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Assay for the dianhydride of diethylenetriaminepentaacetic acid and its major degradation products by capillary electrophoresis

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Abstract

DTPA-DA, the dianhydride of diethylenetriaminepentaacetic acid (DTPA), is a drug intermediate used for preparing functional derivatives of the DTPA metal chelator as well as for coupling DTPA to polypeptides, antibodies and other macromolecules. The DTPA functions as a complexing agent for various types of metal ions or radionuclides for diagnostic as well as therapeutic applications. A capillary electrophoretic (CE) method has been developed to assess the purity of DTPA-DA. The method involves derivatization of the DTPA-DA to form a diamide and subsequent complexation of the diamide with Zn^{2+} followed by free solution CE with UV absorbance detection at 200 nm. The procedure is capable of separating and quantitating the two major degradation products of DTPA-DA, the monoanhydride (DTPA-MA) and the free pentaacetic acid (DTPA). The method was validated with respect to linearity, precision/reproducibility, limit of detection and accuracy. This procedure was shown to be a fast and reliable method to assay this highly reactive drug intermediate.

1. Introduction

The DTPA chelator (diethylenetriaminepentaacetic acid) is an octadentate chelator of the polyamino-polycarboxylate variety. It forms relatively stable complexes with a number of di- and trivalent metal ions [1]. Its pharmaceutical importance lies in its ability to deliver various types of radionuclides or paramagnetic ions for both therapeutic and diagnostic applications. The dianhydride of DTPA (DTPA-DA) is a drug intermediate useful for preparing functional derivatives of DTPA [2,3] and for coupling the DTPA chelator to polypeptides [4,5], antibodies [6] as well as for incorporating DTPA into synthetic polymers [7,8]. The determination of the purity of DTPA-DA is critical to the use of this intermediate in preparing these various derivatives. Due to the high reactivity of this dianhydride compound, samples typically contain from 3 to 10% degradation products. The degradation products consist primarily of the hydrolysis products DTPA-MA (the monoanhydride) and free DTPA. Whereas DTPA-DA is bifunctional, DTPA-MA is monofunctional and DTPA itself is typically unreactive. Some reactions such as polymerizations depend on the bifunctionality of the DTPA-DA and will, therefore, be significantly affected by the purity of the sample.

Due to the rapid hydrolysis of the anhydride functionality, analysis of samples of DTPA-DA "as is" in aqueous solutions is precluded. In addition, these compounds possess weak UV

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chromophores and are quite hydrophilic making chromatographic analysis difficult. CE was chosen as the analysis method because it is well suited for highly charged hydrophilic compounds and is amenable to low-wavelength UV detection. In fact, several reference exist documenting the use of CE for analyzing other types of metal complexes [9,10]. In this method the anhydride functionalities are first converted to the corresponding propylamide derivatives by reaction with propylamine prior to CE analysis. Propylamine was selected from among the lower alkyl amines due to its ease of handling and availability in high purity which is necessary for this purity method. Electrophoretic separation of the derivatized sample is accomplished using the zinc complexes of the various species in the sample. This report summarizes results of validation experiments for this method.

2. Experimental

2.1. Materials

Water was purified by a Barnstead water purification system (Barnstead/Thermolyne). Propylamine (99 + %) was obtained from Aldrich. HPLC grade acetonitrile was purchased form J.T. Baker. $ZnCl_2$ (99.999%) was from Aldrich. All other chemicals were of reagent grade and obtained from J.T. Baker.

Samples of the DTPA-DA were synthesized in the department of Medicinal Chemistry at Sterling-Winthrop.

2.2. CE instrumentation

All separations were conducted on a Spectra-Physics (Thermo Separation Products, San Jose, CA, USA) SpectraPhoresis 1000 system controlled using an IBM Model 70386 PC running SpectraPhoresis version 1.05 software. Data was collected and processed using Fisons Multichrom data system (Danvers, MA, USA). All capillary tubing (70 cm × 50 μm I.D., 63 cm to the detector) was from Polymicro Technologies (Phoenix, AZ, USA). Capillaries were conditioned with 1 *M* NaOH for 15 min prior to use. The buffer was 50 m*M* boric acid adjusted to pH 10.05 with 5 *M* NaOH. Separations were conducted at 30 kV, 30°C using detection at 200 nm. All peak area measurements were corrected for mobilities by dividing by the peak migration time. Injections were accomplished using vacuum for 4 s. The capillary was reconditioned with 1 *M* NaOH for 30 s followed by 3 min with the buffer after every fourth run.

