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# Quantitative determination of methyl 2-benzimidazole-carbamate by bioautography on thin-layer chromatograms

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Methyl 2-benzimidazolecarbamate (M.B.C.) is a fungicidal conversion product of several fungicides, such as benomyl and thiophanate-methyl. Methods for bioautographic detection of fungicides have been described earlier (Weltzien, 1958; Dekhuyzen, 1961). An improved method for direct bioautography on thin-layer chromatograms has been published by Homans and Fuchs (1970). The aim of this paper is to propose a simple mathematical model which describes the relation between spot area and M.B.C. concentration. Further we examine the precision of the above mentioned method.

Amounts of 10 to 200 ng (mostly in steps of 10 or 20 ng) M.B.C., solved in acidified (HCl) ethylacetate p.a. were spotted in fourfold on TLC aluminium sheets (silicagel 60/Kiezelguhr $F_{254}$, Merck n° 5567) with Medical Laboratory Automation Inc. pipettes. Further development of the chromatograms was carried out according to the method by Homans and Fuchs (1970) and Fuchs et al. (1974). Inhibition zones were measured planimetrically.

A change in slope appears on the curve giving the relation between concentration and spot area[1]. Therefore we assume that this relation can be expressed by a twophase linear model (Fig. 1). The optimal position of the two subsystems can be determined by minimizing the sum of the squared deviations for each linear subsystem (Bellman and Roth, 1969). The point of intersection is calculated from the two equations and is situated at a concentration of 64.8 ng of M.B.C. which corresponds with a spot area of 4.6 $cm^2$. The inverse confidence limits (probability 0.95) for this point (64.8 ng) are calculated, applying the Fieller theorem (Finney, 1971) and yield the values: 50.6 and 80.6 ng. Therefore it can be assumed that the first linear system holds between concentrations of 10 and 80 ng. The mathematical model shows what subsystem has to be used for the practical purpose of quantitative analysis of M.B.C.

For examination of the precision an amount of 50 ng M.B.C., which is about the middle of the first linear range, was determined in 50 replicates. Omitting spots with irregular shape, the standarddeviation was 0.46 $cm^2$, the calculated standard deviation of the concentration is 7.97 ng. Knowing the true mean and having an estimate of the standard deviation, Table 1 can be set up by application of the properties of the normal distribution. In this table the limits are given (in % of the mean) between which a

[1]The change in slope is caused probably by the occurence of M.B.C. in different chemical states (Fuchs et al., 1974).

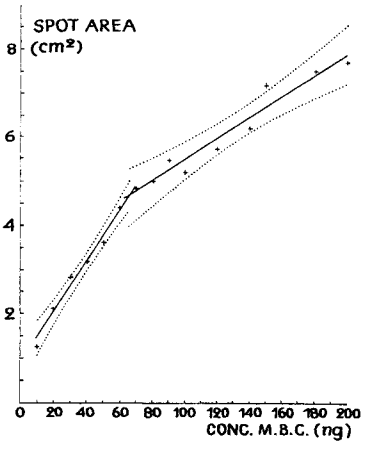


Fig. 1. Regression lines for the range 10–70 ng M.B.C. (Y = 0.875 + 0.0577X), and 80–200 ng M.B.C. (Y = 3.044 + 0.0242X) with confidence belts (probability 0.95) for the whole lines (dotted lines).

*Fig. 1. Regressielijnen voor het gebied 10–70 ng B.C.M. (Y = 0,875 + 0,0577X) en 80–200 ng B.C.M. (Y = 3,044 + 0,0242X) met betrouwbaarheidsgrenzen (waarschijnlijkheid 0,95) op de volledige lijnen (puntlijnen).*

simple determination, or a mean value of n determinations, is expected to be situated with a given probability P. From Table 1 also one can gather the number of spots necessary for a given precision.

Table 1. Symmetrical limits (in %) for a M.B.C. determination of 50 ng in function of a chosen probability (P) and a number of replicates (n).

| n | P | | | | |
|---|---|---|---|---|---|
| | 0.80 | 0.90 | 0.95 | 0.99 | 0.999 |
| 1 | 20.4 | 26.2 | 31.3 | 41.1 | 52.5 |
| 2 | 14.5 | 18.5 | 22.1 | 29.0 | 37.1 |
| 3 | 11.8 | 15.1 | 18.0 | 23.7 | 30.3 |
| 4 | 10.2 | 13.1 | 15.6 | 20.5 | 26.2 |
| 5 | 9.1 | 11.7 | 14.0 | 18.4 | 23.5 |
| 6 | 8.3 | 10.7 | 12.8 | 16.8 | 21.4 |
| 7 | 7.7 | 9.9 | 11.8 | 15.5 | 19.8 |
| 8 | 7.2 | 9.3 | 11.1 | 14.5 | 18.6 |
| 9 | 6.8 | 8.7 | 10.4 | 13.7 | 17.5 |
| 10 | 6.5 | 8.3 | 9.8 | 13.0 | 16.6 |
| 15 | 5.3 | 6.8 | 8.1 | 10.6 | 13.6 |
| 20 | 4.6 | 5.9 | 7.0 | 9.2 | 11.7 |

*Tabel 1. Symmetrische grenzen (in %) op een B.C.M. bepaling van 50 ng in funktie van vooropgestelde waarschijnlijkheden (P) en een aantal waarnemingen (n).*

## Samenvatting

*Kwantitatieve bepaling van B.C.M. door bio-autografie op dunnelaagchromatogrammen*

De bruikbaarheid van direkte bio-autografie op dunnelaagchromatogrammen voor de kwantitatieve bepaling van B.C.M. is onderzocht.

De relatie tussen de hoeveelheid B.C.M. en de oppervlakte der vlekken wordt be-

schreven met een tweeledig lineair model, waarbij als subsystemen deze worden aangenomen die de kwadratensom van de afwijkingen op het totaal minimaal maken. Rekening houdend met de inverse betrouwbaarheidsgrenzen voor het snijpunt bij een vlekoppervlakte van 4,6 $cm^2$ wordt vooropgesteld dat de eerste regressievergelijking bruikbaar is tussen 10 en 80 ng (Fig. 1).

In de omgeving van 50 ng werd de precisie van de methode berekend afhankelijk van het aantal aangebrachte opbrengplaatsen (Tabel 1).

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