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Assay and purity analysis of diatrizoate sodium in drug product by LC

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Abstract

An LC procedure was developed to separate diatrizoate sodium from three known impurities. These impurities are 2,4- and 2,6-diiodo-3,5-diacetamidobenzoic acid (DDZA), and the free amine (5-acetamido-3-amino-2,4,6-triiodobenzoic acid). The separation was achieved using a Hamilton, PRP-X100, anion exchange column. The retention of diatrizoate sodium and the impurities was dependent on pH, potassium chloride concentration and phosphate concentration. Increasing any of these mobile phase modifiers decreased the retention time of all of the components. The eluent for assay and purity determination of drug product consisted of 0.1 M potassium chloride and 0.05 M potassium phosphate dibasic in water/acetonitrile (900:100). The mean concentration of diatrizoate sodium in Hypaque Sodium 50% determined over 3 days was 102.3% of label claim with an R.S.D. of 1.3. The accuracy of the purity method, determined by spiking known amounts of the impurities at five concentrations ranging from 0.025 to 0.06% (w/w) into drug product, was 100.1% for DDZA and 94.2% for the free amine. The decomposition of diatrizoate sodium in 0.1 N potassium hydroxide at 85°C followed pseudo first-order kinetics. The calculated half-life was 2 days. © 1997 Elsevier Science B.V.

Keywords: LC; Diatrizoate sodium; Hypaque; Contrast media

1. Introduction

Diatrizoic acid is a triiodinated benzoic acid derivative and is used as an X-ray contrast agent. It is formulated as an injectable, oral powder and oral liquid. The active is either a sodium salt, meglumine salt or a mixture of both. Diatrizoic acid is stable in neutral solution [1]. It has been estimated that decomposition of the *N*-acyl bond to be 0.1% in 50 years [1]. Decomposition is observed at elevated temperatures in very acidic or basic conditions. The major decomposition product, resulting from acid and base hydrolysis, is the free amine (5-Acetamido-3-amino-2,4,6-triiodobenzoic acid). The free amine is toxic. The specification of the free amine in drug product is no more than 0.05% (w/w) [2]. The known impurities in DTZA Na drug substance are 2,4- and 2,6-diiodo-3,5- diacetamidobenzoic acid (DDZA) and the free amine (Fig. 1). Current USP methods of analysis for assay and purity rely on wet chemical methods [2]. These methods are not selective

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in that they do not differentiate the decomposition products. In a recent publication, a chromatographic procedure was developed which separated the DDZA isomers from diatrizoic acid [3]. In another publication, a proton NMR spectroscopic procedure was developed for measuring diatrizoate in commercial solutions [4].

This paper describes the simultaneous separation of diatrizoate sodium (DTZA Na) from the free amine and DDZA. The validation parameters included determination of accuracy, precision, limits of quantitation and limits of detection in drug product [5]. The specificity of the LC procedure was tested by stressing DTZA Na to strong base and analyzing the reaction mixture for impurities and decomposition products. The separation was achieved on a Hamilton, PRP-X100 anion-exchange column using an eluent of 0.1 M potassium chloride and 0.05 M of potassium phosphate dibasic in water/acetonitrile (900:100). Also discussed is the effect of mobile phase modifiers (pH, phosphate and potassium chloride) on the elution of these compounds.

2. Materials and methods

2.1. Reagents

Diatrizoate sodium, 5-acetamido-3-amino-2,4,6-triiodobenzoic acid, 2,4- and 2,6-diiodo- 3,5-diacetamidobenzoic acid, Hypaque Sodium 50% (diatrizoate sodium injection, USP) were obtained from Nycomed, Wayne, PA. Potassium chloride, reagent grade, was obtained from EM Science, Gibbstown, NJ. Potassium phosphate dibasic, ACS reagent grade, potassium phosphate monobasic, ACS reagent grade, and 0.1 N potassium hydroxide, volumetric solution were obtained from J.T Baker, Phillipsburg, NJ.

