Determination of Some Diuretic Drugs in Pure form and Their Pharmaceutical Formulations with Ammonium Hexanitratocerate (IV)

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ABSTRACT

A quick, convenient and simple titrimetric method for determination of diuretic drugs e.g., acetazolamide, furosemide, hydrochlorothiazide, mannitol and spironolactone in pure form and in their pharmaceutical formulations *viz*., diamox (tab), synomax (tab), tebemid (tab), frusemene (tab), aquazide (tab), xenia (tab), kratol (inj), mannigyl (inj), aldactide (tab) and spilactone (tab) with ammonium hexanitratocerate (IV) reagent have been reported. It is a versatile oxidizing agent of cerium (IV) and is being widely used as an oxidant for several classes of organic compounds. The values of percentage error, coefficient of variation (CV) and standard deviation (SD) prove the method is precise and reproducible. To establish authenticity of the method, percentage recovery experiments were also carried out by standard drug addition method indicating non interference of excipients in the method results.

Keywords: Diuretics, Pharmaceuticals, Ammonium hexanitratocerate (IV), Oxidizing agent, Titration.

INTRODUCTION

A variety of compounds containing cerium (IV) have proved to be versatile reagents capable of oxidizing almost every oxidizable functional group.[1] Extensive work has lead to the development of a good number of such oxidants like cerium mischmetal[2], cerium ammonium sulphate[3,4], cerium sulphate[5,6], cerium nitrate[7], cerium fluoride[8], cerium chloride[9,10], cerium oxide[11,12], cerium metal[13], cerium carbonate[14] and cerium acetate.[15] There is no any titrimetric method is reported till date in literature for the estimation of diuretic drugs used for present study. In Indian pharmacopoeia, determination of acetazolamide, furosemide, hydrochlorothiazide and spironolactone is reported by infrared absorption spectrophotometry while mannitol by liquid chromatography.[16] In British Pharmacopoeia the determination of acetazolamide, furosemide, hydrochlorothiazide, spironolactone and mannitol is reported by thin layer chromatography using silica gel under ultraviolet light at 254 nm.[17]

Industrial demands have led many workers to search for more ideal oxidants with a number of specifications including lower cost, higher yields, better selectivity, milder neutral conditions, easier preparations, high solubility, less toxicity and short reaction times. Among the above mentioned reagents[2–15], ammonium hexanitratocerate (IV) (AHC) has an edge over others for rendering easy manipulation and sharp end points. Therefore we have selected ammonium hexanitratocerate (IV) (AHC) as an oxidizing reagent for our study.

Diuretics are generally referred as the "water pills and are used to treat heart failure, liver cirrhosis, hypertension and certain kidney diseases. Acetazolamide is used to prevent and reduce the

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symptoms of attitude sickness. Furosemide is a powerful diuretic that is used to treat excessive accumulation of fluid and swelling (edema) of the body caused by heart failure, cirrhosis, chronic kidney failure and the naphrotic syndrome. Hydrochlorothiazide is used to treat high blood pressure, decrease swelling of the arms, legs and stomach and to treat a condition known as "water diabetes" (diabetes insipidus) and to help prevent calcium kidney stones. Mannitol is used in the circuit prime of a heart lung machine during cardiopulmonary problems with other diuretics (e.g. Furosemide, Chlorothazide) and intravascular replacement. Spironolactone is used as anti-androgen by binding to the androgen receptor and preventing it from interacting with dihydrotestisterone. Because of great medicinal value of these compounds their estimation has widely been studied. The methods reported involve sophisticated instruments and complicated techniques. In the present paper, we describe a simple and convenient titrimetric method for the determination of some diuretics with ammonium hexanitratocerate (IV) reagent.

MATERIALS AND METHODS

Ammonium hexanitratocerate solution (0.1 M)

13.70 g of ammonium hexanitratocerate (IV) was weighed accurately and dissolved in 250 ml of 0.5 N nitric acid in a 250 ml volumetric flask.

Ferrous ammonium sulphate solution (0.025 M)

2.4508 g of ferrous ammonium sulphate was accurately weighed and dissolved in distilled water in the presence of 10 ml of 4M H_2SO_4 in a 250 ml volumetric flask.

Ferroin indicator (0.001 M)

Solution was prepared by diluting 0.025 M ferroin (1, 10-phenanthroline ferrous sulphate complex) solution (4 ml) with distilled water (96 ml) in a 100 ml volumetric flask.

Sulphuric acid solution (4 M)

Solutions were prepared by diluting concentrated sulphuric acid (37 N, Merck) with distilled water.

