 mg l^{-1} to 800 mg l^{-1} for the high level, respectively. The samples were treated in the same manner as described above.

3. Results and discussion

The quantitative determination of pure DTPA (Fig. 1) by spectrophotometric methods is very insensitive and unselective as the absorption coefficient $E^{1\%}_{1\ cm}$ of DTPA is about 16%$^{-1}$ cm^{-1}, even at a wavelength of 220 nm. DTPA is a strong chelating agent for some metals whereby some of the metal–DTPA complexes show an increased absorption coefficient at higher wavelengths. Several spectrophotometric methods for the quantitative determination of DTPA in aqueous solutions based on these metal–DTPA complexes have been described, e.g., the decrease of the absorption of an iron thiocyanate complex [15] or the direct photometric UV detection with bismuth or lead complexes [16]. A further quantification method is represented by the titration of DTPA with bismuth using xylenol orange as indicator [17]. However, a sensitive, accurate and simple HPLC method for the determination of DTPA in biological fluids is still lacking.

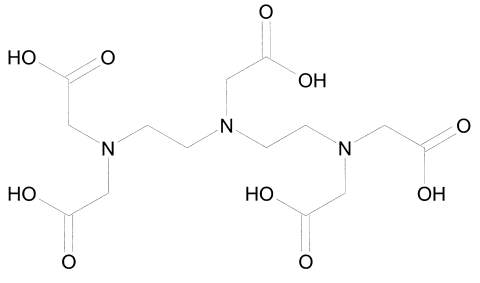

Fig. 1. The molecular structure of the chelating agent DTPA.

As DTPA is a polyanion, a cationic column was selected for the separation in the present study. The elution of DTPA from the column requires a mobile phase with high salt concentrations so that the detection of the pure DTPA at 220 nm was impossible. For this reason, the ability of DTPA to form stable heavy metal complexes with increased absorption coefficients at higher wavelengths was employed. The lead–DTPA complex is very stable so that chromatographic separation is not able to break this strong bond. The lead cation is able to displace other cations like calcium from the complex. This may be the same with bismuth, but the bismuth–DTPA complex has to be analyzed at a pH$<$0.6 [16], too acid for the analytical column used. In contrast to pure DTPA, the lead–DTPA complex shows a maximum absorption at 246 nm and a specific absorption coefficient of about 190%$^{-1}$ cm^{-1}. Because of interference of the gradient with the DTPA detection due to the high salted buffer, the wavelength was fixed to 260 nm.

A typical chromatogram of the DTPA–lead complex with a total retention time of 5.3 min is shown in Fig. 2. The long recovery time after the gradient is necessary to regenerate the column, since otherwise remaining salt would decrease the retention time of the following sample.

A calibration curve of DTPA was obtained that was linear over a concentration range between 10 and 1000 mg l^{-1} DTPA (y=0.1098x−0.5904, SD slope 0.0014, SD intercept 0.6634, standard error 1.5058, r=0.9993, $n\geq$4). The upper limit of quantitative detection in the DTPA solution used for the HPLC assay is 1000 mg l^{-1} DTPA [calculations based on a DTPA-MG=393.35 g mol^{-1} and a lead (II) nitrate-MG=331.2 g mol^{-1}] following addition of 1 mg ml^{-1} lead solution for stoichiometric reasons. The quantification of higher DTPA concentrations may be possible if a more concentrated lead solution is used. However, higher concentrations are not relevant for biological samples. The same applies to the lower concentration limit: the added DTPA solution contains 10 mg l^{-1} DTPA, but the total concentration in the HPLC vial is 5 mg l^{-1} DTPA after addition of the lead solution. If smaller amounts of higher concentrated lead solutions are used, the determination of lower DTPA concentrations may be possible. However, following the

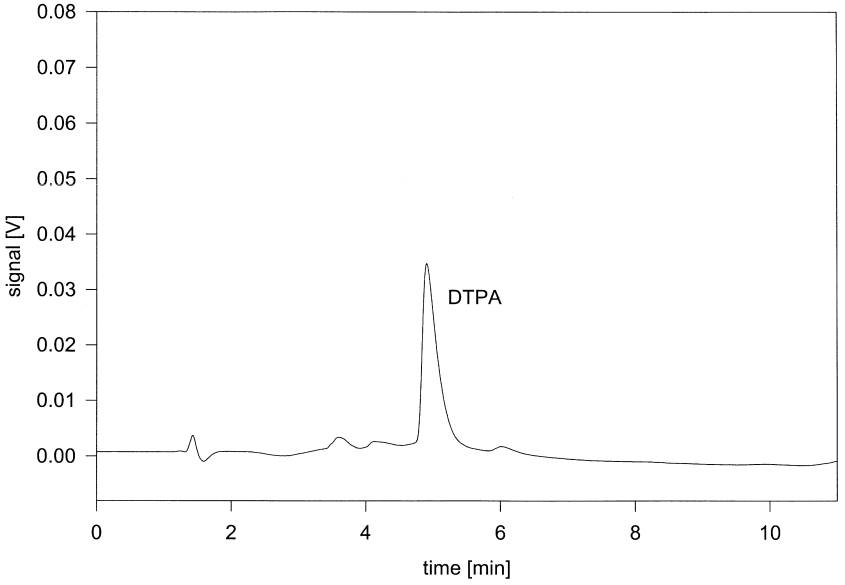

Fig. 2. A typical DTPA chromatogram at a concentration of 200 mg l^{-1} DTPA.

