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Study of the British Pharmacopeia method on methimazole (thiamazole) content in carbimazole tablets

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Abstract

The analysis of carbimazole tablets in The British Pharmacopoeia, 1993, includes a quantitative thin layer chromatography (TLC) determination of methimazole. Repeated analysis of the same samples did not give similar results. The repeatability and reproducibility of the method was studied. It was proved that the residence time of the methimazole spot on the TLC plate is time dependent. © 1998 Elsevier Science B.V.

Keywords: Carbimazole; Methimazole (thiamazole); Thin layer chromatography; High performance liquid chromatography

1. Introduction

Both carbimazole and methimazole (thiamazole) are drugs used in the treatment of hyperthyroid by the production of thyroxin, a hormone excreted by the thyroid gland.

Carbimazole is considered to be metabolized fully to methimazole, which is absorbed by the gastrointestinal tract and concentrates in the thyroid gland [1,2]. Both drugs may cause side effects such as irritation of the skin, allergies and pharyngitis with fever. On rare occasions they may cause more serious side effects such as nephritis and liver cirosis, [3]. According to The British Pharmacopoeia 1993, (BP'93) [4] methimazole is a related substance to carbimazole and is quantitatively determined by thin layer chromatography (TLC). Carbimazole tablets should not contain methimazole in concentrations higher than 1%.

The Pharmaceutical Quality Control Laboratory of the State General Laboratory observed that the above TLC method for methimazole determination had variations in repeatability and reproducibility during duplicate analysis of the same samples. Specifically, it was observed that the spot which corresponded to methimazole, in a carbimazole sample solution, applied first to the plate was of higher intensity than the corresponding methimazole spot of the same carbimazole solution applied last to the plate. This led to the conclusion that the concentration of methimazole was possibly increasing with the residence time of the carbimazole sample spots on the plate, before the plate was developed.

The objectives of this study were:

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1. to prove that carbimazole sample solutions break down to methimazole on TLC plates,
2. to prove that the intensity of the methimazole spots on the TLC plate increases with residence time on the plate thus producing false high results,
3. to investigate whether the breakdown of carbimazole and consequent increase of intensity of the methimazole spot were dependent on the type of TLC plate used,
4. to find an alternative TLC method to the BP methimazole method, and
5. to find an even better and more stable method for the determination of methimazole such as High Performance Liquid Chromatograph (HPLC).

2. Experimental

2.1. Methodology

Two methods were used for the determination of methimazole in carbimazole tablets. The first method was the TLC method described in BP'93 and the second was the modified method which the BP commission intends to include in the BP' Addendum 1997.

The main differences in the two methods are:

1. the use of the solvent dichloromethane instead of chloroform
2. the use of a 20 μl spotting volume of carbimazole solution instead of 10 μl and
3. the examination of the intensity of the spots under a UV lamp without spraying, instead of spraying and producing colour spots.

2.2. Equipment, reagents, reference standards, TLC plates

- CS-900 Shimadzu Dual Wavelength Flying-Spot TLC Scanner Densitometer.
- Reference standards of carbimazole and methimazole of European Pharmacopoeia (EPCRS) and American Pharmacopoeia (USPRS), respectively.
- Solvents were of Analar grade.
- TLC plates:
  - Silica Gel 60 F 254, 20 × 20 cm, Merck,
  - Sil-G-25 UV 254 20 × 20 cm, Macherey Nagel,
  - Sil-GUR-25 UV 254 20 × 20 cm, Macherey Nagel with spotting zone,
  - Silica Gel 60 F 254 with concentrating zone 20 × 20 cm, Merck.

Sample solutions: 500 mg% w/v carbimazole in chloroform, 500 mg% w/v carbimazole in dichloromethane.

Standard solutions: 5 mg% w/v methimazole in chloroform, 5 mg% w/v methimazole in dichloromethane.

Developing solvents: chloroform–acetone (80:20) and dichloromethane–acetone (80:20).

