ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
20 (1999) 727–735

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Spectrodensitometric determination of clorazepate dipotassium, primidone and chlorzoxazone each in presence of its degradation product

A.A. El Bayoumi [a,*], S.M. Amer [a], N.M. Moustafa [a], M.S Tawakkol [a]

[a] *Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt*

Received 26 August 1997; received in revised form 3 December 1997; accepted 16 January 1998

Abstract

The present work describes a quantitative thin layer procedure for estimating primidone, clorazepate dipotassium and chlorzoxazone in bulk powders and in dosage forms, each in the presence of its degradation product. The method consists of dissolving the drug in ethanol (for primidone), or methanol (for clorazepate dipotassium and chlorzoxazone) and then spotting this solution on a thin layer of silica gel G254. Quantitation is achieved by comparing the areas under the peaks obtained from scanning the thin layer chromatographic plates in a spectrodensitometer

Keywords: 2-Amino-5-chlorobenzophenone; 2-Amino-4-chlorophenol; Chlorzoxazone; Clorazepate dipotassium; Phenobarbital; Primidone; Spectrodensitometry

1. Introduction

Clorazepate dipotassium, primidone and chlorzoxazone are members of a class of compounds used as minor tranquillizers, anticonvulsants and central skeletal muscular relaxants, repectively. Several methods have been reported for the quantitative determination of clorazepate dipotassium including, non-aqueous titration [1],chromatographic [2,3], polarographic [4–6], spectrophotometric, [7–9] and spectrofluorimetric [10,11] methods. Analysis of pimidone includes polarographic [12,13], colorimetric [14–16], spectrophotometric [17–19], titrimetric [20] and chromatographic methods [21–27]. The methods of analysis of chlorzoxazone include spectrofluorimetric [28], spectrophotometric [29,30], colorimetric [31] and chromatographic [32–34] methods.

* Corresponding author.

0731-7085/99/$ - see front matter
PII: S0731-7085(98)00043-0

2. Experimental

2.1. Samples

- Clorazepate diapotassium, reference standard powder, obtained from Nile Pharmaceuticals, A.R.E.
- Primidone, reference standard powder obtained from Kahira Pharmaceutical/Chemical, A.R.E.
- Chlorzoxazone, reference sample obtained from Amon Pharmaceutical, Cairo A.R.E.
- 2-amino, 5-chlorobenzophenone, Aldrich.
- Phenobarbital, Merck.
- Tranxene capsules labeled to contain 5 mg of dipotassium batch No. 123 and 104 manufactured by the Nile Pharmaceuticals.
- Mysoline tablets labeled to contain 250 mg of primidone batch No. 426, 424 manufactured by Kahira Egypt, under licence of ICI, England.
- Myolgin capsules labeled to contain 250 mg of chlorzoxazone batch No. 114, 113 manufactured by Amon Pharmaceutical.

2.2. Reagents

- Ethanol, Prolabo.
- Methanol, Merck.
- Benzene, Prolabo.
- Ethyl Acetate, Prolabo.
- Acetic, Acid, Prolabo.
- TLC developing system consists of ethyl acetate: benzene: acetic acid (1:1:0.05).
- Plates for thin layer chromatography, precoated with silica gel G254. (Merck), conditioned with the mobile phase prior to use.
- 2-Amino-4-chlorophenol: prepared by refluxing 0.25 g of chlorzoxazone bulk powder with 25 ml of 20% sodium hydroxide for 2 h then cooling and neutralizing using 25 ml 2 M hydrochloric acid. 2-Amino-4-chlorophenol is extracted with diethyl ether and dried under vacuum at 60°C.

2.3. Apparatus

- Shimadzu spectrodensitometer CS-9000, dual wave length scanning densitometer.
- Hamilton syringe (10 μl).

3. Procedures

3.1. Densitometric determination of clorazepate dipotassium and its degradation product, 2-amino-5-chlorobenzophenone

0.02, 0.03, 0.4, and 0.05 g of both clorazepate dipotassium reference sample and 2-amino-5-chlorobenzophenone were weighed accurately and quantitatively transferred to a series of 100 ml volumetric flasks and dissolved in methanol. Ten μl of each solution was applied to a thin layer chromatographic (TLC) plate (20 × 20 cm) using a 10 μl syringe.

