Journal of Thermal Analysis and Calorimetry, Vol. 88 (2007) 2, 383–387

DEVELOPMENT OF THERMOGRAVIMETRIC METHOD FOR QUANTITATIVE DETERMINATION OF METRONIDAZOLE

*Ana Paula Barrêto Gomes**, *Lidiane Pinto Correia, Mônica Oliveira da Silva Simões and R. O. Macêdo*

Federal University of Paraíba – UFPB, Unified Development and Medicine Assays Laboratories – LUDEM
Cidade Universitária, Campus I – CCS, ZIP code: 58051-970 João Pessoa, Paraíba, Brasil

The objective of this work was to develop and validate a fast and reproducible method to determine the concentration of metronidazole in drug substance and tablets. The samples were analyzed by dynamic thermogravimetry, using 10, 20, 40, 60 and 80°C min^{-1} heating rates in nitrogen and in nitrogen with synthetic air. Obtained data were used in the Antoine and Langmuir equations in order to have the pressure curves. Vapor pressure curves of drug and tablet of metronidazole were evaluated using the mathematical indexes of difference factor, f_1, and similarity factor, f_2, to compare their profiles. The data showed that there is no significant difference between the vapor pressure profiles of drug and tablet of metronidazole in both environmental conditions, which confirms that the process is really vaporization. The concentration of metronidazole was determined in the raw material and tablets of the drug.

Keywords: *Antoine equation, Langmuir equation, metronidazole, thermogravimetry, vapor pressure*

Introduction

Metronidazole is a nitroimidazole antiprotozoal agent; it also has a powerful antibacterial activity against anaerobics including the species *Bacterioides* and *Clostridium* [1]. Thermal techniques are used for several purposes, e.g. for thermal characterization [2–4], study the stability of drugs [5–9], for pre-formulation studies [10, 11]. Recently, thermogravimetric method was used to measure the vapor pressure [12–15]. The vaporization pressure process of substances obeys a zero order kinetic reaction, which is a requirement to determine the kinetic vaporization of drugs [16]. The objective of this work was to develop a sensitive and fast method for quantitative analysis of metronidazole using thermogravimetry.

Experimental

Material and method

Methylparaben metronidazole drug substance and tablets containing 250 mg of metronidazole were used in this study.

Calorimetric curves of methylparaben, metronidazole drug substance and its respective product were obtained using a Shimadzu, model DSC-50 calorimeter in nitrogen with 50 mL min^{-1} flow rate of purging at different heating rates (10, 20, 40, 60 and 80°C min^{-1}) up to 500°C. Non-isothermal thermogravimetric curves of metronidazole drug substance and tablets of 250 mg were obtained using a Shimadzu model TGA-50H thermobalance at 10, 20, 40, 60 and 80°C min^{-1} heating rates up to 900°C (n=3) in a synthetic air (flow rate: 20 mL min^{-1}) and in nitrogen (flow rate: 50 mL min^{-1}). Non-isothermal thermogravimetric curves of methylparaben were recorded in the same Shimadzu thermobalance using the same heating rates up to 400°C when mass loss process is complete (n=3). 8.0±0.5 mg of initial sample mass was used in alumina crucible. Curves were analyzed using TASYS program from Shimadzu to characterize the mass loss stages.

Arrhenius equation

Non-isothermal thermogravimetric data were used to determine the reaction order applying the Arrhenius equation [17].

$$k_{vap} = A e^{-E_{vap}/RT}$$

where E_{vap} is the vaporization energy, A is the pre-exponential factor, R is the universal gas constant, T is the absolute temperature and k_{vap} is the evaporation coefficient.

* Author for correspondence: apaula.barreto@gmail.com

1388–6150/$20.00

Akadémiai Kiadó, Budapest, Hungary
Springer, Dordrecht, The Netherlands

Antoine and Langmuir equations

Data obtained from thermogravimetric experiments of methylparaben were used to construct the vapor pressure curves using Antoine equation and after determining the value of '*k*', which will be used to construct the vapor pressure curves of drug substance and tablets of metronidazole, using Langmuir equation, as follows:

The Antoine equation [17]:

$$\ln P = \frac{A - B}{T + C}$$

where P is the vapor pressure, T is the absolute temperature and A, B and C are the Antoine's constants in a temperature interval [18]. Antoine's constants for methylparaben are: A=5.23662, B=1159.34 and C= –220.03 in the 446–517 K temperature interval [18].