2.3. Working procedure

For confirmation and better evaluation of the experimental results, carbimazole tablets were used along with carbimazole raw material (RM) and reference carbimazole standard (EPCRS).

Having in mind the possible degradation of carbimazole to methimazole, all the necessary precautions were taken. These precautions included the use of: freshly prepared solutions at low temperature and protected from light. Moreover all precautions were taken to avoid possible evaporation of the volatile solvents leading to more concentrated solutions. TLC spotting was carried out at specific time intervals. The intensity of the spots was examined under a UV lamp and confirmed with a Scanning Densitometer.

3. Results and discussion

3.1. Stability and linearity of methimazole as a reference standard

The stability of methimazole standard was investigated in relation to its residence time on the various plates used before development in the TLC tank. The results of the experiment indicated that the intensity of the methimazole standard was independent of residence time on the plate, and therefore showed no variations (Fig. 1).

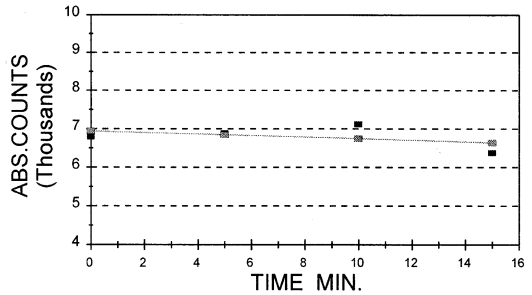

Fig. 1. Methimazol in $CHCl_3$. Silica gel UV plate–METHRF2.

An investigation of different methimazole concentrations in relation to the densitometer measurements was carried out. This relation was found to be linear. Solutions of 2.5, 5, 7.5 and 10 mg% were used for the linearity graph which had a correlation coefficient value of 0.979–0.999 (Fig. 2).

3.2. Evaluation of the results on carbimazole samples

Linear graphs for methimazole were obtained from solutions of carbimazole tablets, carbimazole raw material and carbimazole reference material in relation to their residence time on the plate. Both methods of intensity measurement described above were used.

The linear graphs obtained with plates silica gel UV 254 are depicted in Figs. 3–8. The graphs show the critical time beyond which, the methimazole concentration, appears to be higher than the maximum allowable concentrations in carbimazole tablets, according to BP. This critical time (ranging from 3 to 6 min) seems to be much shorter than the time actually needed to manually apply the TLC spots to a plate. This phenomenon could lead to false results especially in the analysis of carbimazole tablets in which small quantities of methimazole could pre-exist. False results could also be obtained in cases where a number of carbimazole samples are simultaneously analysed and the TLC spotting time takes longer on a 20×20 plate than when analysing a single sample with a 10×20 plate.

Table 1 depicts the average critical times for the different samples under investigation. The difference in the critical times observed between the two TLC methods is considered negligible.

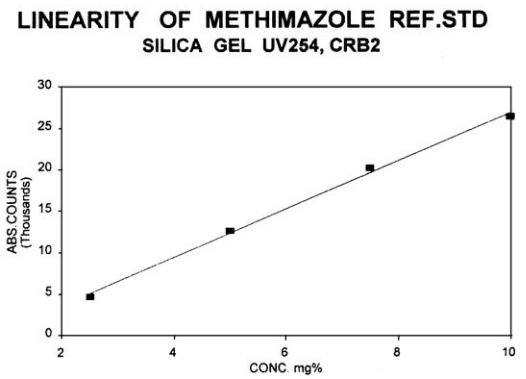

Fig. 2.

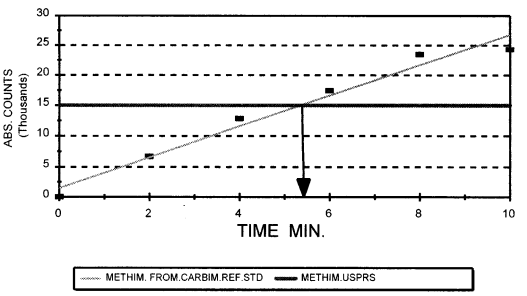

Fig. 3. Quantity of methimazole in a sample of carbimazole in relation to its residence time on a silica gel UV 254 plate: carbimazole reference standard; BP 93 method; silica gel UV; RF14.