Spots were spaced 2 cm apart from each other and 2 cm from the edge of the plate. The plate was placed in a chromatographic tank previously saturated for 1 h with mobile phase, which consists of ethyl acetate:benzene:acetic acid (1:1:0.05).

The plate was developed by ascending chromatography through a distance of 18 cm, drying at room temperature and detecting the spots under UV. The results were scanned under the following conditions:

- Photo mode: reflection
- Scan mode: zigzag
- Chart speed: 50 mm min^{-1}

The wavelengths for clorazepate dipotassium ($R_f = 0.56$) and benzophenone ($R_f = 0.76$) were 228 and 385 nm, respectively.

3.2. Recorder parameters

- Abscissa scale × 1
- Result output Chromatogram and area under the peak

Peak detection:

- Minimum width 10 mm
- Minimum area 600
- Swing width 16 mm
- Drift line 100

The calibration curve representing the relationship between the recorded area under the peak and the corresponding concentration was plotted. Linear relationships were obtained. The regression equations were computed for both compounds and found to be:

$A = 3.41C + 0.52$ (for clorazepate dipotassium)

$r = 0.999$

$A = 7.28C - 0.40$ (for benzophenone)

$r = 0.997$

where: A is the area under the peak and C is the corresponding concentration in mcg spot^{-1}

The concentration of clorazepate dipotassium bulk powder and its degradation product (benzophenone) were calculated, each from it's corresponding computed regression equation. The results obtained are shown in Table 1.

3.3. Densitometric determination of primidone and its degradation product, phenobarbital

0.1–0.25 g of both the primidone reference sample and of phenobarbital were accurately weighed and quantitatively transferred to a series of 100 ml volumetric flasks and dissolved in ethanol. The experiment proceeded as described previously (Section 3.1), where the wavelengths of 222 and 218 nm were used for densitometric determination of primidone ($R_f = 0.46$) and phenobarbital ($R_f = 0.75$), respectively.

The area under the peaks was plotted against the respective concentration of primidone and phenobabital. The regression equations were also computed and found to be:

$A = 0.84C - 0.24$ (for primidone) $r = 0.999$

$A = 1.02C + 0.37$ (for phenobabital) $r = 0.998$

Where: A is the area under the peak and C is the corresponding concentration in mcg spot^{-1}. The concentration of primidone bulk powder and phenobarbital were calculated from it's corresponding computed regression equation. The results obtained are shown in (Table 1).

3.4. Densitometric determination of bulk powder of chlorzoxazone and its degradation product, 2-amino-4-chlorophenol

0.05–0.2 g of both the chlorzoxazone reference sample and of its degradation product, 2-amino-4-chlorophenol were weighed, then quantitatively transferred to a series of 100 ml volumetric flasks and dissolved in methanol. This proceeded as before (Section 3.1)using wavelengths of 282 and 285 nm for the densitometric determination of chlorzoxazone ($R_f = 0.75$) and 2-amino-4-chlorophenol ($R_f = 0.43$), respectively.

A calibration curve was constructed by plotting the area under the peak against the respective concentration of reference sample of chlorzoxazone and 2-amino-4-chlorophenol. Linear relationships were obtained.

The regression equations were computed for both compounds and found to be:

$A = 0.76C - 0.25$ (for chlorzoxazone)

$r = 0.998$

$A = 0.61C - 0.09$ (for chlorophenol)

$r = 0.999$

where: A is the area under the peak and C is the corresponding concentration in mcg spot^{-1}. The concentrations of chlorzoxazone and 2- amino, 4-chlorophenol were calculated from the corresponding regression equations. The results obtained are shown in Table 1.

4. Assessment of the accuracy of the proposed method when applied to mixtures of the reference drugs and their degradation products

The following laboratory mixtures were prepared in different proportions:

1-clorazepate dipotassium and 2-amino-5-chlorobenzophenone;

2-primidone and phenobarbital;

3-chlorzoxazone and 2-amino-4-chlorophenol.

