A contribution to the determination of aflatoxin B_1, quinine hydrochloride and L(+)-ascorbic acid in foodstuffs by quantitative in situ thin-layer chromatographic analysis.

P. R. Beljaars

Aan mijn ouders

Aan Ina
Edmond en Bernice

Dit proefschrift met stellingen van Paul Raymond Beljaars, chemisch doctorandus, geboren te Maastricht op 29 september 1941, is goedgekeurd door de promotor, dr. W. Pilnik, hoogleraar in de levensmiddelenleer.

De Rector Magnificus van de Landbouwhogeschool
H. A. Leniger

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P. R. Beljaars

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Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus, prof. dr. ir. H. A. Leniger,
hoogleraar in de technologie,
in het openbaar te verdedigen
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The material submitted in this thesis is based on the following publications:

1970 D. H. Liem, P. R. Beljaars
Note on a rapid determination of aflatoxins in peanuts and peanut products.
JAOAC.: 53, 1064 – 1066.

1972 P. R. Beljaars, F. H. M. Fabry
Quantitative fluorodensitometric measurements of aflatoxin B_1 with a flying-spot densitometer.
I. Fluorodensitometric study of the behavior of aflatoxin B_1 standard spots on different types of silica gel.
JAOAC.: 55, 775 – 780.

1972 P. R. Beljaars, F. H. M. Fabry, M. M. A. Pickott, M. J. Peeters
Quantitative fluorodensitometric measurements of aflatoxin B_1 with a flying-spot densitometer.
II. Comparative study of B_1 measurements in spiked and naturally contaminated peanut products.
JAOAC.: 55, 1310 – 1315.

1972 Dutch Mycotoxin Committee
Determination of aflatoxin B_1 in peanuts and peanut products. Official Dutch Method. – – Proposed method, May 1972.

1973 P. R. Beljaars, P. J. Koken
Quantitative fluorodensitometric determination of quinine in soft drinks.
JAOAC.: 56, 1284 – 1289.

1973 P. R. Beljaars, C. A. H. Verhülsdonk, W. E. Paulsch, D. H. Liem
Collaborative study on the two-dimensional TLC analysis of aflatoxin B_1 in peanut butter extracts, using the "anti-diagonal" spot application technique.
JAOAC.: 56, 1444 – 1451.

1974 P. R. Beljaars, W. van Steenbergen Horrocks, T. M. M. Rondags
Assay of L(+)-ascorbic acid in buttermilk by densitometric transmittance measurement of the dehydroascorbic acid osazone.
JAOAC.: 57, January issue in press.

Stellingen

1
De argumentatie van Frei, dat spleet-densitometrie te prefereren is boven flying-spot densitometrie, is niet overtuigend en derhalve aanvechtbaar.

R. W. Frei, J. Chromatogr. 64, 285 – 295 (1972).

2
Vooralsnog zijn er onvoldoende aanwijzingen om de aanwezigheid van aflatoxine M_1 in melk en melkprodukten als ontoelaatbaar te kwalificeren; desalniettemin dient men voor melkprodukten bestemd als grondstof voor babyvoeding uit oogpunt van de volksgezondheid bijzondere contrôle maatregelen te overwegen.

R. Allcroft, B. A. Roberts, M. K. Lloyd, Food Cosmet. Toxicol., 6, 619 – 625 (1968).
R. Allcroft, B. A. Roberts, Vet. Record., 82, 116 – 118 (1968).
R. O. Sinnhuber, D. J. Lee, J. H. Wales, M. K. Landers, A. C. Keyl, Fred. Proc. Amer. Soc. Exptl. Biol., 29 (2) Abstract 1800 (1970).

3
De veronderstelling van Mendoza, dat de toepassing van in situ spectrodensitometrie in combinatie met de enzymremmings techniek op dunnelaag platen geschikt is voor de kwantitatieve analyse van bestrijdingsmiddelen, is onvoldoende gefundeerd; als kwalitatieve methode daarentegen is deze techniek geschikt als bewijsmiddel voor de toxiciteit van pesticiden.

C. E. Mendoza, Residue Reviews, 43, 105 – 142 (1972).
C. E. Mendoza, J. Chromatogr. 78, 29 – 40 (1973).

4
De verklaring van Barton en medewerkers voor de vorming van α-(methylthio)-stilbeen bij inwerking van diazomethaan op 2,3,4-trifenyl-2-mercaptoöxetaan is aanvechtbaar.

D. H. R. Barton, M. Bolton, P. D. Magnus, P. J. West, J. Chem. Soc. Perkin I 1580 – 1583 (1973).

5
Bij het aantonen van caseïne in vlees- en vleeswaren volgens de immuno-diffusietest van Ouchterlony moet men er op bedacht zijn, dat het gebruik van het anti-serum tegen melkeiwit vals positieve resultaten kan geven.