2.3. Sample preparation

The derivatization solution was propylamine–acetonitrile–water (4.4:69.6:26, v/v). The sample of DTPA-DA (10 ± 0.5 mg) was weighed into a 15-ml polypropylene capped test tube. While vortex-mixing at high speed, 460 μl of the derivatization solution were added to the sample until dissolved (about 5 s) followed by 100 μl of 1 *M* HCl. This was followed by 410 μl of a 10.0 mg/ml solution of $ZnCl_2$ (pH ~3 with 1 *M* HCl). The sample is then brought to a total of 4 ml by the addition of 3030 μl of water. This solution is then directly analyzed by CE.

3. Results and discussion

3.1. Separation conditions

Fig. 1 shows a typical electropherogram obtained from a typical sample of DTPA-DA that has been derivatized according to the conditions prescribed in the Experimental section. Electrophoretic separation of the derivatized sample is accomplished using the zinc complexes of the various species. Separation of the zinc complexes was found to be easier and more reproducible than directly analyzing the free acid form of the compounds. Fig. 2 shows the UV spectra obtained on the CE peaks of DTPA-DA and its degradation products DTPA-MA and DTPA (see Fig. 3 for structures) in a stressed sample of DTPA-DA. DTPA-MA and DTPA were the only significant degradation products detected in

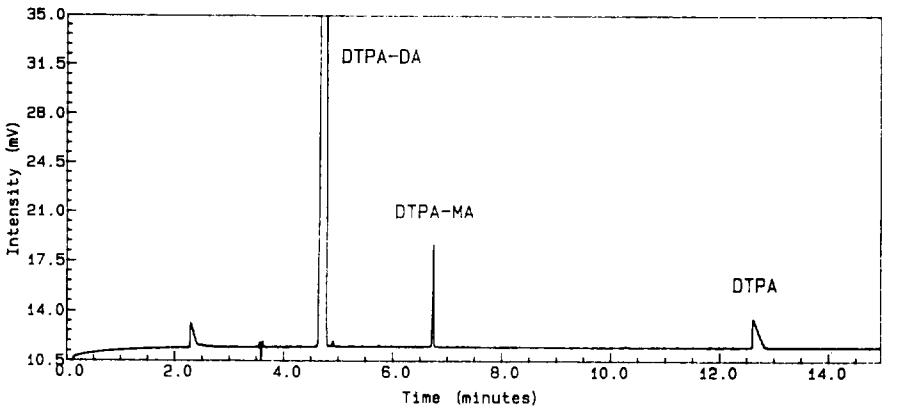

Fig. 1. Typical CE electropherogram obtained for the purity determination of DTPA-DA. Separation conditions given in the Experimental section.

stressed samples of DTPA-DA. All three compounds display similar spectral properties which is necessary for the successful implementation of peak-area percent calculations. No pure samples of DTPA-DA and DTPA-MA were available and, therefore, comparison of molar absorptivities of all three compounds could not be accomplished. However, it is anticipated that these compounds will have similar absorptivities supporting the use of peak-area normalization (corrected for mobilities). A wavelength of 200 nm was chosen for maximum sensitivity.

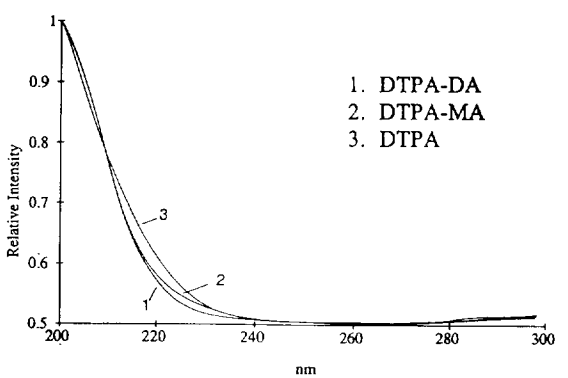

Fig. 2. UV spectra of the CE peaks of DTPA-DA, DTPA-MA and DTPA in a stressed sample of DTPA-DA. Conditions as given in the Experimental section with the instrument operated in the scanning mode.