(see Sections 3.1, 3.3 and 3.4 for the determination of the above mentioned compounds). The area under the peak was recorded and the concentration of each compound was calculated from the corresponding computed regression equation. The results obtained are shown in Table 2.

Table 1
Spectrodensitometric determination of authentic samples of clorazepate dipotassium, primidone and chlorzoxazone each with its degradation product

Clorazepate dipotassium			2-Amino-5-chlorobenzophenone			Primidone			Phenobarbital			Chlorzoxazone			2-Amino-4-chlorophenol		
Taken (mcg spot^{-1})	Found (mcg spot^{-1})	Recovery %	Taken (mcg spot^{-1})	Found (mcg spot^{-1})	Recovery %	Taken (mcg spot^{-1})	Found (mcg spot^{-1})	Recovery %	Taken (mcg spot^{-1})	Found (mcg spot^{-1})	Recovery %	Taken (mcg spot^{-1})	Found (mcg spot^{-1})	Recovery %	Taken (mcg spot^{-1})	Found (mcg spot^{-1})	Recovery %
2	1.98	99.00	2	1.97	98.50	10	9.88	98.80	10	9.98	98.90	5	4.97	99.40	5	4.95	99.00
3	2.98	96.33	3	2.90	96.66	15	14.83	98.86	15	14.85	99.00	10	9.89	98.90	10	9.88	98.80
4	3.98	99.50	4	3.98	97.25	20	19.93	99.65	20	19.66	98.30	15	14.85	99.00	15	15.05	100.33
5	4.88	97.60	5	4.96	99.20	25	24.85	99.40	25	24.91	99.64	20	19.95	99.75	20	19.98	99.90
Mean ± R.S.D.		98.10 ± 1.45			97.90 ± 1.17			99.13 ± 0.35			98.96 ± 0.79			99.26 ± 0.39			99.30 ± 1.30

Table 2
Assesment of the accuracy of the proposed method when applied to mixtures of the reference drugs and their degradation products

Clorazepate dipotassium			2-Amino-5-chlorophenone			Primidone			Phenobarbital			Chloroxazone			2-Amino-4-chlorophenol		
Taken (mcg spot^{-1})	Found (mcg spot^{-1})	Recovery %	Taken (mcg spot^{-1})	Found (mcg spot^{-1})	Recovery %	Taken (mcg spot^{-1})	Found (mcg spot^{-1})	Recovery %	Taken (mcg spot^{-1})	Found (mcg spot^{-1})	Recovery %	Taken (mcg spot^{-1})	Found (mcg spot^{-1})	Recovery %	Taken (mcg spot^{-1})	Found (mcg spot^{-1})	Recovery %
2	1.97	98.50	2	1.99	99.50	10	9.80	98.00	10	9.90	99.00	5	4.94	98.80	10	9.90	99.00
3	2.85	95.00	2	1.98	99.00	15	14.92	99.46	10	9.75	99.00	10	9.96	99.60	15	14.80	98.66
2	1.98	99.00	4	3.97	99.25	10	9.90	99.00	20	19.98	97.50	10	9.90	99.00	10	10.05	100.50
4	3.97	99.25	3	2.89	96.33	20	19.97	99.85	15	14.83	98.86	15	14.90	99.33	5	4.80	96.00
5	4.87	97.40	2	1.95	97.50	25	24.85	99.40	10	9.91	99.10	20	19.97	99.85	20	20.2	101.00
5	4.95	99.00	5	4.98	97.80	25	24.95	99.80	25	24.98	99.92	20	19.80	99.00	15	15.10	100.66
Mean ±R.S.D.		98.02 ±1.65			98.23 ±0.68			99.25 ±0.68			99.04 ±0.89			99.26 ±0.4			99.30 ±1.88

Table 3
Spectrodensitometric determination of clorazepate dipotassium, primidone and chlorzoxazone in their pharmaceuitical formulations