O. Wyler, J. J. Siegrist, Mitt. Gebiete Lebensm. Hyg., 56, 299 – 303 (1965).
H. O. Günther, Z. Lebensm. Unters. Forsch., 149, 98 – 99 (1972).

6
Het onvermijdelijk gebruik van moderne instrumentele analysetechnieken voor het onderzoek van en de contrôle op levensmiddelen vraagt om aanpassing van de Officiële methoden van onderzoek in de Warenwet; in dit verband is het specificeren en aanbevelen van instrumenten, hulpmiddelen, chemicaliën etc met fabrieks- of merknaam noodzakelijk.

7
De afspraak om een maximum gehalte aan grondnotenschroot in mengvoeders toe te laten dient uit volksgezondheidsoverwegingen uitgebreid te worden met een eis aan het maximum gehalte aan aflatoxine B_1 in het gerede produkt.

Produktschap voor veevoeders, Verordening veevoeder, Paragraaf 2, Artikel 7 sub. 2 (1970).

8
Onvoldoende kommunikatie en gebrek aan koördinatie tussen internationale organisaties onderling, werken belemmerend op de harmonisatie van Officiële methoden van onderzoek voor levensmiddelen.

9
Indien de overheid zou toestaan om op beperkte schaal vleesvreemde eiwitten als bindmiddel in vleeswaren te verwerken, dan moet de eis gesteld aan het vocht/eiwit gehalte niet zonder meer betrekking hebben op het vlees-eiwit.

10
De toenemende veranderingen van de eetgewoonten en de bereidingswijzen van maaltijden in Nederland maken het gewenst, dat de overheid wettelijke normen en methoden van onderzoek opstelt m.b.t. het micro-nutriëntengehalte van bepaalde levensmiddelen zoals o.a. dieetwaren, babyvoeding, convenience foods, substitutie produkten etc.

11
Alleen al op ekonomische motieven is inschakeling van kennis en outillage van de Keuringsdiensten van Waren bij het toezicht op de milieu verontreiniging van bodem, water en lucht gewenst; hiervoor is een organisatorische vervlechting van deze diensten een absolute noodzaak.

12
Bij de keuring van nieuwe auto's dient de afstelling van de uitlaatgasemissies volgens de normen van de Europese rijcyclus niet direkt bij in gebruikneming te geschieden, maar na b.v. duizend km òf meer.

13
Het streven van de Commissie Voorbereiding Medische Faculteit Maastricht om de aktiviteit van de Rijksuniversiteit Limburg (in oprichting) zovéél mogelijk in te bedden in het lokale- en regionale levenspatroon, speelt in op één van de hoofdaspekten van het huidige denken m.b.t. de strukturele ontwikkeling van deze regio op zowel onderwijskundig-, ekonomisch- als kultureel gebied.

Proefschrift van P. R. Beljaars
Wageningen, 10 januari 1974.

Acknowledgments

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Last but not least I am grateful to Dipl.Ing. J. Daenen, Director of the Food Inspection Service Maastricht, who gave me the opportunity to publish this study and who kindly afforded me time and research facilities to carry out my experiments.

Abstract

Beljaars, P. R., (1974). A contribution to the determination of aflatoxin B_1, quinine hydrochloride and L(+)-ascorbic acid in foodstuffs by quantitative in situ thin-layer chromatographic analysis. Doctoral thesis, Wageningen; 23 p, 2 Tables, 76 refs, Dutch summary.

The application of quantitative thin-layer chromatography (TLC) involving in situ spectrodensitometric measurements with a flying-spot densitometer is described in this study for the analysis of aflatoxin B_1, quinine hydrochloride and L(+)-ascorbic acid (vitamin C) in various food and foodstuffs.

Several aspects dealing with the quantitative analysis of aflatoxin B_1 in peanuts and peanut products were investigated: isolation, thin-layer chromatographic conditions, visual estimations and fluorodensitometric measurements. The results of this study contributed to the development of the Official method of analysis for aflatoxin B_1 in the Netherlands. A new spotting procedure for aflatoxin assay was introduced ("anti-diagonal" technique) improving the accuracy for visual B_1 estiminations for samples with low contamination levels (0–10 μg/kg).

An in situ fluorodensitometric procedure was developed for the quantitative determination of quinine hydrochloride in soft drinks containing quinine whereas in situ densitometric transmittance measurements were applied for the quantitative determination of L(+)-ascorbic acid (vitamin C) in buttermilk.

It was generally concluded that in situ spectrodensitometric procedures were suitable for quantitative routine determinations in food control work, for specific purposes.