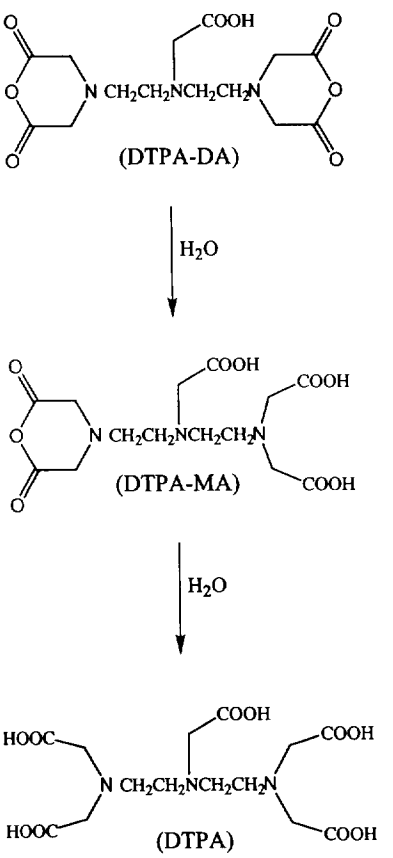

Fig. 3. Structures for DTPA-DA and its two hydrolysis products DTPA-MA and DTPA.

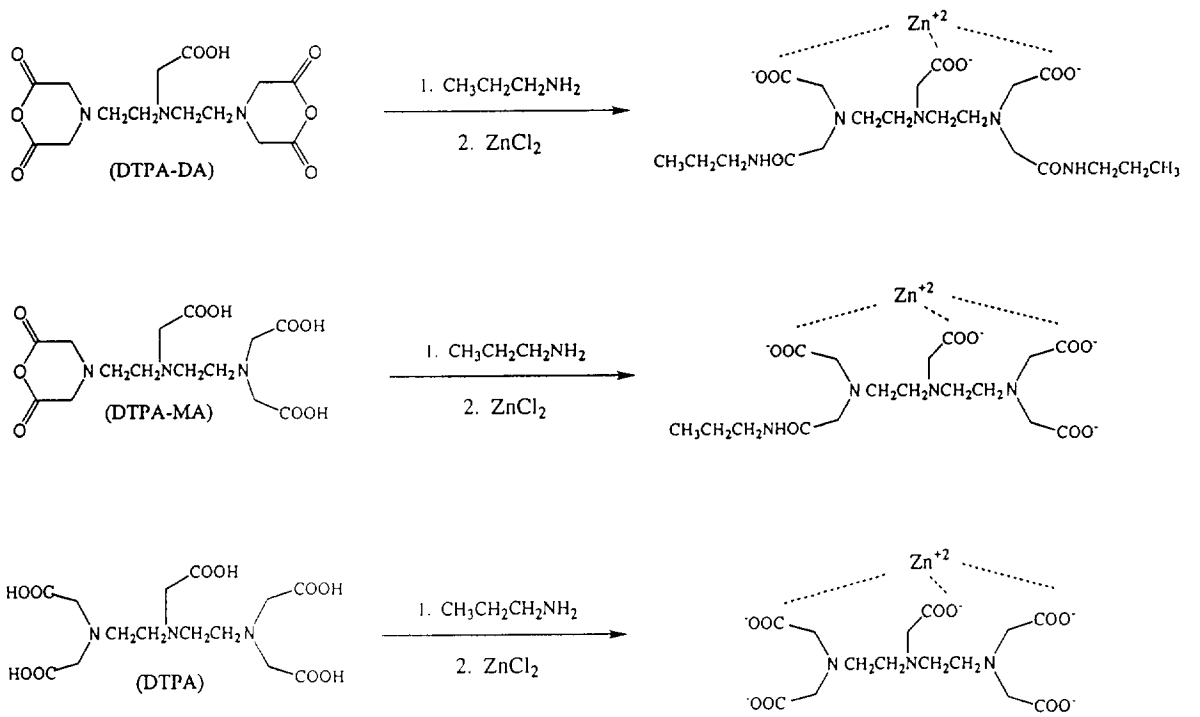

Fig. 4. Derivatization scheme for the CE purity assay for DTPA-DA.