Clorazepate dipotassium			Primidone			Chlorzoxazone		
Taken (mcg)	Found (mcg)	Recovery %	Taken (mcg)	Found (mcg)	Recovery %	Taken (mcg)	Found (mcg)	Recovery %
2	1.97	97.50	10	9.00	98.8	5	4.85	97.00
3	2.85	96.33	1	14.71	98.06	10	10.00	100.00
2	1.98	98.75	20	19.95	99.75	15	14.75	98.33
4	3.97	99.40	25	24.85	99.40	20	19.70	98.50
Mean ± R.S.D.		97.99 ± 1.38			99.00 ± 0.747			98.45 ± 1.23

5. Applications of the proposed methods for the determination of the mentioned drugs in their pharmaceutical formulations

5.1. Tranxene capsules

The contents of 20 capsules were evacuated, mixed thoroughly, weighed and the average weight of each capsule was calculated. A quantity of powder equivalent to 0.02–0.05 g of clorazepate dipotassium was transferred to a series of 100 ml volumetric flasks. 75 ml methyl alcohol was added and shaken for 10 min and completed to volume with methyl alcohol. This was filtered and proceeded as mentioned prevously (Section 3.3) for the determination clorazepate dipotassium ($R_f = 0.56$) at 228 nm.

5.2. Mysoline tablets

Twenty tablets of mysoline were finely powdered, mixed and weighed and the average weight of a tablet was calculated. A quantity of powder equivalent to 0.1–0.25 g of primidone was transferred to a series of 100 ml volumetric flasks. This proceeded as mentioned in Section 3.1 from the line 'add 75 ml of ethyl alcohol, shake for 10 min and dilute to 100 ml ethyl alcohol'. This was filtered as mentioned in the determination of primidone ($R_f = 0.46$) at 222 nm.

5.3. Myolgin capsules

The contents of 20 capsules were evacuated, mixed thoroughly, weighed and the average weight of each capsule was calculated. A quantity of powder equivalent to 0.05–0.20 g of chlorzoxazone was transferred to a series of 100 ml volumetric flasks. This proceeded as mentioned in Section 3.1 starting from the word 'add 75 ml methyl alcohol'. Chlorzoxazone ($R_f = 0.75$) at 282 nm was determined using the condition mentioned in Section 3.4.

6. Results and discussion

The present work is concerned with the application of densitormetric techniques for simultaneous determination of primidone, clorazepate dipotassium and chlorzoxazone, each in the presence of its degradation product. The method depends on the difference of the R_f values of the drug and its degradation product. Complete separation of clorazepate dipotassium ($R_f = 0.56$) and 2-amino, 5-chlorobenzophenone ($R_f = 0.76$) were separated by the same mobile phase mentioned above and the two separate chromatograms were scanned densitometrically at 228 nm for clorazepate dipotassium and at 385 nm for 2-amino-5-chlorobenzophenone.

Also primidone ($R_f = 0.46$) and its degradation product phenobarbital ($R_f = 0.75$) was obtained using the mobile phase consisting of (ethyl acetate: benzene: acetic acid 1:1:0.05). The two separate chromatograms scanned densitometrically on the same plate at 222 nm for primidone and 218 nm for phenobarbital.

Chlorzoxazone ($R_f = 0.75$) and 2-amino-4-chlorophenol ($R_f = 0.43$) were separated by the

Table 4
Application of the standard addition technique to the spectrodensitometric determination of clorazepate dipotassium, primidone and chlorzoxazone in their pharmaceuitical formulations

Clorazepate dipotassium				Primidone				Chlorzoxazone			
Claimed amount tablet (mcg)	Authantle added (mcg)	Found added (mcg)	Recovery %	Claimed amount tablet (mcg)	Authantle added (mcg)	Found added (mcg)	Recovery %	Claimed amount tablet (mcg)	Authantle added (mcg)	Found added (mcg)	Recovery %
2	1	1.00	100.00	10	5	4.95	99.00	5	5	4.96	99.20
2	2	1.97	98.50	10	10	9.98	99.80	5	10	9.95	99.50
2	3	2.65	98.33	10	15	14.85	99.00	5	15	15.20	101.33
Mean ±R.S.D.			98.94 ±0.928				99.26 ±0.464				100.01 ±1.15