Contents

1. Introduction

1.1. Dutch Food Legislation

The surveillance activities of the 16 Food Inspection Services in the Netherlands are statutorily determined by several acts indicated as Royal Decrees òr KB acts (Koninklijke Besluiten). Most of the legal regulations related to food and foodstuffs are laid down in the Dutch Food Law. This Law was adopted in 1919 and slightly amended in 1935. The main principles of the Law are the protection of public health and the promotion of fairness in trade. This means that merchandise as indicated by the Law has to be innocuous to human health and must be of the prescribed composition and condition when offered for sale. At present the Food Inspection Services are not only dealing with regulations of the Food Law but also with the application of the Pesticide Act and the Residue Order which provide for control of the level of harmful pesticide residues in food and foodstuffs. The Services also administer a task under the Nuclear Energy Act and the Statutory Trade Organizations Act, the latter in cooperation with Produce Boards to goods within the meaning of the Food Law.

In order to effect such surveillance and to trace offences against the Law, the Food Inspection Services are forced to apply Official methods of analysis which are regulated by Royal Decrees (KB-methods), if available. These methods are developed, tested and approved by experts and members of the Advisory Committee of Dutch Food Law who are appointed by the Crown. Revisions of inadequate procedures together with the introduction of new KB-methods are continually carried out by this Committee, resulting in widening of the scope of òr changes to the Official methods of analysis. Adaption of modern instrumental methods òr techniques to the Law is inevitable due to development of new products by industry, broadening new areas of concern, harmonisation of international legislation (e.g. Benelux, E.E.C.) etc.

1.2. Purpose of the study

It is evident that the official analytical food chemist will be faced in the near future with more numerous and complex problems. This explains the use of modern instrumental methods by the chemists of the Food Inspection Services as screening procedures in order to exert more effective control for determining composition and condition of food and foodstuffs subjected to legal control. (Details of current legislation pertaining to the compounds dealt within this thesis are given in Appendix on page 22). In addition the suitability of modern instrumental analysis to automation enables the Services to expand their surveillance activities.

It is the purpose of this study to investigate the validity of quantitative thin-layer chromatographic (TLC) analysis involving in situ densitometric measurements for constituents of food and foodstuffs. The results of the densitometric determinations of aflatoxin B_1, quinine hydrochloride and L(+)-ascorbic acid (vitamin C), substances which are subjected to legal inspection, are described herein. The findings in this study were compared with those of previous investigators using different techniques.

2. General aspects of in situ spectrodensitometry

2.1. General methodology for quantitative thin-layer chromatography

Thin-layer chromatography (TLC) has become a separation technique widely employed by many chemists because of its simplicity and convenience. TLC has mainly been applied as a qualitative òr semi-quantitative procedure for the analysis of chemical compounds that are low in volatility òr thermal instable.

During the past 20 years various methods were developed for the quantitative evaluation of thin-layer chromatograms. The application of the quantitative TLC techniques can roughly be divided in 6 groups:

1. visual estimation of the separated spots on TLC plates
2. measurement of spot areas by photographic- òr graphical techniques
3. removal of spots from plate by mechanical means and extraction of sorbent layer, followed by spectrophotometric- òr fluorimetric determinations
4. zonal flaming of the chromatoplate followed by gas-liquid chromatographic (GLC) analysis
5. radio-active labeling of compounds followed by measurement of radio-activity of the separated spots on the TLC plates
6. in situ densitometric measurement of the separated spots on the chromatoplates using spectrophotometric- òr fluorometric techniques.

Methods 1–3 are less accurate, though suitable for semi-quantitative analysis only. Visual comparison of spots and measurement of spot areas with photographic- òr graphical techniques can prove misleading and are subjected to individual interpretation. Scraping of spots from plate followed by extraction with a suitable solvent is not only time-consuming but also not particularly accurate.

Methods 4–6 are more accurate and thus suitable for quantitative analysis. Zonal flaming suffers the drawback that the sample is destroyed during the measurement and can not be re-run. The use of radio-active tracers is effective but also tedious and cumbersome especially for routine use.

In situ densitometric measurements of compounds separated by TLC are convenient and widely applied in many laboratories to perform quantitative TLC analysis. At present densitometry is accepted as an analytical procedure in various branches of science e.g. biochemistry, biology, pharmacy, medicine etc. seen the many publications in these fields.

The number of publications for this technique in food science is rather small. An exception can be made for the quantitative evaluation of mycotoxins in agricultural products introduced by Ayres (1966) and Pons (1966) resulting in the Official method of analysis of the Association of Official Analytical Chemists (AOAC., 1970) using the fluorodensitometric procedure for the determination of aflatoxins in cottonseed products. (see also 3.2).

During the last 3 years studies have been reported on the fluorodensitometric analysis of pesticides and fungicides (Frei et al., 1973 a+b), preservatives (Rios, 1972; Brunink et

al., 1972; Duden et al., 1973) and coffeine (Washüttl et al., 1970, Baltes et al., 1971 and 1973) in food and foodproducts. It was found that these fluorescent procedures were rather accurate, yielding coefficients of variation of 2–5% for the complete procedures.