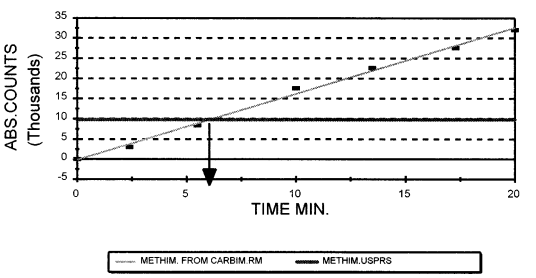

Fig. 4. Quantity of methimazole in a sample of carbimazole in relation to its residence time on a silica gel UV 254 plate: carbimazole raw material; BP 93 method; silica gel UV; RM4.

Table 1
Average value of critical time for the various sample solutions —chromatographic plates: silica gel UV254

Sample solutions of carbimazole	Critical time (min)	
	Method BP'93	Suggested method
Reference standard (EPCRS)	5.5	6.0
Raw material	4.6	5.5
Tablets	3.0	4.0

Linear graphs were also obtained in relation to the methimazole residence time on the plate using silica gel UV 254 with a concentrating zone plate. What was distinctly different this time, while using the above plate, was the critical time which was about 15 min on average. This means that the methimazole concentration does not change on the plate before a residence time of 15 min on average This time is considered sufficient for manual spotting of a number of samples on a 20×20 plate. The results are shown in Figs. 9–14.

The above experimental results prove that:

1. carbimazole breaks down to methimazole on TLC plates when applying both the BP'93 method and the modified BP method for carbimazole tablets,
2. this breakdown is time dependent and depends on the residence time of the carbimazole samples on the plate, before the plate is developed in the TLC tank, and

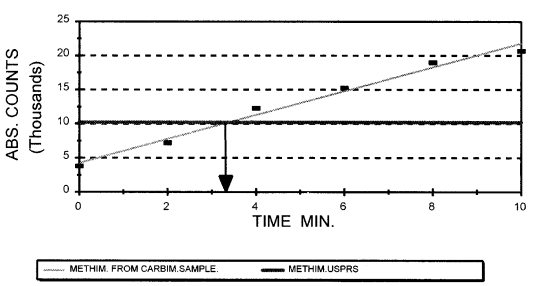

Fig. 5. Quantity of methimazole in a sample of carbimazole in relation to its residence time on a silica gel UV 254 plate: carbimazole sample; BP 93 method; silica gel UV, SMPL13.

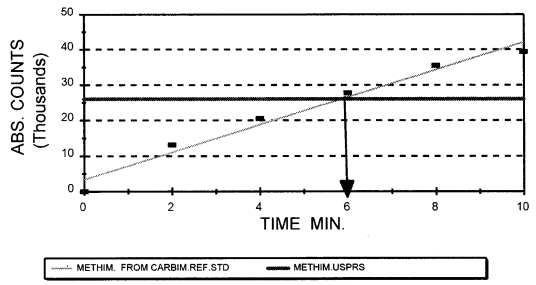

Fig. 6. Quantity of methimazole in a sample of carbimazole in relation to its residence time on a silica gel UV 254 plate: carbimazole reference standard; suggested method; silica gel UV; RF17.

3. the breakdown rate is subject to the type of plate used.

4. High performance liquid chromatography

High performance liquid chromatography (HPLC) was investigated as an alternative method to the TLC method for the quantitative determination of methimazole in carbimazole tablets.