The Langmuir equation [17] is presented as follows:

$$dm/dt = P\alpha(M/2\pi RT)$$

where (dm/dt) is the mass loss rate per area unit, P is the vapor pressure, α is the vaporization constant and M is the molecular mass of evaporate.

Langmuir equation can be modified to obtain the vapor pressure values of many simple components. The modification is described below [17]:

$$P=[\alpha^{-1}(2\pi R)^{1/2}][(T/M)^{1/2}(dm/dt)]=k\nu$$

where $k=\alpha^{-1}(2\pi R)^{1/2}$ and $\nu=(T/M)^{1/2}(dm/dt)$.

ν can be obtained from the T curves. If k is constant and it is independent from the used material, P *vs*. ν plot gives the value of k.

The Ozawa method

Activation energy of non-isothermal TG curves of drug substance and tablet of metronidazole was determined by Ozawa method [19].

f_1 and f_2 equations

f_1 and f_2 equations are recommended by FDA as an acceptable method to compare the dissolution profiles; however, this method was used because it compares straight line parallel bars, in which f_1 defines the difference between the straight lines and f_2 defines the similarity between them. Data from vapor pressure curve of the pure drug and the metronidazole tablet in both experimental conditions were applied in the f_1 and f_2 equations. The acceptance limits for two samples belonging to the same population are: f_1 less than 15% and f_2 bigger than 50% [20].

$$f_1 = \left\{ \frac{\sum_{t=1}^{n} |R_t - T_t|}{\sum_{t=1}^{n} R_t} \right\} \cdot 100\%$$

$$f_2 = 50 \log \left\{ \left[1 + \frac{1}{n} \sum_{t=1}^{n} \omega_t (R_t - T_t)^2 \right]^{-0.5} \cdot 100\% \right\}$$

Quantitative determination of metronidazole

Thermoanalytical curves of metronidazole drug substance diluted in microcrystalline cellulose were recorded at a heating rate of 40°C min^{-1}. The mass/mass ratios of metronidazole:microcrystalline cellulose were of 210:199, 230:179, 250:159, 270:139 and 290:119 in nitrogen and nitrogen with synthetic air. The raw material and the metronidazole tablets were analyzed by the calibration curve, using the straight line equation.

Determination of metronidazole according to Brazilian's Pharmacopoeia [21]

Powdering and putting 20 tablets (equivalent with 0.2 g of metronidazole) to a sintherized glass funnel followed by an extraction with 6 portions of 10 mL of warm acetone. After cooling the extract addition of 50 mL of anhydride acetic and 0.1 mL of 1-naftolbenzenae (*m*/*v*) solution in glacial acetic acid is the next step. The resulted liquid has to titrated with 0.1 M perchloric acid solution until the color change. Each mL of perchloric acid solution 0.1 M is equivalent to 0.01712 g of metronidazole.

Results and discussion

Calorimetric characterization of metronidazole's drug and tablets

Calorimetric curves of metronidazole drug substance showed two endothermic processes (Fig. 1a). At 10°C min^{-1} heating rate the first endothermic process begins at 159°C, with a peak maximum at 163°C indicating the melting of the metronidazole drug. The second endothermic process begins at 216°C, exhibiting a peak maximum at 246°C, which probable corresponds to the vaporization and decomposition of the drug. According to The United States Pharmacopoeia, melting of metronidazole drug substance occurs between 159–163°C which is in a good agreement with our findings. Metronidazole tablet showed two endothermic processes (Fig. 1b). The first endothermic process begins at 159°C, with a peak maximum at 163°C and the second process