The application of in situ densitometric procedures involving transmittance measurements has been described for sugars (Welch et al., 1972; Bush et al., 1973), antioxidants (Dobies 1969), organic acids (Bourzeix et al., 1970) non-meat proteins (Freimuth et al., 1970 a+b) and phenols (Miskov et al., 1970) in various foodproducts. Coefficient of variation values ranging from 5 to 15% were established for the complete procedures.

2.2. Theoretical principles of in situ densitometry

In situ quantitation of separated compounds on TLC plates by densitometry is commonly performed by:

1. measurement of **light absorption** using transmission techniques
2. measurement of **light absorption** using reflection techniques
3. measurement of **fluorescence**
4. measurement of **fluorescence quenching.**

Various theoretical aspects related to these measurements are summarized below.

2.2.1. Light absorption measurements by transmittance

This method is applicable for measurement of coloured compounds in the visible region. The theoretical relationship between the absorbance (A) of light through absorbing material and its concentration (C) is formulated by Lambert-Bouguer-Beer's Law in equation [1]:

$$A = -\log T = KCX \quad \ldots\ldots [1]$$

where

A = absorbance of light absorbing material
T = transmittance of light
K = absorption coefficient in $cm^2\ mmol^{-1}$.
X = thickness of absorbent layer in cm.
C = concentration of light absorbing material in mol l^{-1}.

The validity of Beer's Law is restricted to some conditions such as use of monochromatic light, homogeneous diluted solutions, absence of scattering due to coagulated particles, absence of interaction with solvent molecules by chemical effects etc.

In situ densitometric measurement of thin-layer chromatograms by means of transmission techniques is not a simple matter since:

1. the separated spots show concentration gradients in 3 dimensions
2. the thin-layer absorbs the incident light depending on the wavelength used
3. the path of light through the silica gel is made longer than normal caused by scattering effect of the particles.

The most accepted theory concerning radiative transfer of light through scattering and light absorbing media was developed by Kubelka-Munk (1931, 1948). According to this theory an empirical relationship was proposed, yielding a simplified and adequate approach of light transmission c.q. light reflection in highly scattering media such as

thin-layers. The Kubelka-Munk equation for light transmission measurements through opaque layers is given by expression [2]:

$$T = \frac{b}{a \sinh b.SX + b \cosh b.SX} \quad \ldots\ldots [2]$$

where

T = transmittance of thin-layer with infinite thick layer
S = scattering coefficient of thin-layer in cm^{-1}
X = thickness of sorbent layer in cm

$$a = \frac{S + k}{S} \quad \text{and} \quad b = \sqrt{a^2 - 1}$$

k = absorption coefficient of thin-layer in cm^{-1}

The description of radiative transfer of light through light scattering and light absorbing media by equation [2] is rather complex due to the appearence of many variables when compared to equation [1] derived from Beer's Law. When $S \rightarrow o$ indicating absence of light scattering by the thin-layer, Lambert-Beer's Law holds ideally by:

$$T = T_o = e^{-kX} \quad \ldots\ldots [3]$$

Within limits an overlap between Beer's Law and the Kubelka-Munk equation is possible at low concentration levels of the absorbing material. At higher concentration levels a non-linear response is recorded according to Kubelka's function. The curve resembles more a hyperbola than the straight line given by Beer's Law. Therefore simplified empirical relationships between the mass of the absorbing compound (k X) and the measured quantities of T òr A were developed by several investigators transforming the Kubelka-Munk equation.

A simplified Kubelka-Munk equation expressing the absorption coefficient in terms of two variables (background absorbance and scattering of the medium) was developed and tested by Goldman and Goodall (1968, 1969 and 1970), for in situ transmittance measurements of thin-layer chromatograms. Pollak and Boulton (1970 a+b; 1971) deduced empirical equations for both transmittance and reflectance measurements by theoretical approachments of the Kubelka-Munk theory. The authors tested the validity of their empirical equations using electronic circuit arrangements in order to model their expressions. Studies were also performed to minimize the influence of optical noise the magnitude of which is determined by fluctuations of light transfer through the medium due to non-homogenity of the substrate. It was concluded that reflectance was less succeptible to optical noise than transmittance measurements.

An empirical relationship between Lambert-Beer's Law and the Kubelka-Munk equation was designed by Treiber et al (1971, 1972 a+b) resulting in the construction of an electronic analog converter in order to obtain a linear detector system for the quantitative in situ evaluation of chromatograms for both transmittance and reflectance measurements.