2.2. Chromatographic conditions

The LC system consisted of a Perkin Elmer Series 200 autosampler, a Perkin Elmer Series 200 LC pump and an Applied Biosystems, 785A programmable absorbance detector. Detection was at 232 nm. The injection volume was 10 μl. The flow

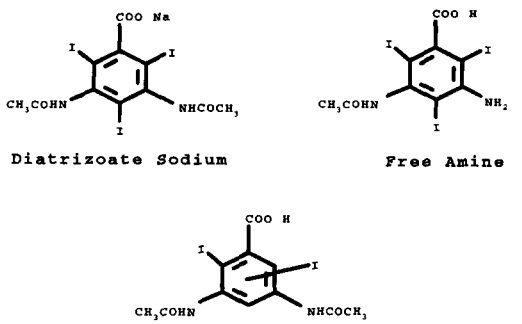

Fig. 1. Chemical structures of diatrizoate sodium and its related compounds

rate was 1.0 ml min^{-1}. Samples were separated on a 100 × 4.1 mm, PRP-X100 stainless steel column (Hamilton, Reno, NV). The mobile phase, for assay and purity analysis, consisted of potassium chloride (0.1 M) and potassium phosphate dibasic (0.05 M) into 900 ml of water and 100 ml of acetonitrile. For photodiode array analysis, a Shimadzu, SPD-MSA Photodiode Array UV-Vis Detector was used.

To determine the effect of apparent pH on the elution of the free amine, DDZA and DTZA Na, four different mobile phases were prepared at pHs of 4.7, 6.5, 7.9 and 9.3. To prepare these mobile phases, two solutions were prepared. The first solution contained 0.05 M of potassium phosphate dibasic, 0.1 M potassium chloride in 900 ml of water and 100 ml of acetonitrile. The second solution contained 0.1 M of potassium phosphate monobasic and 0.15 M of potassium chloride in 900 ml of water and 100 ml of acetonitrile. The calculated ionic strength of each solution was 0.25. The two mobile phases were mixed together to achieve the appropriate pH. After column equi-

Table 1
The effect of mobile phase pH on the elution of DTZA Na and its impurities

Apparent (pH)	k′ (DDZA)	k′ (DTZA Na)	k′ (FA[a])
4.7	1.6	3.7	12.8
6.5	1.3	2.9	9.8
7.9	1.0	2.4	7.9
9.3	0.9	2.3	7.4

[a]FA stands for free amine.

Table 2
The effect of phosphate concentration on the elution of DTZA Na and its impurities

Phosphate concentration [M]	k′ (DDZA)	k′ (DTZA Na)	k′ (FA)[a]
0.04	2.4	5.5	21.1
0.08	1.9	4.3	16.8
0.11	1.7	3.9	15.1
0.15	1.5	3.5	13.7

[a]FA stands for free amine.

libration, a resolution mixture consisting of DDTA, the free amine and DTZA Na, diluted in mobile phase, were injected onto the column. The concentration of each analyte was 0.125 mg ml^{-1}.

To determine the effect of phosphate concentration, four mobile phases were prepared at four different phosphate concentrations by varying the concentration of potassium phosphate, dibasic. The concentration of potassium phosphate dibasic in each of the mobile phases was 0.038, 0.075, 0.112 and 0.15 M prepared in 900 ml of water and 100 ml of acetonitrile. After column equilibration, the resolution mixture was injected onto the column.

To determine the effect of chloride ion on the elution of the three analytes, four mobile phases containing different concentrations of potassium chloride were prepared in 900 ml of water and 100 ml acetonitrile. The concentration of potassium chloride in each of the four mobile phases was 0, 0.05, 0.1 and 0.15 M. The concentration of potassium phosphate dibasic, to maintain a constant ionic strength of 0.3, was 0.1, 0.083, 0.066 and 0.05 M, respectively. The resolution mixture was injected after column equilibration.

Table 3
The effect of potassium chloride on the elution of DTZA Na and its impurities

K Cl [M]	k′ (DDZA)	k′ (DTZA Na)	k′ (FA)[a]
0	1.7	3.9	15.3
0.05	1.0	2.4	7.9
0.10	0.9	2.1	6.7
0.15	0.8	1.8	5.7

[a]FA stands for free amine.

Drug substance was stressed in strong base at a temperature of 85°C. Into a 100 ml volumetric flask, 2.5 g of drug substance was dissolved and diluted to volume with 0.1 N potassium hydroxide. Aliquots, approximately 5 ml, were transferred to vials and placed into an 85°C oven. At specific time intervals, a vial was removed from the oven. An aliquot was removed and diluted 100-fold for LC analysis.

To determine intermediate precision of the assay procedure, Hypaque Sodium 50% was assayed on 3 separate days. For each days analysis, a new vial was opened. Mobile phase and standard solution of DTZA Na, at a concentration of 0.25 mg ml^{-1}, were prepared fresh for each day. Three serial dilutions, using mobile phase as the diluent, were made such that the final concentration of diatrizoic acid was 0.25 mg ml^{-1}. The concentration of DTZA Na was determined by using bracketed standards.