3.2. Optimization of the derivatization conditions

Fig. 4 summarizes the derivatization scheme for this method. Several of the parameters important for the derivatization of DTPA-DA were optimized to ensure complete and reproducible reaction. A series of experiments were performed to demonstrate that DTPA-DA and DTPA-MA were not hydrolyzed in the derivatization matrix prior to their conversion to the corresponding amides. This included an optimization of the mole ratio of the propylamine derivatizing agent to anhydride functionalities, optimization of the amount of propylamine relative to water in the reaction matrix and an evaluation of the appropriate sample weight.

Table 1 summarizes the results for the optimization of the mole ratio of propylamine to anhydride. The reaction of the anhydride with propylamine is evidently very rapid relative to any hydrolysis as evidenced by the relatively low ratio of propylamine to anhydride needed to achieve reproducible results in terms of peak-area percentages. The high level of consistency in the peak-area percent results achieved with ratios from 3.4:1 to 14:1 indicates completeness of the derivatization process within this range. The ratio used in the method is 4.4:1.

Table 1
Optimization of the derivatization procedure with respect to mole ratio of propylamine to anhydride

Propylamine/anhydride ratio	Area (%)		
	DTPA-DA	DTPA-MA	DTPA
1.14	73.35	16.88	9.78
2.33	94.04	1.87	4.09
3.43	94.31	1.92	3.77
4.41[a]	94.27	1.91	3.82
9.25	94.44	1.84	3.73
14.1	94.40	1.83	3.77

[a] Ratio used in method.
Derivatization conditions as given in the Experimental section with the ratios of propylamine to anhydride as indicated. Area % results corrected for migration times.

Table 2
Optimization of the derivatization procedure with respect to mole ratio of water to propylamine

Water/ propylamine ratio	Area (%)		
	DTPA-DA	DTPA-MA	DTPA
18.6	93.82	2.01	4.17
27.2[a]	94.38	1.94	3.68
40.8	94.25	1.84	3.92
54.4	94.31	1.82	3.87

[a] Ratio used in method.
Derivatization conditions as given in the Experimental section with the ratios of water to propylamine as indicated. Area % results corrected for migration times.

Table 2 summarizes the results obtained with different mole ratios of water to propylamine in the derivatization matrix. Within the range examined here, which encompasses the level used in the method, the results were also fairly consistent. Table 3 summarizes the results obtained using different sample weights with an appropriately scaled amount of derivatization matrix. Again, the results were quite consistent in terms of peak-area percentages for all sample weights examined. These results support the conclusion that the derivatization reaction is complete and that there is no significant competitive hydrolysis occurring during the reaction using the conditions specified in the method.

As a final test for any competitive hydrolysis during the derivatization reaction, a different sample of higher purity (96.7%) was derivatized in one case using the conditions described in the Experimental section and in the other case in a non-aqueous matrix of propylamine in methanol. The results from this experiment are given in Table 4. Excellent agreement was obtained using the two different approaches proving that no hydrolysis is occurring.

Table 3
Optimization of the derivatization procedure with respect to the sample weight

Sample size	Area (%)		
	DTPA-DA	DTPA-MA	DTPA
5 mg	94.45	1.88	3.67
10 mg[a]	94.41	1.97	3.62
15 mg	94.49	1.98	3.53
20 mg	94.45	1.97	3.60

[a] Sample weight used in method.
Derivatization conditions as given in the Experimental section with the sample sizes as indicated. Area % results corrected for migration times.

3.3. Precision

The precision of injection in terms of migration times and peak-area percent results for DTPA-DA and its two hydrolysis products was evaluated for a typical sample preparation. Six replicate injections were made on each of two separate days using the same sample preparation. The data summarized in Table 5 demonstrate good precision in terms of both migration times and peak-area percentages for DTPA-DA and its two hydrolysis products. These data also demonstrate that the sample preparation is stable for at least one day.