2.2.2. Light absorption measurements by reflectance

This method is applicable for the measurement of coloured compounds in the visible

region. When applied to infinitely thick opaque layers the Kubelka-Munk equation for reflectance measurements may be written as:

$$F(R_\infty) = \frac{(1 - R_\infty)^2}{2R_\infty} = \frac{k}{S} = \frac{\varepsilon C}{S} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots [4]$$

where

R_∞ = monochromatic reflectance of thin-layer with infinite thick sorbent layer
k = absorption coefficient in cm^{-1}
S = scattering coefficient of thin-layer in cm^{-1}
ε = molar absorptivity of light absorbing material in cm^2 $mmol^{-1}$
C = concentration of light absorbing material mol l^{-1}

The degree of reflectance from the coloured chromatogram spot is determined in relation to the reflectance from the adsorbent on the plate. Thus, the greater the amount of substance on the spot, the smaller the degree of reflectance.

Frei (1965) and Jork (1966) found that adsorbents commonly used in TLC-analysis are applicable with respect to the Kubelka-Munk equation [4]. A linear relationship was observed between the degree of reflectance $F(R_\infty)$ and the concentration C of the absorbing spots situated on infinite thick layers. Jork (1968 a+b) investigated the relationship between layer thickness of the adsorbent and the amount of light through the thin-layer. Modifications of the Kubelka-Munk equation [4] for reflectance measurements on transparant thin-layers involved the development of empirical functions, which was used by Frei (1972) to obtain linear calibration curves. The author concluded that reflectance spectrodensitometry for the evaluation of thin-layer chromatograms is to be preferred to transmittance techniques.

2.2.3. Fluorescence measurements

In situ fluorodensitometric measurements can be considered as a special case of reflectance where the incident light consists of UV light (primary radiation), which is converted to light of different wave length (secondary radiation) usually in the visible region. The advantage of fluorodensitometric measurements is that, normally, a linear relationship exists between the intensity of the emitted fluorescent light and the amount of fluorogenic material situated on the chromatoplates at low concentrations (ng range) (Seiler 1963; Klaus 1971). The existence of this linear relationship was in agreement with the theoretical expectation of Braunsberg (1952) resulting in equation [5]:

$$F = \frac{I_f}{I_0} = p \cdot C \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots [5]$$

where

F = coefficient of fluorescence
I_f = intensity of emitted fluorescent light
I_0 = intensity of incident light
p = constant in l mol^{-1}
C = concentration of fluorescence substance in mol l^{-1}

The value of F depends on the proportion of primary radiation I_0 that is converted at

any point inside the medium to secondary radiation I_f of different wavelength.

In situ fluorodensitometric measurements are sensitive and capable of detecting quantities down to the nanogram range using calibration curves prepared with standards of known concentration range and chromatographed on the same plate. A drawback of this sensitivity is that the measurements are susceptible to "random noise" of the sorbent layer. Pollak and Boulton (1972) mentioned that the optical background noise was of no effect for fluorodensitometric measurements due to the absence of fluorescence from the substrate, when compared to other optical procedures.

The application of fluorodensitometry has been greatly extended by the introduction of fluorogenic labeling techniques in order to detect non-fluorescent compounds such as amino acids, amines, steroids, phenols etc. Sensitive and specific procedures involving dansylderivatives prepared from dansyl chloride (1-dimethylaminonaphthalene-5-sulfonyl chloride) have been described by Seiler et al (1966).

2.2.4. Fluorescence quenching measurements

In situ fluorescence quenching measurements are based on the decrease in fluorescence of the substrate depending on the degree of quenching caused by the separated substances. The method is applicable to compounds that diminish the fluorescence of the chromatoplate, which is impregnated with a suitable "fluorescence" indicator. Quenching effects of these compounds are caused by absorption of the excited radiation or by radiationless energy transfer.

Sawacki et al (1966) and Jänchen et al (1968) demonstrated the existence of a linear relationship between the fluorescence of the substrate and the concentration of the quenching compounds on the TLC plates. Quenching procedures are less sensitive when compared with fluorescent procedures, as described in 2.2.3. This is explained by the increase of the optical noise caused by interfering influences of the background fluorescence of the chromatoplate.

2.3. Instruments for quantitative thin-layer chromatography

2.3.1. Description of instruments

In situ quantitation of thin-layer chromatograms is carried out with instruments commonly indicated as "scanners" or "densitometers". The scanning devices range from simple filter transmission instruments up to more complicated machines linked to highly sophisticated recording equipment. Most of the instruments are designed to perform either transmission – or reflection measurements or both.

Scanning of the separated spots or zones on the chromatoplates may be realized by employing two different scanning methods i.e. the conventional *slit-scanning* procedure or the *flying-spot* procedure.

2.3.2. Slit-scanning vs. flying-spot scanning

Spectrodensitometry of chromogenic compounds on thin-layer chromatograms depends on several factors such as spatial distribution of the absorbing compound in the thin-layer, deviations in layer thickness, scattering of sorbent layer and others.