Table 5
Statistical comparision between the results of the proposed method and the Pharmacopoeial method

Compound	Proposed method	Pharmacopoeial method
Primidone		
Mean ± S.D.	99.00 ± 0.74	100.02 ± 1.46
n	4	7
Variance	0.547	2.131
Student's $t_{(0.95)}$-test	1.336 (2.132)	
F ratio (0.05)	3.685 (6.1)	
Clorazepate dipotassium		
Mean ± S.D.	97.00 ± 1.36	99.97 ± 0.71
n	4	9
Variance	1.849	0.504
Student's $t_{(0.99)}$-test	3.72 (3.747)	
F ratio (0.05)	3.66 (6.4)	
Chlorzoxazone		
Mean ± S.D.	98.45 ± 1.22	99.98 ± 0.79
n	4	6
Variance	1.488	0.624
Student's $t_{(0.975)}$-test	2.5 (2.77)	
F ratio (0.05)	2.38 (6.2)	

same mobile phase mentioned above and the two separate chromatograms were scanned densitometrically at 282 nm for chlorzoxazone dipotassium and at 285nm for 2-amino-4-chlorophenol.

By applying this technique a linear relation correlation was obtained between the area under the peak and the concentration (10–25 mcg spot^{-1}), for primidone and phenobarbital, (2–5 mcg spot^{-1}) for clorazepate dipotassium and 2-amino-5-chlorobenzophenone and (5–20 mcg spot^{-1}), for chlorzoxazone and 2-amino-4-chlorophenol, .

Linear regression equations were computed and used for the determination of the above mentioned compounds under the specified conditions. Proposed procedures for the determination of chlorzoxazone have the advantage of being selective without interference of the paracetamol which is added to myolgin capsules to give more analgesic action. Paracetamol was separated by the same mobile system having $R_f = 0.32$ which is widely separated from chlorzoxazone ($R_f = 0.75$).

To assess the efficiency of the proposed method phenobarbital was mixed with reference sample of primidone, 2-amino-5-chlorobenzophenone was mixed with reference sample of clorazepate dipotassium and 2-amino-4-chlorophenol was mixed with reference sample of chlorzoxazone in different ratios. The three mixtures were analyzed, each compound with its optimum conditions (see Table 2). It is obvious that primidone and phenobarbital were determined with an accuracy of 99.25 ± 0.685, and 99.04 ± 0.893, respectively. Clorazepate dipotassium and benzophenone were determined with an accuracy of 98.02 ± 1.650, and 98.23 ± 1.252, respectively. Also chlorzoxazone and 2-amino-4-chlorophenol were determined with an accuracy of 99.26 ± 0.40, and 99.30 ± 1.88, respectively.

The proposed method is applied for the determination of primidone in Mysoline tablets, clorazepate dipotassium in Tranxene capsules and chlorzoxazone in myolgin capsules with an accuracy of 99.20 ± 0.456, 98.12 ± 1.25, and 98.45 ± 1.23, respectively (Table 3).

The validity of the proposed method was checked by applying the standard addition technique, whereby the authentic added of drugs was determined with an accuracy of 99.26 ± 0.464 for primidone, 98.94 ± 0.928 for clorazepate dipotassium and 100.01 ± 1.15 for chlorzoxazone (see Table 4). Table 5 shows a statistical comparison between the results of the spectrodensitometric method and the pharmacopoeial method.

The calculated '*t*' and '*F*' values were less than the corresponding theoretical values and there was no significant difference between the two methods with respect to both precision and accuracy.

References

[1] F. Barbate, C. Grieco, C. Silipo and A. Vittoria, Farmaco, 345 (1979) 187–201 through C.A. 91 (1979) 9540n.
[2] A. Viala, J.P. Cano, P Angeltti, J. Eur. Toxicol. 3 (1971) 109.
[3] A.H. Stead, R. Giu, T. Wright, P.J. Gibbs, Analyst 107 (1982) 1106.
[4] United States Pharmacopeia XXII United States Pharmacopeial convention, Inc. 12601, Rockville, 1995.