The following HPLC method applied to three different pharmaceutical preparations of carbimazole, gave reproducible and reliable results.Equipment and materials:

HPLC	Waters, 501 pump
Injector	Rheodyne, injection loop: 20 μl
Flow rate	1.2 ml min^{-1}
Column	Symmetry Waters, 5 μ, 25 cm × 4.6 mm

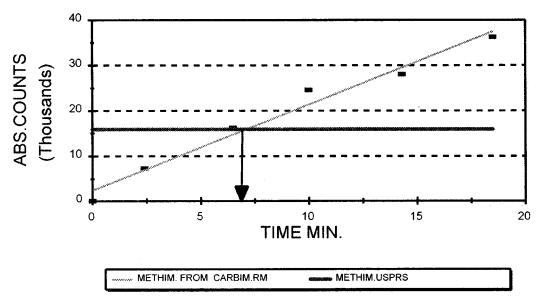

Fig. 7. Quantity of methimazole in a sample of carbimazole in relation to its residence time on a silica gel UV 254 plate: carbimazole raw material; suggested method; silica gel UV; RM1.

Table 2
Average amount of methimazole in the three different pharmaceutical preparations

Sample	Determined immediately after its preparation (%)	Determined at approximate 18 h after its preparation (%)
A	0.13	0.14
B	0.28	0.29
C	1.91	1.91

Detector	Waters 486, UV at 240 nm
Mobile phase	Methanol–water (40:60)
Reference standards	Carbimazole, methimazole (as before)
Solvents	HPLC grade

4.1. Methods

A quantity of the powdered tablets containing 5 mg of carbimazole was diluted in 10 ml dichloromethane and then centrifuged. The supernatant liquid was then filtered through a 0.22 μm membrane filter.

A solution containing both 0.5 mg% w/v methimazole and 50 mg% w/v carbimazole in dichloromethane was used as a reference standard.

Duplicate solutions were prepared both for samples and reference.

The different solutions were injected several times over a period of approximately 18 h to monitor possible degradation over time. The calculated amount of methimazole in each carbimazole tablet determined at different intervals of time from the time these tablets were dissolved in dichloromethane is shown in Table 2.

4.2. Quality control

The linearity of methimazole was investigated in the range from 0.25 to 1.25 mg% w/v versus absorbance and showed a correlation coefficient of 0.9975. This was shown using a 1% w/v methimazole standard solution relative to carbimazole (Fig. 15). The relative retention times of methimazole and carbimazole are 0.6 and 1 respectively.

The R.S.D. for duplicate injections was not more that 2%.

4.3. Comparison of the HPLC with the TLC method

To evaluate the HPLC results, the same samples were also examined with the TLC method (method BP Addendum 1997). The procedure followed was the same described in Section 2. The solutions and plates used are:

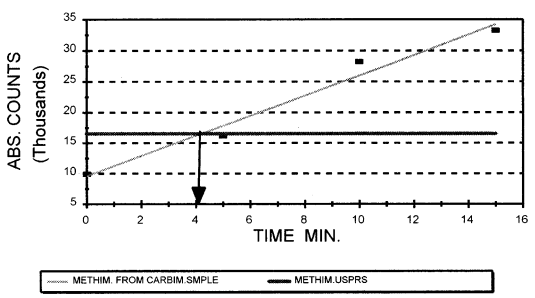

Fig. 8. Quantity of methimazole in a sample of carbimazole in relation to its residence time on a silica gel UV 254 plate: carbimazole sample; suggested method; silica gel UV, SMPL2.

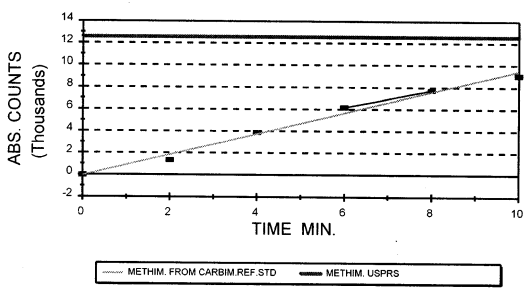

Fig. 9. Quantity of methimazole in a sample of carbimazole in relation to its residence time on a silica gel UV 254 plate with a concentration zone: carbimazole reference standard; BP 93 method; silica gel UV with concentration zone; RF15.