If spots are scanned with a slit-type densitometer, a mean transmission value $\overline{T}$ is measured. The derived absorbance is therefore: $\overline{A} = -\log \overline{T}$, instead of measuring the mean absorbance $\overline{A} = -\overline{\log T}$, which is required. Large errors may occur with slit scanners when spots are measured with an appreciable variation of concentration over the area of scan, since $-\overline{\log T} \neq -\log \overline{T}$ is while $A \neq \overline{A}$. Only if the absorbing material is spread uniformly across the slit $A = \overline{A}$ since, in this case, the transmission is identical at all points of the illuminating slit. The errors, which contribute to irregular spot geometry

and non-uniform concentration profile, vanish if the illuminated slit is made small enough so that variations of concentration over that area can be neglected.

The problems of the uneven distribution of material in the spots can be eliminated by employing a flying-spot system. In that case spots are scanned with a lightbeam of a very small cross-section, performing a zig-zag sinewave movement over the separated TLC spot in such a way that all parts are measured successively. For transmittance measurements the photomultiplier signal is transformed continuously to an inverse logarithm. The resulting signal is averaged over the width of the scanning stroke L (in cm). The derived absorbance is therefore:

$$\bar{A} = -\overline{\log T} = \frac{1}{L} \int_0^L (-\log T)\, dl \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots [6]$$

The absorbance value A obtained with the flying-spot densitometer can therefore be said to be equal to the mean absorbance value $\bar{A}$.

The advantage of the flying-spot principle is the independence of transmittance measurements in relation to the spatial distribution of the separated compounds, providing a new validity of Lambert-Bouguer-Beer's Law. An additional advantage of flying-spot scanning is the reduction of optical noise due to the fact that integration by the photomultiplier is carried out after linearization of the signal, which is more efficient in combatting noise. For slit-type measurements the direct optical integration is affected by non-linearity of the transfer function of the medium. Suppression of the optical noise and reduction of internal fluctuations are also obtainable by the application of double beam densitometers as described by Salganicoff (1967) and Pollak and Boulton (1971) for slit scanners.

3. Present study

3.1. Methods

From the features mentioned in 2.3.2 of this study, it was decided to perform scanning with a flying-spot densitometer. The suitability of the flying-spot principle was tested for the quantitative in situ TLC analysis of some compounds present in food and foodstuffs. Densitometric results recorded in this study were obtained with a Vitatron TLD–100*) flying-spot densitometer. Thin-layer chromatographic analyses were carried out under normal routine conditions using standard equipment and techniques. The methods developed for the quantitative densitometric analysis for aflatoxin B_1, quinine hydrochloride and L(+)-asorbic acid are itemized below.

3.1.1. Aflatoxin B_1 in peanuts and peanut products

1. *Isolation and thin-layer chromatography*

   Aflatoxin B_1 in peanuts and peanut products was extracted from homogenized samples according to the Official Dutch (KB) method (1966; defatting with petroleumether, Soxhlet extraction with a mixture of chloroform-methanol (90+10) followed by liquid-liquid extraction of extracts with chloroform), IUPAC method (1968; liquid extraction of samples with chloroform followed by defatting and column chromatographic clean-up on silica gel with resp. petroleumether, diethyl ether and a mixture of methanol-chloroform (3+97)) and the Methanol method (Liem & Beljaars 1970; methanolic extraction of samples followed by liquid-liquid extraction of extracts with chloroform).

   KB and IUPAC extracts were separated on silica gel G plates with a mixture of chloroform-acetone (90+10) whereas Methanol extracts were chromatographed on silica gel H plates using chloroform-trichloroethylene-n-amylalcohol-formic acid (80+15+4+1) for a developing solvent as described by Engstrom (1970). After TLC development the amount of B_1 in samples on a given plate was estimated òr calculated by means of visual òr fluorodensitometric measurements, referring to standard spots.

2. *Quantitative analysis*

   In situ fluorodensitometric reflectance measurements were used for the quantitative evaluation of B_1 spots on TLC plates. The effects of some variables that influence both separation and fluorescence of aflatoxin B_1 by TLC such as properties of silica gel, composition of solvent mixture, stability and concentration of B_1 spots on plates etc were studied (Beljaars et al.; 1972 a).

   The efficiency and precision of both the extraction and TLC techniques for the densitometric B_1 determinations in spiked and naturally contaminated peanut samples were investigated by comparing the KB, IUPAC and Methanol methods (Beljaars et al.; 1972 b).

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*) Vitatron Scientific B.V., P.O. Box 76, NL 6210 Dieren, The Netherlands.

3. *Semi-quantitative analysis*

Visual estimations of the fluorescent intensities of the separated aflatoxin B_1 spots from samples and standards on same plates were used for the semi-quantitative analysis of B_1 in peanuts and peanut products. In order to pinpoint and facilitate visual comparison in the case of two-dimensional TLC, a new spotting procedure (called the "anti-diagonal" technique) was developed for aflatoxins (Beljaars et al., 1973 a). The validity of this technique was tested in a collaborative study (20 participants) for spiked peanut butter extracts prepared and chromatographed according to the proposed Official Dutch (KB) method (1972) representing low contamination levels (0–12 μg B_1/kg).