The accuracy of the purity method was determined by spiking known amounts of DDZA and the free amine into Hypaque Sodium 50% at nominal concentrations of 0.025, 0.03, 0.04, 0.05 and 0.06% (w/w). Solutions were prepared as follows. A primary stock solution of the DDZA and the free amine were prepared each at a nominal concentration of 125 μg ml^{-1}. In 25 ml volumetric flasks, 2.5 ml of the Hypaque Sodium 50% was added. Into these same flasks, a single aliquot of the DDZA and free amine primary stock was added: 2.5, 3, 4, 5 or 6 ml. The samples were diluted to volume with mobile phase. The final dilution was made by taking a 2.5 ml aliquot into a 25 ml flask, diluting to volume with mobile phase. The concentration of DTZA Na after the last dilution was 5 mg ml^{-1}. Intermediate preci-

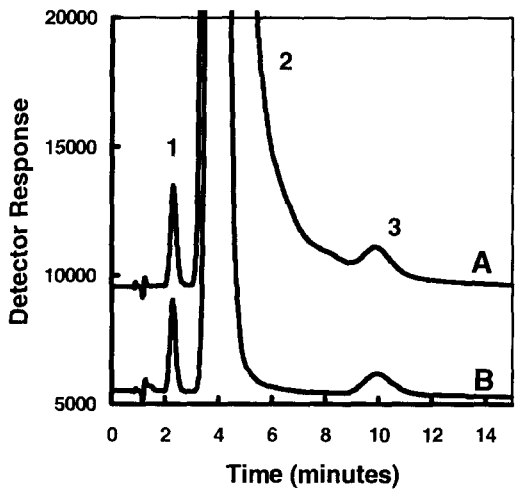

Fig. 2. Typical chromatograms where A is drug product spiked with DDZA and free amine each at 0.05% (w/w) and B is a standard mixture: 1, DDZA; 2, DTZA Na and 3, free amine.

sion was determined over 3 days at a nominal spiking concentration of 0.05% (w/w). The standard mixture contained DDZA and the free amine, each at a concentration of 2.5 μg ml^{-1}.

3. Results

Separation was achieved using a Hamilton, PRP X-100 column. The order of elution was the DDZA isomers, DTZA Na, and the free amine. The DDZA isomers were found to coelute. Both isomers have similar molar extinction coefficients. The PRP-X100 is a strong base anion exchanger containing *N,N,N,*-trimethyl-ammonium methyl-styrene- divinylbenzene (0.2 mEq g^{-1}) [6]. According to the literature, approximately one in every seven phenyl sites is functionalized. This stationary phase has been shown to function as an ion exchange phase for organic anions, while retaining some of the original reverse-phase characteristics [6]. The effect of mobile phase pH on the elution of DTZA Na and its impurities are shown in Table 1. Mobile phases were prepared in phosphate buffer. Ionic strength was kept constant at 0.25 by the addition of potassium chloride. The results show that increasing pH decreased retention times. All three analytes are weak acids and should be ionized in the prepared mobile phases. The pKa of DTZA Na is 3.4 [1]. Hence, the retention of these analytes is perhaps partially due to ion pairing of the carboxylate ion to the ion exchanger on the column. The free amine was retained longer than the other compounds, because in addition to the negative charged carboxylate, there is a lone pair of electrons on the free amine which may interact with the exchanger to form perhaps a stronger ion pair. Separation of DDZA from DTZA Na is probably due to the reverse-phase characteristic of the column. The DDZA is less lipophilic due to one less iodine and elutes before the DTZA Na. The effect of phosphate ion concentration is shown in Table 2. Mobile phases were prepared in potassium phosphate, dibasic. The apparent pH range was 9.3 ± 0.2. It was shown that increasing phosphate concentration, decreased the observed retention times. The effect of potassium chloride concentration is shown in Table 3. The ionic strength was kept constant at 0.3 by the addition of potassium phosphate, dibasic. The results show that increasing chloride ion concentration decreased the observed retention times. The observed retention time changes due to pH, chloride ion concentration and phosphate ion concentration are in accordance to theoretical consideration [7]. Chloride