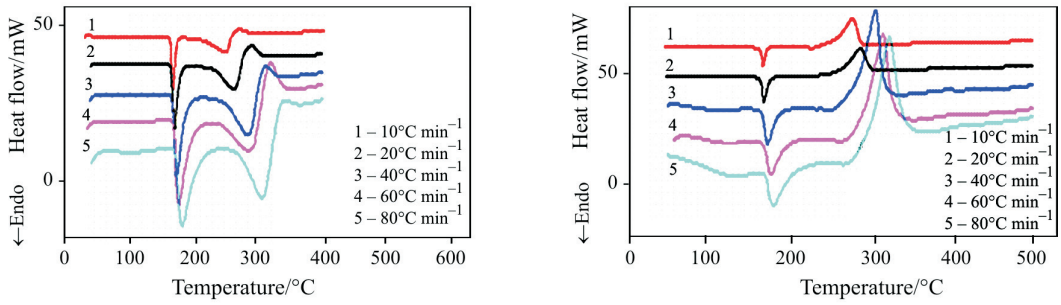

Fig. 1 DSC curves of a – metronidazole drug and b – tablet

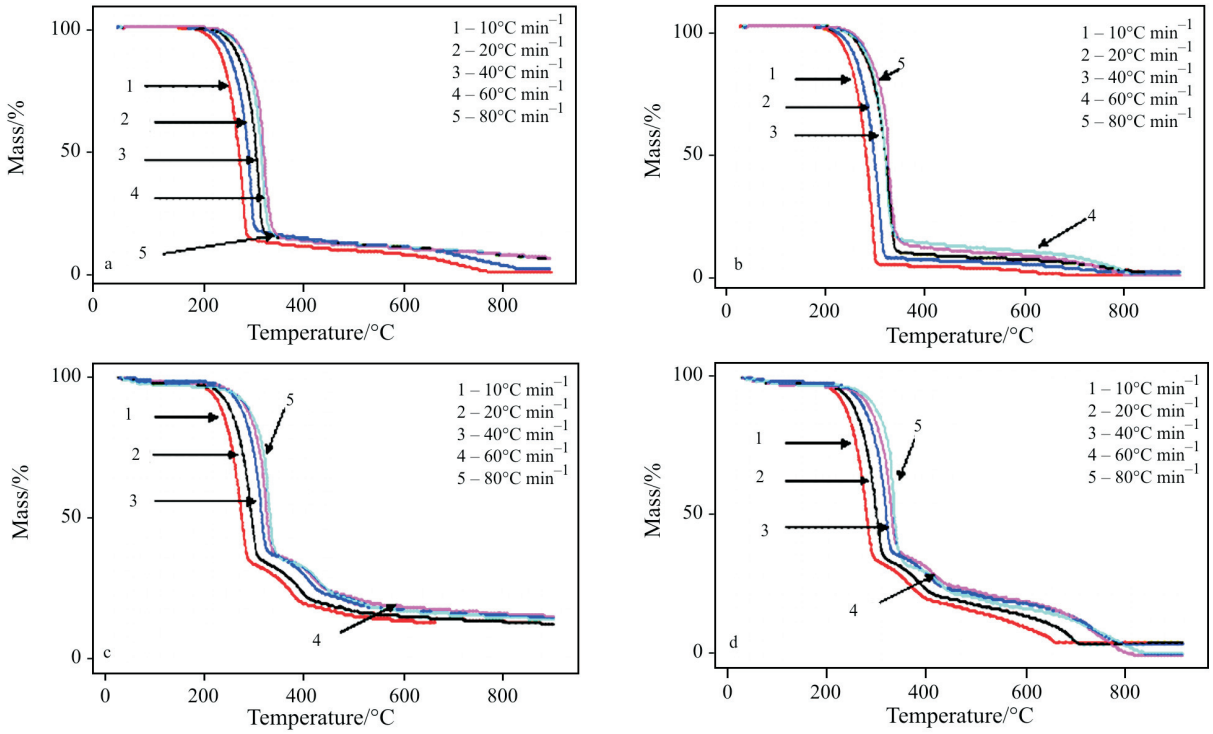

Fig. 2 TG curves in varying heating rates of a – metronidazole drug in nitrogen, b – nitrogen with synthetic air atmospheres, c – metronidazole tablet in nitrogen and d – nitrogen with synthetic air atmosphere

begins at 254°C with a peak maximum at 274°C at 10°C min^{-1} heating rate (melting and then evaporation and decomposition, respectively), which overlaps with the literature data.