3.1.2. Quinine hydrochloride in soft drinks

In situ fluorodensitometric reflectance measurements were used for the quantitative analysis of quinine hydrochloride in soft drinks. For this purpose soft drinks were treated with an alkaline mixture of methanol-25% ammonia (485+15) and then separated by TLC using an acid developing system of chloroform-methanol-acetic acid (80+20+3) in saturated tanks. Quantitative determinations for quinine hydrochloride were established in tonic waters, lemonades and fruit juices containing quinine (Beljaars et al.; 1973 b).

3.1.3. L(+)-Ascorbic acid (vitamin C) in buttermilk

In situ densitometric transmittance measurements were used for the quantitative analysis of L(+)-ascorbic acid in buttermilk. The method is based on oxidation of L(+)-ascorbic acid to dehydroascorbic acid followed by reaction with 2,4 – dinitrophenylhydrazone (DNPH). The red-brown osazones of the vitamin were separated by TLC with a mixture of chloroform-ethylacetate-acetic acid (60+35+5) in unsaturated tanks ensuring loss of interfering substances from sample (Beljaars et al.; 1974).

3.2. Results and discussion

3.2.1. Aflatoxin B_1 in peanuts and peanut products

1. *Isolation and thin-layer chromatography*

The Official Dutch (KB) method (1966) of analysis for aflatoxin B_1 in peanuts and peanut products consists of a time-consuming Soxhlet extraction procedure (extract preparation 4.5 hrs) together with a cumbersome TLC analysis. Poor resolutions are obtained yielding unreliable and inaccurate results. The method was recently revised (proposed KB method., 1971) by only modifying the chromatographic procedure to improve TLC separation.

Meanwhile Liem & Beljaars (1970) introduced a direct extraction procedure applying methanol as the main extracting solvent (Methanol-method). According to this procedure preparation of the extracts reduced time of analysis (extraxt preparation 0.5 hrs) making it suitable for series analysis of peanut butter òr roasted peanut products (levels $\geqslant$ 8 μg B_1/kg) and raw peanuts òr peanut shavings (levels $\geqslant$ 3 μg B_1/kg). In addition less influence was experienced to background interferences on chromatoplates due to presence of fluorescent impurities from samples.

Combination of the methanolic extraction procedure and the two-dimensional TCL technique proposed by Schuller et al (1970) resulted in a refined and sophisticated method, which was recently recommended by the Dutch Mycotoxin Committee as the proposed KB method (1972) for the quantitative analysis of aflatoxin B_1 in peanuts and peanut products. This procedure proved to be suitable for both visual- and fluorodensitometric determinations and applicable to routine determinations (detection limit: 5 μg B_1/kg).

2. *Quantitative analysis*

Preliminary densitometric experiments were performed to study the behaviour of aflatoxin B_1 standard spots chromatographed on different types of silica gel (Beljaars et al., 1972 a). Different procedures were applied to develop TLC plates. Coefficients of variation of 5–7% were calculated for single in situ measurements (50–80 observations) for a series of standard B_1 spots (1–4 ng/spot)on silica gel H and MN–G–HR plates representing the combined errors of spot application, TLC development and scanning technique. Coefficients of variation of 11–12% were calculated for G plates (85 observations). The results obtained with H and MN–G–HR plates were consistent with precision values varying from 2 to 7% for standard B_1 spot measurements published by different authors using slit-type instruments (Pons et al. 1966, 1968 a+b, 1969, 1971; Peterson et al. 1967; Stubblefield et al., 1967; Toth et al., 1970; Childs et al., 1970). A linear relationship between integrated peak areas and aflatoxin B_1 concentrations (0.4–20 ng/spot) was present for H– and MN–G–HR plates but absent for silica gel G plates. The stability of the fluorescence of B_1 spots was investigated for different types of silica gel.

The efficiency and precision of the extraction- and TLC techniques were investigated in this study (Beljaars et al., 1972 b) for B_1 determinations applying standard routine procedures. Spiked peanut butter extracts and samples (5–40 μg B_1/kg) were analyzed together with a great number of naturally contaminated peanut products by the proposed KB (1971), Methanol and IUPAC procedures. The average recoveries for spiked peanut butter extracts were 99–105% while coefficients of variation of 11–13% (6 samples; 15–18 observations) were found, representing the combined errors of spot application, background interference, TLC development and scanning. For spiked samples variation values of 13–15% (4 samples; 12 observations) were calculated resulting from the combined errors of sampling, extraction procedure, spot application, background interference, TLC development and scanning. Recovery values of 69% (proposed KB method) and 84% (Methanol– and IUPAC methods) were obtained for spiked peanut butter samples. A statistical analysis of variance was carried out determining the contribution of each component of variability.