Sample solutions (1 mg/ml)

Accurately weighed (100 mg) pure samples as well as pharmaceutical formulation of acetazolamide and mannitol were dissolved in min. amount of distilled water and furosemide was dissolve in min. amount of alcohol. Hydrochlorothiazide and spironolactone were in min amount of acetone in a 100 ml volumetric flask and then made up to the mark with distilled water to give a concentration of 1 mg/ml. While diluting the solution every care was taken to keep the solution homogeneous.

Tablets solution

Twenty tablets of a particular sample were crushed to a fine powder and powder equivalent to 100 mg of sample was taken in 100 ml volumetric flask and dissolved as above to get a concentration of 1 mg/ml. No residue was noted in any of the samples.

Injections solution

The injection volume equivalent to 100 mg of the pure sample were taken and dissolved as usual to get a concentration of 1 mg/ml.

General procedure

Aliquots containing 1–5 mg of the samples were taken in 100 ml stoppered conical flask followed by the addition of 5 ml ammonium hexanitratocerate (IV) (AHC) reagent. The reaction mixture was shaken well and allowed to react for required reaction time (10–15 min) at room temperature (25–30°C). After the reaction was over it was quenched by adding 10 ml of 4 M sulphuric acid. The unconsumed Ce (IV) was titrated against 0.025 M ferrous ammonium sulphate solution using two drops of ferroin indicator (0.001 M). A blank experiment was also performed under identical conditions using all the reagents except the sample. The amount of drug in pure samples and pharmaceutical formulations was calculated by following expression:

$$\text{mg of sample} = \frac{M \times N(B - S)}{n}$$

Where, M = Molecular weight of sample, N = Normality of ferrous ammonium sulphate solution, B = Volume of ferrous ammonium sulphate for blank, S = Volume of ferrous ammonium sulphate for sample, n = Stoichiometry of the reaction.

For testing quantitative validity of the recommended method, standard deviation (SD) and coefficient of variation (CV) were also calculated for each sample size. At least nine determinations were carried out and the average results were noted. To justify the validity of the

proposed method, recovery experiments were carried out by the standard drug addition method (Table 2–6). A known amount of the pure compound was taken and to this, varying amounts of the pharmaceutical preparations of the same compounds were added the total amount of the compound was determined by the usual method.[18]

$$\%\text{Recovery} = \frac{N(\Sigma XY) - (\Sigma X)(\Sigma Y)}{N(\Sigma X^2) - (\Sigma X)^2} \times 100$$

Where, N = ΣN = Total number of observations, X = Amount of drug added, Y = Amount of drug obtained by calculation.

Standard deviation (SD) was calculated by the expression

$$SD = \sqrt{\frac{(X_1 - \bar{X})^2 + (X_2 - \bar{X})^2 \cdots (X_n - \bar{X})^2}{(n-1)}}$$

From the value of SD, Coefficient of variation (CV) was calculated by the following expression.

$$CV = \frac{SD \times 100}{\bar{X}}$$

Where, $\bar{X}$ = Average value of amount obtained by calculations, X_1, $X_2 \cdots X_n$ = Amount obtained by calculations in different observations, n= Number of observations

The determinations were done with varying sample size (i.e. 1–10 mg) but for convenience, results have been shown only with 1, 3 and 5 mg sample size (Table 1).

RESULTS AND DISCUSSION

Although in British Pharmacopoeia the determination of acetazolamide, furosemide, hydrochlorothiazide, spironolactone and mannitol is reported by thin layer chromatography using silica gel under ultraviolet light at 254 nm[17] but in a routine analytical laboratory it if difficult to perform tests reported by Them. In our present investigation, we started with simple titrimetric method which is economical as well as precise and can be performed in an ordinary laboratory. Investigation results indicate that the stoichiometric ratio between ammonium hexanitratocerate (IV) (AHC) reagent and diuretics varies, and different stoichiometric ratio is obtained in different diuretics such as acetazolamide (1:2), furosemide (1:2), hydrochlorothiazide (1:4), mannitol (1:2) and spironolactone (1:4) in pure form and in their pharmaceutical preparations. The ratio remains constant even under varying reaction conditions i.e. change in reaction time, concentration of the reagent, reaction medium, reaction temperature etc. It was observed that all the compounds studied need 15 min to complete the reaction except acetazolamide and mannitol which require only 10 min. By allowing more reaction time (more than 10–15 min) there is no improvement in the results. A lesser reaction time (less than 10 min) than the described limit gives higher percentage of error because of incomplete reaction. The effect of concentration of ammonium hexanitratocerate reagent (0.01–0.16 M) was also studied and it was found that the recommended concentration (0.10 M) was suitable for accurate and concordant results.