Thermogravimetric characterization of metronidazole drug and tablets

Metronidazole drug in nitrogen (Fig. 2a) and nitrogen with synthetic air (Fig. 2b) presented two mass loss stages in all studied heating rates. The mass loss was 89.5 and 6.3%, respectively in the first and second stages in nitrogen; and 90.7 and 8.1%, respectively in the first and second stages in nitrogen with synthetic air.

Metronidazole tablet in nitrogen (Fig. 2c) and in nitrogen with synthetic air (Fig. 2d) showed four- and five-step processes, respectively. Metronidazole tablet in nitrogen showed 1.3, 62.0, 12.9 and 10.7%, mass losses, respectively in the first, second, third and fourth stages. Metronidazole tablet in nitrogen with synthetic air showed 1.5, 63.5, 12.3, 9.6 and 17.4%, mass losses in the first, second, third, fourth and five stages, respectively.

Determination of reaction order, activation energy and respective vapor pressure curves of the drug and metronidazole tablet

Methylparaben was used as sample and presented a zero order thermal process as foreseen to compounds that present vaporization process, according to [12]. The obtained values of 'k' for methylparaben at 10, 20, 40, 60 and 80°C min^{-1} with nitrogen and nitrogen with synthetic air are in Table 2. The activation energy of metronidazole drug obtained by Ozawa method in nitrogen and nitrogen with synthetic air

were 73 kJ mol^{-1} in the 180–300°C range and 71 kJ mol^{-1}, between 185–300°C, respactively. The activation energy of metronidazole tablet in nitrogen and nitrogen with synthetic air atmosphere were 84 kJ mol^{-1}, between 170–300°C and 87 kJ mol^{-1} between 190–300°C.

The drug substance and the metronidazole tablet presented a zero order reaction kinetic, confirming the vaporization process. The *k* values from methylparaben were used to obtain the vapor pressure values and its respective curves to metronidazole drug and tablet in nitrogen and in nitrogen with synthetic air, at a heating rates of 10, 20, 40, 60 and 80°C min^{-1} (Figs 3 and 4).

Table 1 shows that f_1 and f_2 values of the drug and metronidazole tablet are in the accepted limits in all heating rates. f_1 is lower than 15% and f_2 is higher than 50%, confirming that there is no difference in the behaviour of the drug and metronidazole tablet in both experimental conditions, confirming the process of volatilization.

Determination the metronidazole content in the tablets

Data of average vapor pressure from drug and metronidazole tablet at 10, 20, 40, 60 and 80°C min^{-1}

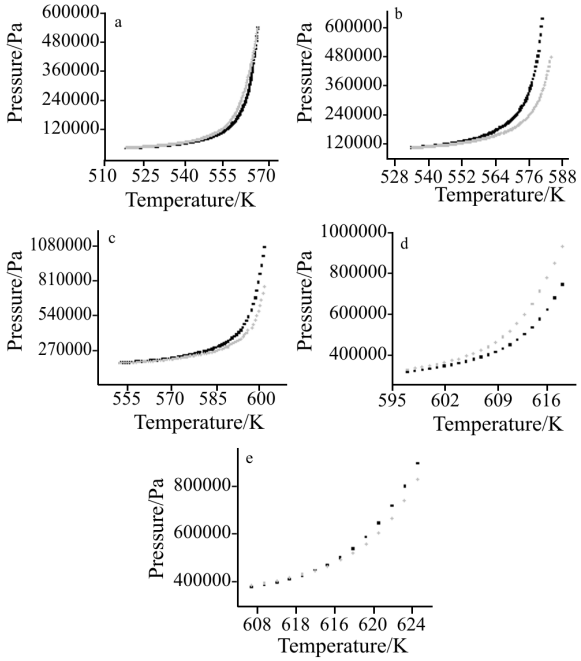

Fig. 3 Vapor pressure curves of metronidazole's drug in the heating rates of: a – 10, b – 20, c – 40, d – 60 and e – 80°C min^{-1}, in black circle – nitrogen and grey circle – nitrogen with synthetic air atmospheres