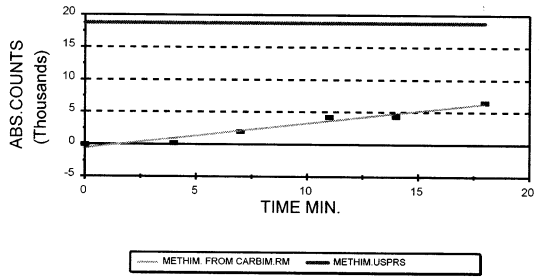

Fig. 10. Quantity of methimazole in a sample of carbimazole in relation to its residence time on a silica gel UV 254 plate with a concentration zone: carbimazole raw material; BP 93 method; silica gel UV with concentration zone; RM6.

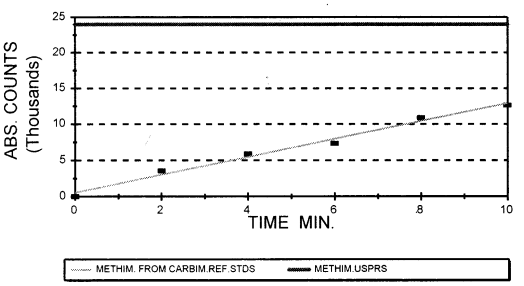

Fig. 12. Quantity of methimazole in a sample of carbimazole in relation to its residence time on a silica gel UV 254 plate with a concentration zone: carbimazole reference standard; suggested method; silica gel UV with concentration zone; RF16.

1. sample solutions: equivalent 500 mg% w/v carbimazole in dichloromethane were prepared in duplicate for each sample,
2. standard solutions: 5 mg% w/v methimazole RS in dichloromethane,
3. TLC plates: SIL G-25 HR/UV254, 20×20 cm, Machery Nagel,
4. developing solvents: dichloromethane–acetone (80:20)

To prevent possible breakdown of carbimazole a separate plate was used for each sample. Three spots were applied on each plate. The first spot was from reference standard and then the following spots were the duplicate spots from the sample.

The plates were placed immediately after the application of the last spot in the developing tank.

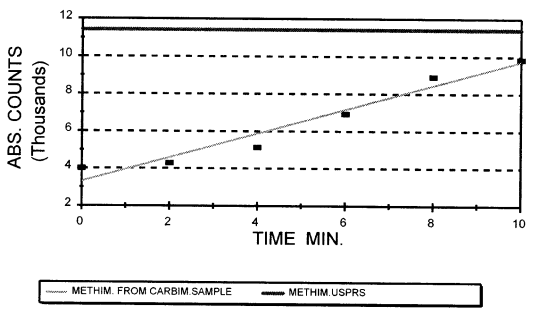

Fig. 11. Quantity of methimazole in a sample of carbimazole in relation to its residence time on a silica gel UV 254 plate with a concentration zone: carbimazole sample; BP 93 method; silica gel UV with concentration zone; SMPL12.

The intensity of the spots was measured with a Scanning Densitometer as before. The results obtained are given in Table 3.

4.4. Evaluation

The results from the HPLC method indicate that this method is a lot more stable than the TLC method given in both the original BP method as well as the Addendum 97.

The results obtained with the TLC (Table 3) indicate one more time that this method is time dependent and which is clearly shown by the two different values for spots A_1 and A_2, B_1 and B_2 and C_1 and C_2. This observation gives time limitations to the TLC method applied for quantitation of methimazole in carbimazole tablets. In contrast to the above observation the HPLC method as

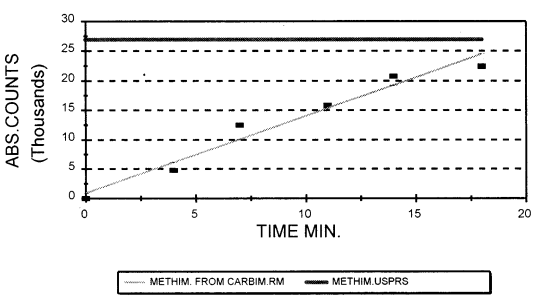

Fig. 13. Quantity of methimazole in a sample of carbimazole in relation to its residence time on a silica gel UV 254 plate with a concentration zone: carbimazole raw material; suggested method; silica gel UV with concentration zone; RM5.