Effect of variation in reaction temperature was studied. The reactivity of the sample is very slow at ice cold temperature but increases with the rise in temperature up to room temperature (25–30°C). Beyond this temperature, no improvement over the results has been noticed.

On the basis of available literature and stoichiometry, a possible course of reaction may also be suggested. Since the isolation and identification of final reaction product was not possible, it is assumed that the diuretic drugs were oxidized to their corresponding oxidized products.

Interferences

Excipients like up to be present in pharmaceutical formulations are starch, calcium carbonate, sodium carbonate, cellulose, magnesium trisilicate, tricalcium phosphate and gum acacia. The percentage recovery was found to be between 99.19% to 99.81% which indicated the non-interference of excipients in the described titrimetric method.

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Table 1: Determination of Some Diuretic Drugs in Pure form and in Their Pharmaceutical Preparations with (0.1 m) Ammonium Hexanitratocerate (IV) Reagent

S. No.	Sample	Aliquots taken (ml)	Amount present* (mg)	Reaction time (min)	Molecularity	Amount obtained by calculation** (mg)	Error (%)	SD	CV
1	Acetazolamide (Pure Sample)	1.00	0.987	10	2	0.977	–1.01	0.0035	0.3582
		3.00	2.961	10	2	2.940	–0.71	0.0034	0.1156
		5.00	4.935	10	2	4.911	–0.48	0.0047	0.0977
A	Diamox (Tab) Wyeth	1.00	0.911	10	2	0.901	–1.08	0.0043	0.4772
		3.00	2.733	10	2	2.713	–0.73	0.0033	0.1216
		5.00	4.555	10	2	4.533	–0.48	0.0028	0.0618
B	Synomax (Tab) Syntho Pharma	1.00	0.886	10	2	0.877	–1.02	0.0030	0.3421
		3.00	2.658	10	2	2.640	–0.68	0.0036	0.1364
		5.00	4.430	10	2	4.411	–0.43	0.0042	0.0952
2	Furosemide (Pure Sample)	1.00	0.973	15	2	0.963	–1.03	0.0051	0.5296
		3.00	2.919	15	2	2.896	–0.79	0.0036	0.1243
		5.00	4.865	15	2	4.842	–0.47	0.0052	0.1074
A	Tebemid (Tab) Neon Labs	1.00	0.893	15	2	0.884	–1.01	0.0020	0.2262
		3.00	2.679	15	2	2.657	–0.82	0.0032	0.1204
		5.00	4.465	15	2	4.438	–0.60	0.0030	0.076
B	Frusemene (Tab) GSK Pharma	1.00	0.903	15	2	0.893	–1.10	0.0025	0.2799
		3.00	2.709	15	2	2.691	–0.66	0.0022	0.0818
		5.00	4.515	15	2	4.496	–0.42	0.0027	0.0600
3	Hydrochloro-thiazide (Pure Sample)	1.00	0.962	15	4	0.952	–1.04	0.0023	0.2416
		3.00	2.886	15	4	2.868	–0.62	0.0026	0.0907
		5.00	4.810	15	4	4.794	–0.33	0.0030	0.0626
A	Aquazide (Tab) Sun Pharma	1.00	0.917	15	4	0.907	–1.09	0.0031	0.3418
		3.00	2.751	15	4	2.733	–0.65	0.0029	0.1061
		5.00	4.585	15	4	4.564	–0.46	0.0031	0.0679
	Xenia (Tab) USV Pharma	1.00	0.898	15	4	0.889	–1.00	0.0025	0.2812
		3.00	2.694	15	4	2.673	–0.78	0.0030	0.1122
		5.00	4.490	15	4	4.467	–0.51	0.0038	0.0851
4	Mannitol (Pure Sample)	1.00	0.944	10	2	0.934	–1.06	0.0036	0.3854
		3.00	2.832	10	2	2.811	–0.74	0.0027	0.0961
		5.00	4.720	10	2	4.695	–0.53	0.0031	0.0660
A	Kratol (Inj) Molekule	1.00	0.887	10	2	0.878	–1.01	0.0016	0.1822
		3.00	2.661	10	2	2.639	–0.83	0.0012	0.0455
		5.00	4.435	10	2	4.412	–0.52	0.0014	0.0317
B	Mannigyl (Inj) C B Pharma	1.00	0.923	10	2	0.913	–1.03	0.0022	0.2410
		3.00	2.769	10	2	2.749	–0.72	0.0024	0.0873
		5.00	4.615	10	2	4.591	–0.52	0.0030	0.0653
5	Spironolactone (Pure Sample)	1.00	0.959	15	4	0.949	–1.04	0.0030	0.3161
		3.00	2.877	15	4	2.857	–0.70	0.0022	0.0770
		5.00	4.795	15	4	4.773	–0.46	0.0025	0.0524
A	Aldactide (Tab) RPG Pharma	1.00	0.926	15	4	0.916	–1.08	0.0026	0.2838
		3.00	2.778	15	4	2.757	–0.76	0.0027	0.0979
		5.00	4.630	15	4	4.605	–0.54	0.0032	0.0695
B	Spilactone (Tab) Sun Pharma	1.00	0.908	15	4	0.898	–1.10	0.0028	0.3118
		3.00	2.724	15	4	2.704	–0.73	0.0027	0.0999
		5.00	4.540	15	4	4.515	–0.55	0.0031	0.0687