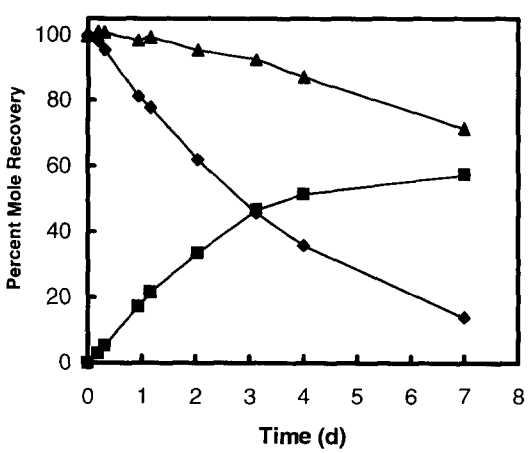

Fig. 3. Decomposition of DTZA Na in 0.1 N potassium hydroxide at 85°C where (▲) is mass balance; (◆) is DTZA Na and (■) is the free amine

Table 4
Accuracy determination of DDZA

Wt% of hypaque	Spike amount (μg)	Spike[a] concentration (μg ml^{-1})	Peak area	Amount found	%Recovery
0.025	313	1.25	37 167	319	101.9
0.025	313	1.25	36 008	308	98.4
0.025	313	1.25	36 774	316	101.0
0.03	376	1.50	42 609	383	101.9
0.03	376	1.50	41 796	374	99.5
0.03	376	1.50	42 355	380	101.1
0.04	501	2.00	53 633	508	101.4
0.04	501	2.00	53 654	508	101.4
0.04	501	2.00	53 364	505	100.8
0.05	627	2.51	64 230	627	100.0
0.05	627	2.51	65 979	646	103.0
0.05	627	2.51	65 047	636	101.4
0.05	627	2.51	64 213	628	100.2
0.05	627	2.51	62 188	605	96.5
0.05	627	2.51	61 646	599	95.5
0.06	752	3.01	76 030	757	100.7
0.06	752	3.01	74 585	741	98.5
0.06	752	3.01	74 874	745	99.1
				Mean	100.1
				S.D.	1.9
				R.S.D.	1.9

[a]The represented spiked amount was diluted 1 in 250 for analysis.

ion and phosphate ion act as counter ions. The observed pH effect is perhaps due to the more dissociated phosphate anion being a better counter ion.

The mobile phase selected for assay and purity analysis of Hypaque Sodium 50% consisted of 0.1 M potassium chloride, 0.05 M potassium phosphate dibasic in water/acetonitrile (900:100). The concentrations of potassium chloride and potassium phosphate dibasic were chosen because of better peak shape and decreased retentions. The resolution between DDZA and DTZA Na typically ranged from 2.1 to 2.4. The capacity factor (k') for DDZA was in all cases greater than 0.7. A typical chromatogram is shown in Fig. 2. The observed retention times of DDZA, DTZA Na, and the free amine were 2.3, 3.9 and 10.9 min, respectively. For purity analysis, DTZA Na is diluted to a concentration of 5 mg ml^{-1}; the same sample is diluted 20-fold for DTZA Na assay. The validation parameters included determination of accuracy, intermediate precision and stressing of drug substance to strong base at a temperature of 85°C. Drug substance, prepared at a concentration of 25 mg ml^{-1} in 0.1 N potassium hydroxide was stressed at 85°C. The reaction was followed over a period of 7 days. At specific time points, shown in Fig. 3, an aliquot was removed, diluted and chromatographed. The major decomposition product, by base hydrolysis, was the free amine. The decomposition of DTZA Na, the formation of the free amine and the calculation of mass balance (mM DTZA Na + mM free amine divided by the initial mM of DTZA Na multiplied by a 100) is shown in Fig. 3. Mass balance recoveries decreased over time. At day 7, mass balance was only 71%. Also formed during the course of the reaction were two unknowns. The first had a retention time of 2 min and the second, 6.5 min. The UV scan of the unknown peak at 2 min by PDA was dissimilar to that of the DDZA. Reports have suggested that DDZA is a decomposition product [3]. But in this study no DDZA was observed after stressing drug substance to strong base. It is perhaps better to say that DDZA is a synthetic impurity resulting from in-