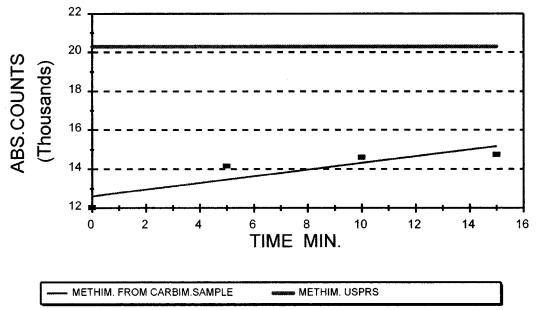

Fig. 14. Quantity of methimazole in a sample of carbimazole in relation to its residence time on a silica gel UV 254 plate with a concentration zone: carbimazole sample; suggested method; silica gel UV with concentration zone; SMPL3.

shown in Table 2 gives stable results over a period of 18 h. The methimazole concentration in the same pharmaceutical preparation was investigated even up to 36 h and was not found to increase substantially.

The results from the TLC method which can be safely correlated with the HPLC results are the ones from the last spotting on the plate. This is because, as discussed earlier, carbimazole suffers degradation on the plate as time goes by and therefore the first spots on the plate produce false high results.

The correlation of the results from the two methods is shown in Table 4.

In the case of samples A and B a good correlation is observed. In both cases the concentration of methimazole is within the acceptable BP limits (max 1%). However, in the case of sample C, the results obtained from each method do not correlate so well because the methimazole content in this particular pharmaceutical preparation happened to be well above the maximum acceptable limits and outside the investigated range of our method.

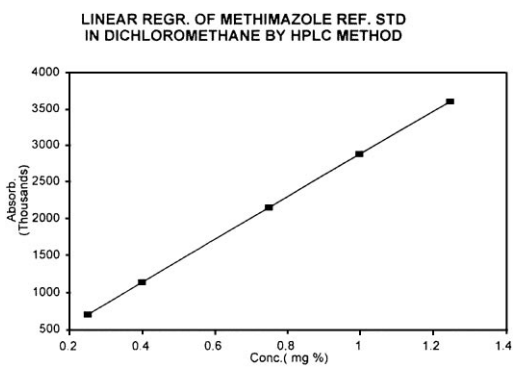

Fig. 15.

Table 3
Concentration of methimazole in carbimazole tablets determined by the TLC method

Sample	Spot of duplicate	Amount of methimazole found (%)
A	A_1	0.66
	A_2	0.19
B	B_1	0.61
	B_2	0.28
C	C_1	1.79
	C_2	1.56

A_1, B_1, C_1 are spots that were applied first on the TLC plates. A_2, B_2, C_2 are spots that were applied last on the TLC plates.

5. Conclusion and suggestions

The BP 93 method for the determination of methimazole in carbimazole tablets, as well as the modified BP method which has been included in the Addendum 1997 may lead to false results because of degradation of carbimazole to methimazole indicating that the TLC method is clearly unsatisfactory. To avoid this, the method should be modified.

Two different modifications were investigated. The first modification was a change in the type of TLC plates being used in the BP methods. This change involved the use of a concentrating zone silica gel UV 254 plate as well as the critical time required for spotting before plate development.

The second modification, which proved to be more reliable than the above method, involved the use of HPLC. This method for determining methimazole in carbimazole tablets is not time dependent (at least in the time range of 18 h)

Table 4

Sample	Amount of methimazole obtained from the HPLC method (%)	Methimazole obtained by TLC (last spot) (%)
A	0.13–0.14	0.19
B	0.28–0.29	0.28
C	1.91–1.91	1.56

and can be used by quality control laboratories to safely evaluate their results.

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