The precision of the assay was investigated by performing six independent assays on another typical sample of DTPA-DA. The data from this experiment is given in Table 6. The R.S.D. for the overall purity estimate (peak area %) of DTPA-DA for these six assays was 0.145%. The R.S.D. for the estimate of DTPA-MA, which was present in this sample at the 1.94% level, was 2.52%. For DTPA, which in this sample was

Table 4
Comparison of the purity results for a sample of DTPA-DA derivatized with propylamine in an acetonitrile–water matrix and a methanol matrix

	Area (%)		
	DTPA-DA	DTPA-MA	DTPA
Sample matrix of acetonitrile–water	96.76	1.94	1.30
Sample matrix of methanol	96.64	2.07	1.30

Area % results corrected for migration times.

Table 5
Two-day precision of injection results for the CE purity assay of DTPA-DA

	DTPA-DA		DTPA-MA		DTPA	
	Time (min)	Area (%)	Time (min)	Area (%)	Time (min)	Area (%)
Initial (day 1)						
Mean ($n = 6$)	4.64	94.37	6.47	1.94	11.51	3.68
R.S.D. (%)	0.190	0.021	0.236	0.430	0.443	0.374
24 Hours (day 2)						
Mean ($n = 6$)	4.65	94.39	6.48	1.94	11.52	3.66
R.S.D.	0.220	0.057	0.344	0.800	0.610	1.04

Table 6
Precision of assay results for the CE purity assay of DTPA-DA

	DTPA-DA		DTPA-MA		DTPA	
	Time (min)	Area (%)	Time (min)	Area (%)	Time (min)	Area (%)
Mean ($n = 6$)	4.81	96.76	6.78	1.94	12.69	1.30
R.S.D.	0.300	0.145	0.370	2.52	0.680	11.1

present at the 1.30% level, the R.S.D. was 11.1%.

3.4. Recovery

As mentioned previously, no pure samples of DTPA-DA or DTPA-MA were available to perform spiking experiments. Therefore, to get an assessment of accuracy, various amounts of a stressed sample of DTPA-DA were spiked into the unstressed sample to produce samples encompassing a range of levels of DTPA-DA and its hydrolysis products. These different compositions were then assayed using this method and the assay results for each component were compared to the expected values based on the individual assays of the stressed and unstressed sample and the known proportions of the two materials comprising the spiked samples. The results of this recovery experiment are summarized in Table 7. Excellent correlation was observed between the percent theory and percent

Table 7
Recovery results for DTPA-DA, DTPA-MA and DTPA

Sample	DTPA-DA		DTPA-MA		DTPA	
	Theory (%)	Found (%)	Theory (%)	Found (%)	Theory (%)	Found (%)
1	92.30	92.30	4.08	4.08	3.62	3.61
2	87.93	87.91	5.44	5.45	6.62	6.64
3	84.37	86.08	6.55	6.03	9.08	7.88
4	80.92	81.02	7.62	7.59	11.45	11.40

found results for all three components in the samples.

3.5. Linearity

The linearity of the response of the DTPA-DA peak in a derivatized sample (97% pure) was evaluated over the concentration range 0.002–5 mg/ml. This corresponds to 0.08–200% of the nominal 2.5 mg/ml target sample concentration. An R^2 value of 0.99974 and a y-intercept at the origin were obtained. These data support the peak-area normalization approach used to calculate the assay.

3.6. Limit of detection

The limit of detection, determined using the response from the derivatized DTPA-DA peak, was estimated to be 0.5 μg/ml or 0.02% of the target sample concentration. Although the detection limits for the two hydrolysis products are likely to be higher due to their longer migration times, a detection limit of at least 0.1% is anticipated to be achievable for these two degradation products.

4. Conclusions

A free solution CE method has been developed to assess the purity of DTPA-DA in the presence of its two major degradation products DTPA-MA and DTPA. The method was shown to be highly reproducible and linear with an estimated limit of detection of at least 0.1%.

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