Tab = Tablet, Inj = Injection, *Average of nine determinations

Table 2: Recovery Studies of Acetazolamide by Standard Drug Addition Method

S.No.	Number of observations (N)	Amount present (Pure) (mg)	Amount of drug added (mg) X	Total amount of drug obtained by calculation (mg)	Amount of drug obtained by calculation (mg) Y	XY	X^2	Recovery (%)
1	3	0.987	0.983	1.958	0.995	0.978	0.966	
2	3	0.987	1.969	2.930	1.977	3.893	3.877	
3	3	0.987	2.960	3.836	2.862	8.472	8.762	99.19
4	3	0.987	3.965	4.964	3.973	15.753	15.721	
	∑N = 12		∑X = 9.877		∑Y = 9.807	∑XY = 29.096	$\sum X^2$ = 29.326	

Table 3: Recovery Studies of Furosemide by Standard Drug Addition Method

S. No.	Number of observations (N)	Amount present (Pure) (mg)	Amount of drug added (mg) X	Total amount of drug obtained by calculation (mg)	Amount of drug obtained by calculation (mg) Y	XY	X^2	Recovery (%)
1	3	0.973	0.987	1.867	0.898	0.886	0.974	
2	3	0.973	1.976	2.972	1.967	3.887	3.905	
3	3	0.973	2.966	3.940	2.970	8.809	8.797	99.72
4	3	0.973	3.989	4.874	3.975	15.856	15.912	
	∑N = 12		∑X = 9.918		∑Y = 9.810	∑XY = 29.438	$\sum X^2$ = 29.588	

Table 4: Recovery Studies of Hydrochlorothiazide by Standard Drug Addition Method

S. No.	Number of observations (N)	Amount present (Pure) (mg)	Amount of drug added (mg) X	Total amount of drug obtained by calculation (mg)	Amount of drug obtained by calculation (mg) Y	XY	X^2	Recovery (%)
1	3	0.962	0.985	1.953	0.953	0.939	0.970	
2	3	0.962	1.985	2.960	1.968	3.906	3.940	
3	3	0.962	2.984	3.968	2.974	8.874	8.904	99.81
4	3	0.962	3.968	4.970	3.972	15.761	15.754	
	∑N = 12		∑X = 9.922		∑Y = 9.867	∑XY = 29.480	$\sum X^2$ = 29.559	

Table 5: Recovery Studies of Mannitol by Standard Drug Addition Method

S. No.	Number of observations (N)	Amount present (Pure) (mg)	Amount of drug added (mg) X	Total amount of drug obtained by calculation (mg)	Amount of drug obtained by calculation (mg) Y	XY	X^2	Recovery (%)
1	3	0.944	0.974	1.961	0.991	0.965	0.949	
2	3	0.944	1.992	2.968	1.936	3.857	3.968	
3	3	0.944	2.970	3.968	2.960	8.791	8.821	99.38
4	3	0.944	3.989	4.984	3.992	15.924	15.912	
	∑N = 12		∑X = 9.925		∑Y = 9.8789	∑XY = 29.537	∑X2 = 29.650	

Table 6: Recovery Studies of Spironolactone by Standard Drug Addition Method

S. No.	Number of observations (N)	Amount present (Pure) (mg)	Amount of drug added (mg) X	Total amount of drug obtained by calculation (mg)	Amount of drug obtained by calculation (mg) Y	XY	X^2	Recovery (%)
1	3	0.959	0.954	1.959	0.947	0.903	0.910	
2	3	0.959	1.912	2.964	1.893	3.619	3.656	
3	3	0.959	2.867	3.978	2.845	8.157	8.220	99.24
4	3	0.959	3.821	4.972	3.794	14.497	14.600	
	∑N = 12		∑X = 9.554		∑Y = 9.479	∑XY = 27.176	$\sum X^2$ = 27.386	

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