described sample preparation the limit for quantitative recording of the drug was determined to be 10 mg l^{-1} DTPA. The limit for a qualitative recording of the drug was about 1 mg l^{-1}. The data of the DTPA recovery for three concentration levels in water are shown in Table 1.

In human plasma most of the plasma proteins were separated by ultrafiltration with Microcon 30.000 microconcentrators. Preliminary experiments revealed, that the highly water soluble DTPA showed no tendency to adsorb to the ultrafiltration unit (data not shown). The filtrate was added to the lead solution and the DTPA content determined using the described HPLC method. Fig. 3 shows the separation of DTPA from remaining plasma components. Because of a time lag between the two experiments, a second calibration curve was prepared ($y=0.1090x-0.6031$, SD slope 0.0011, SD intercept 0.5389, standard error 1.1935, $r=0.9996$ and $n\geq 4$), that only differed less than 0.7% in slope and 2.1% in intercept from the original calibration curve. The results of the recovery in plasma at three concentration levels and the confidence interval of the mean are shown in Table 1. The data reveals that there is a sufficient recovery of DTPA in plasma over the total calibration range.

Table 1
Recovery data for the HPLC assay of DTPA in water and in human plasma at three different concentration levels

	DTPA in water			DTPA in plasma		
	Low (40–60 mg l^{-1})	Medium (200–300 mg l^{-1})	High (700–900 mg l^{-1})	Low (20–40 mg l^{-1})	Medium (100–200 mg l^{-1})	High (600–800 mg l^{-1})
Recovery (%)	101.39	96.52	105.16	100.81	102.72	100.20
SD (%)	0.87	6.04	2.86	4.83	4.59	1.18
Confidential interval ($P=95\%$)						
Upper limit (%)	102.30	102.86	108.22	105.64	107.31	101.38
Lower limit (%)	100.48	90.12	102.16	95.98	98.13	99.02
Maximum (%)	102.55	104.44	108.42	105.73	107.27	101.69
Minimum (%)	100.42	87.86	101.96	93.88	97.73	98.36
Number of data	6	6	6	10	7	9

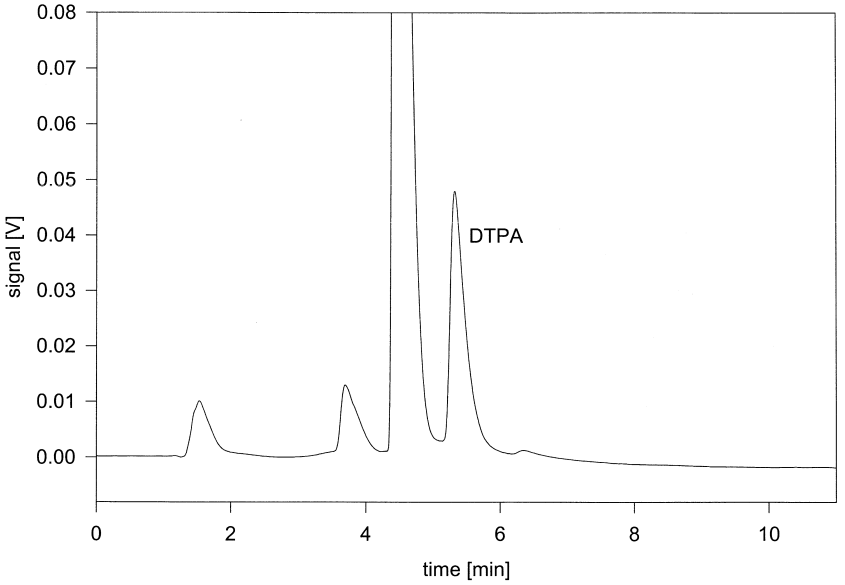

Fig. 3. A typical chromatogram of 200 mg l^{-1} DTPA in plasma. Previously the plasma proteins were removed by ultrafiltration.

In conclusion the new method based on the formation of a highly stable DTPA–lead complex with a drastically increased absorption coefficient and a bathochromic shift of the absorption maximum for the quantitative determination of DTPA can be used for the determination of DTPA plasma levels in clinical trials. The concentration range for the quantitative detection of DTPA was shown to be between 10 and 1000 mg l^{-1} DTPA. The separation of DTPA from components of human plasma was possible. The recovery of DTPA in plasma samples was efficient over the total concentration range of the calibration curve.

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