[5] W.F. Smyth, J.M. Clifford, Analyst 99 (1974) 241.
[6] S. Hanna, F. Dianna, J Sievinski, K. Veronich, L.J. Lachman, Pharm. Sci. 67 (1978) 1723.
[7] J. Laquleau, R. Crockett and P. Mesnard, Bull. Soc. Pharm. Bordeaux, 110 (1971) 10 through Anal. Profile 4 (1975) 91 A. James and E.P. Victor.
[8] F.A. Elyazbi, M.H. Abdel Hay, Int. J. Pharm. 27 (2-3) (1985) 139–144.
[9] M. Guardia, M.V.V. Galdu, J. Monzo, A. Salvador, Analyst 114 (1971) 509.
[10] J. Troschuetz, Arch. Pharm. 314 (3) (1981) 204–209.
[11] P. Lafarque, J. Meunier, Y. Lemontey, J. Chromatogr. 62 (1971) 423.
[12] G. Bozsai, G. Vastagh, Pharm. Zentralhalle 103 (1964) 403–8 through C.A. 61 (1965) 10537h.
[13] W. Weigreble and L. Wehrhahn, Arzneim-Forsch, 25 (1975) 517 through Anal. Profile, 17 (1988).
[14] J.W. Huisman, Clin. Chim. Acta 13 (1966) 323–328.
[15] A. Spinks, W.S. Waring, Progr. Med. Chem. 3 (1963) 286.
[16] P. Cooper, Pharm. J. 177 (1956) 495–496.
[17] J. Wysokowski and W. Rewerski, Diss. Pharm. Pharmacol. 20 (1968) 369–72 through C.A. 70 (1967) 10319v.
[18] M.T. Bush, E. Helman, Life Sci. 4 (1965) 1403–1409.
[19] S.G. Tiraspolskaya, E.V. Komptantseva, A.S. Brill and N.A. Kanivels, Khim. Farm. Zh. 11 (1977) 130 through Anal. Profile 17 (1988) 749 by A.A. Abdullah and A.E. Humeida.
[20] Z.E. Kalinowska, L. Podkowska and K.J. Mieszcza, Farm. Polska 19 (1963) 329–31 through C.A. 60 (1964) 10478g.
[21] R.G. Cooper, M.S. Greaves, G. Owen, Clin. Chem. 18 (1972) 1343.
[22] C.V. Abraham, H.D. Joslin, J. Chromatogr. 128 (1976) 281.
[23] S. Vanderwal, S.J. Bannister, L.R. Snyder, J. Chromatogr. Sci. 20 (1982) 60.
[24] S.J. Soldin, J.G. Hill, Clin. Chem. 22 (1976) 856–859.
[25] N. Wad, E. Hanifl, H. Rosenmund, J. Chromatogr. 143 (1) (1971) 89.
[26] M. Murphy, W.H. Mason, J.M. Fujimoto, J. Pharmacol. Exp. Ther. 159 (2) (1968) 79–88.
[27] C. Gardner, M.J. Parsonage, C. Toothill, Clin. Chim. Acta 35 (1971) 39.
[28] J.T. Stewart, C.W. Chan, Anal. Lett. B11 (9) (1978) 667.
[29] E.L. Kirschner, McNeil pharmaceutical, spring house (1986), personal communication through Anal. Profile 16 (1987) 119 by T.S. James.
[30] E.M. Hassan, A.M. El Walily, S.F. Belal, Bull. Fac. Pharm. 32 (1994) 1.
[31] N.M. Sanghoi, N.G. Joshi, K.S. Dudal, Indian Drugs 18 (1981) 299–302.
[32] B. Kaempe, Arch. Pharm. Chemi. Sci. Ed. 2 (1974) 145.
[33] D.E. Cadwallader, I.L. Honigberg, J. Chromatogr. 46 (1970) 211–216.
[34] J.T. Stewart, I.L. Honigberg, J.W. Coldren, J. Pharm. Sci. 68 (1979) 253–255.