Table 1 Data obtained from equations of f_1 and f_2 to the drug and metronidazole tablet in comparison with vapor pressure profiles in nitrogen and nitrogen with synthetic air atmospheres in varying heating rates

Metronidazole drug										
Heating rate/ °C min^{-1}	10		20		40		60		80	
	f_1	f_2	f_1	f_2	f_1	f_2	f_1	f_2	f_1	f_2
	2.54	53.88	1.70	62.34	1.07	62.68	3.37	101.00	1.09	81.37
	3.16	60.65	3.07	77.07	1.52	72.06	4.45	109.12	0.97	79.11
	3.92	68.08	5.61	95.78	2.39	85.38	7.05	123.40	1.43	92.11
	5.42	80.49	11.55	119.62	3.89	100.21	10.90	136.64	3.49	121.06
	9.82	104.41	9.19	119.88	6.89	118.96	15.09	146.69	4.65	128.50
Metronidazole tablet										
Heating rate/ °C min^{-1}	10		20		40		60		80	
	f_1	f_2	f_1	f_2	f_1	f_2	f_1	f_2	f_1	f_2
	3.65	62.35	4.05	82.16	3.94	95.10	3.43	100.10	1.26	82.85
	4.71	70.17	4.30	84.33	4.24	97.41	3.67	102.75	1.39	85.96
	6.04	78.40	4.66	87.41	5.53	106.55	3.97	105.55	1.68	91.76
	7.44	86.21	5.73	96.38	6.87	113.29	4.41	109.16	2.09	98.40
	8.15	89.46	6.13	98.45	8.19	118.75	5.37	116.06	3.03	110.59

Table 2 Values of *k* for methylparaben at different heating rates in nitrogen and nitrogen with synthetic air

Purging gas	Heating rate/°C min^{-1}				
	10	20	40	60	80
Nitrogen	125413	246932	413676	597515	709030
Nitrogen with synthetic air	125555	245191	414034	605841	714416

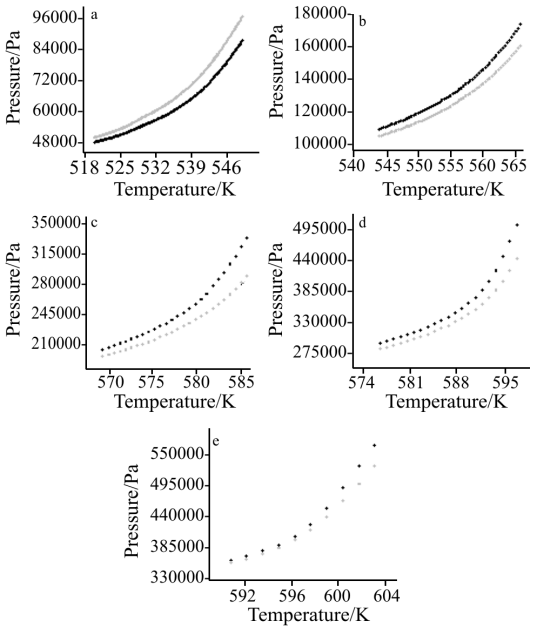

Fig. 4 Vapor pressure curves of metronidazole's tablet in the heating rates of: a – 10, b – 20, c – 40, d – 60 and e – 80°C min^{-1}, in black circle – nitrogen and grey circle – nitrogen with synthetic air atmospheres