Table 5
Accuracy determination of the free amine

Wt% of Hypaque	Spike amount (μg)	Spike[a] concentration (μg ml^{-1})	Peak area	Amount found (μg)	%Recovery
0.025	314	1.26	28 817	312	99.4
0.025	314	1.26	27 674	296	94.3
0.025	314	1.26	27 050	290	92.4
0.03	377	1.51	33 720	362	96.0
0.03	377	1.51	32 415	348	92.3
0.03	377	1.51	33 762	363	96.3
0.04	502	2.01	43 951	473	94.2
0.04	502	2.01	43 121	464	92.4
0.04	502	2.01	43 684	470	93.6
0.05	628	2.51	55 193	597	95.1
0.05	628	2.51	55 589	602	95.9
0.05	628	2.51	55 589	602	95.9
0.05	628	2.51	54 812	593	94.4
0.05	628	2.51	53 902	583	92.8
0.05	628	2.51	53 202	575	91.6
0.06	753	3.01	66 752	710	94.3
0.06	753	3.01	65 152	693	92.0
0.06	753	3.01	65 845	700	93.0
				Average	94.2
				S.D.	2.0
				R.S.D.	2.1

[a]The represented spiked amount was diluted 1 in 250 for analysis.

complete iodination of the 3,5-dinitro-benzoic acid, a precursor of diatrizoic acid [1]. The decomposition of DTZA Na followed first order reaction kinetics and was determined by the direct plot method by plotting the natural log of DTZA Na concentration remaining versus time. The resulting plot was linear with a correlation coefficient of 0.9978. The reaction rate constant, from the slope of the curve, was 0.2793 d^{-1}. The calculated half-life was 2.5 days.

Intermediate precision of the assay method, that is reproducibility, was determined by assaying drug product on 3 different days. The standard solution and mobile phase were prepared fresh on each of the 3 days. Two separate, Hamilton PRP-X100, 100 mm columns and two different analysts were used for this determination. A total of 13 samples were prepared: five on day 1, five on day 2 and three on day 3. The mean value over 3 days was 102.3% of label claim with an R.S.D. of 1.27%. The mean values ranged from 100 to 104.3%.

Accuracy, defined as closeness of the measured analyte value to the true value, was determined by spiking known amounts of DDZA and the free amine into Hypaque Sodium 50%. Spiking levels ranged from 0.025 to 0.06% (w/w) of active. The concentration of DTZA Na after final dilution was 5 mg ml^{-1}. The amounts recovered and percent recoveries appear in Table 4 and Table 5. The average recoveries of DDZA and the free amine were 101.1 and 94.2%, respectively. The limits of quantitation (LOQ) and limits of detection (LOD) were determined using Eq. (1) and Eq. (2) [8]; where m is slope and $s_{y/x}$ is the standard deviation of Y value distribution around the regression line. $s_{y/x}$ and slope were determined, using Excel software, by regressing the amount spiked in μg ml^{-1} (x-axis) versus peak area (y-axis).

$$\mathrm{LOQ} = 10^{*}(s_{y/x})/m \quad (1)$$

$$\mathrm{LOD} = 3^{*}(s_{y/x})/m \quad (2)$$

The resulting correlation coefficients were 0.9947 for DDZA and 0.9968 for the free amine. The DDZA LOQ was 0.47 μg ml^{-1} or 0.009%, calculated by dividing the LOQ by 5 mg ml^{-1} multiplied by 100. The DDZA LOD was 0.14 μg ml^{-1} (0.003%). The LOQ and LOD for the free amine were 0.37 (0.007%) and 0.11 μg ml^{-1} (0.002%), respectively. In a separate experiment DDZA and the free amine were spiked into Hypaque Sodium 50% at concentrations close to the calculated LOQ's. Triplicate samples were prepared. The final concentrations after dilution of DDZA and the free amine were 0.398 and 0.403 μg ml^{-1}, respectively. The average recovery of DDZA was 97.8% with an R.S.D. of 3.4. The average recovery of the free amine was 100.9% with an R.S.D. of 3.1%.

The intermediate precision of the purity method was determined over 3 days at a spiking level 0.05% (w/w). A total of 14 samples were prepared: three on day 1, five on day 2 and six on day 3. The average recovery for DDZA was 98.4% with an R.S.D. of 3.7% with values ranging from 88.5 to 103.1%. The average recovery of the free amine was 91.4% with an R.S.D. of 4.5% with values ranging from 80.4 to 95.8%.

4. Conclusion

The retention of DTZA Na and its impurities was found to be dependent on chloride ion concentration, phosphate ion concentration and pH. The LC procedure for Hypaque Sodium 50% appears adequate. Two separate methods were validated: one for the assay of diatrizoate sodium and the other for purity. Both methods utilize the same chromatographic conditions. For purity, DTZA Na in Hypaque Sodium Injection is diluted to a concentration of 5 mg ml^{-1}. This is further diluted 20-fold for assay. These methods were found to be stability indicating, selective, precise and accurate. The methods should prove useful as an alternative to the labor intensive USP procedures.

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