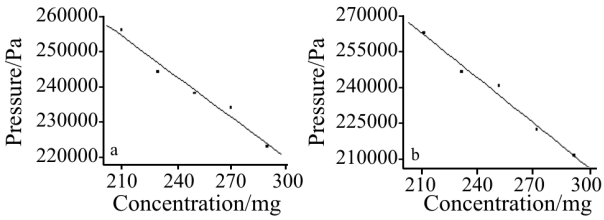

Fig. 5 Calibration curve of metronidazole+MC101 in a – nitrogen and b – nitrogen with synthetic air atmospheres

heating rates and in the two experimental conditions were analyzed to obtain a factor, which was recorded as the reason between the pressure of the drug and metronidazole tablet in the two experimental conditions. This factor was used as an analytical parameter to select the best heating rate that defines the method, which was 40°C min^{-1}. A calibration curve in nitrogen (Fig. 5a) and in nitrogen with synthetic air (Fig. 5b) was constructed and their linear regression allowed to obtain the following equations and respective variation coefficient: y=302908.25+(–257.34)x and R=0.9940, y=475155.61+(–940.40)x and R=0.9998. Utilizing the calibration curve, the calculated amount of the drug was 250.7 mg of metronidazole in nitrogen and 256.6 mg in nitrogen with synthetic air, which are respectively equivalent to 100.3 and 102.6% of labelled value. Data were compared to the obtained values according to [21], which was 103.2%.

Conclusions

Data analysis showed that metronidazole presented the same constants of vapor pressure in both atmosphere (nitrogen or nitrogen with synthetic air). The mounts of metronidazole are similar obtained by thermogravimetric method or according to the method written in Brazilian Pharmacopoeia.

References

1 B. G. Katzung, Farmacologia Básica & Clínica, Guanabara Koogan, Rio de Janeiro 2003, pp. 709–715, 778.
2 A. C. Schmidt, J. Therm. Anal. Cal., 81 (2005) 291.
3 F. I. Kanaze, E. Kokkalou, I. Niopas, M. Georgarakis, A. Stergiou and D. Bikiares, J. Therm. Anal. Cal., 86 (2006) 283.
4 F. S. Souza, A. P. B. Gomes and R. O. Macedo, J. Therm. Anal. Cal., 64 (2001) 739.
5 B. D. Glass, Cs. Novák and M. E. Brown, J. Therm. Anal. Cal., 77 (2004) 1013.
6 B. Marciniec, M. Kozak and K. Dettlaff, J. Therm. Anal. Cal., 77 (2004) 305.
7 R. O. Macedo, T. G. do Nascimento, C. F. S. Aragão and A. P. B. Gomes, J. Therm. Anal. Cal., 59 (2000) 1.
8 A. C. D. Medeiros, N. A. B. de Cervantes, A. P. B. Gomes and R. O. Macedo, J. Therm. Anal. Cal., 64 (2001) 745.
9 N. A. B. de Cervantes, A. C. D. Medeiros, A. F. O. Santos and R. O. Macedo, J. Therm. Anal. Cal., 72 (2003) 535.
10 P. Basu, K. S. Alexander and A. Riga, J. Therm. Anal. Cal., 83 (2006) 19.
11 G. G. G. Oliveira, H. Ferraz and J. S. R. Matos, J. Therm. Anal. Cal., 79 (2005) 267.
12 K. Chartterjee, A. Hazra, D. Dollimore and K. S. Alexander, Eur. J. Pharm. Biopharm., 54 (2002) 171.
13 L. Burnham, D. Dollimore and K. Alexander, Thermochim. Acta, 367–368 (2001) 15.
14 T. V. Sorokina, D. Dollimore and K. S. Alexander, Thermochim. Acta, 392–393 (2002) 315.
15 S. Vecchio, A. Catalani, V. Rossi and M. Tomassetti, Thermochim. Acta, 420 (2004) 99.
16 K. Chaterjee, D. Dollimore and K. Alexander, Int. J. Pharm., 213 (2001) 31.
17 A. Hazra, K. Alexander, D. Dollimore and A. Riga, J. Therm. Anal. Cal., 73 (2004) 317.
18 R. M. Stephenson and S. Malamowski, Handbook of the Thermodynamics of Organic Compounds, Elsevier, New York, p. 263.
19 T. Ozawa, Bull. Chem. Soc. Jpn., 38 (1965) 1881.
20 V. P. Shah, Y. Tsong, P. Sathe and L. Jen-Pie, Pharm. Res., 15 (1998) 6.
21 Farmacopéia Brasileira, 4th Ed., São Paulo, Atheneu 1988.

DOI: 10.1007/s10973-006-8007-2