A coefficient of variation of 13.6% (14 samples, 60 observations) was calculated for fluorodensitometric measurements of aflatoxin B_1 in naturally contaminated peanut products (levels 10–80 μg B_1/kg; 3 procedures averaged) whereas a variation value of 36% (14 samples; 43 observations) was found for visual estimations in same samples (3 procedures averaged). The densitometric results for aflatoxin B_1 obtained in this study together with the results published by other investigators involving different analytical procedures are summarized in **Table 1**.

It was concluded that both the Methanol- and IUPAC methods yielded extracts which were suitable for densitometric analysis; the proposed KB method (1971) was less appropriate, due to the presence of interfering fluorescent materials in the extracts, caused by the poor extraction procedure and TLC conditions. The densitometric method was more accurate when compared to visual estimations indicating the applicability of in situ densitometry for the quantitative analysis of aflatoxins (or mycotoxins in general) in food and foodproducts.

3. *Semi-quantitative analysis*

The influence of the fluorescent background interference was investigated for visual estiminations of the fluorescent intensities of B_1 spots from samples and standards. Apart from general factors the results were seen to depend mainly on contamination level of samples and method of analysis.

A recent collaborative study (33 participants; Beljaars et al. 1972 b) demonstrated

Table 1.
Review of statistical results for in situ fluorodensitometric measurements of aflatoxin B_1 in peanuts and peanuts products.

Method	Contamination level in μg B_1/kg	Coefficient of variation in %
one-dimensional TLC development		
Celite (1) [c]	21.5	20
CB-IUPAC (1, 2, 3) [a, b]	10 - 70	8 - 17
BF (1) [d]	10 - 20	19 - 26
KB 1966, 1971 (3, 4) [e]	10 - 40	13 - 15
Methanol (3)	10 - 40	13 - 15
two-dimensional TLC development		
KB 1966 (4, 5) [e]	6 - 24	4 - 6 *)

1. Waltking. (1970)
2. Beckwith et al. (1968)
3. Beljaars et al. (1972 b)
4. Official Dutch method (1966)
5. Schuller et al. (1970)

Legend: *) spiked peanut butter extracts.

a) CB = AOAC Official first action method I designated as the CB method (Contamination Branche, Division of Food Chemistry, Food and Drug Administration).

b) IUPAC = Official method of analysis of the International Union of Pure and Applied Chemistry (identical with CB-method).

c) Celite = AOAC Official first action method II using acid washed Celite for column chromatographic clean-up of extracts.

d) BF = AOAC Official first action method III designated as BF method (developed by A. E. Waltking, Research Laboratories of Best Food (BF) Division, CPC International Inc.).

e) KB = Official Dutch method of analysis (Koninklijk Besluit).

Table 2.
Review of statistical results for visual estimations of aflatoxin B_1 in peanuts and peanut products.

Method	Contamination level in μg B_1/kg	Coefficient of variation in %
one-dimensional TLC development		
Celite (1) [c]	10 - 110	89 - 40
CB-IUPAC (2, 3, 4) [a, b]	10 - 200	64 - 33
BF (5) [d]	10 - 15	65 - 39
KB 1971 (6) [e]	10 - 20	33 - 37 *)
All methods (7, 8)	5 - 45	85 - 63
two-dimensional TLC development		
Anti-diagonal (9)	3 - 6	35 *)

1. Campbell et al. (1966)
2. Beckwith et al. (1968)
3. Eppley et al. (1968)
4. IUPAC (1968)
5. Waltking (1970)
6. Beljaars et al. (1972 b)
7. Goldblatt (1969)
8. Coon et al. (1972, 1973)
9. Beljaars et al. (1973 a)

Legend: *) spiked peanut butter extracts.
For a - e see Table 1.

that coefficients of variation of 33–37% (66 observations) were established for visual estiminations of spiked peanut butter extracts (2 samples; levels 10 and 20 μg B_1/kg) prepared and chromatographed according to the proposed KB method (1971) using one-dimensional TLC. Variation values of 24% (66 observations) were calculated for standard B_1 solutions (levels 10 and 20 μg B_1/kg) indicating the absence of background interference. These findings were in agreement with variation values of 20–28% mentioned by Goldblatt (1969) and Beckwith (1968) for visual estimations under ideal conditions.

The interfering fluorescent compounds did not have a marked influence on the readings by using two-dimensional TLC. By this method spots originated from samples were free from background interference. The advantage of this procedure is that crude extracts can be analyzed, omitting cumbersome and time-consuming clean-up procedures.

The results obtained with the "anti-diagonal" technique (Beljaars et al., 1973 a) for spiked peanut butter extracts (levels 0–12 μg B_1/kg) demonstrated that no experience was necessary to locate and identify B_1 spots from sample and standards when chromatographed in both directions (this in contrary with the 1-dimensional TLC techniques).

An approximate coefficient of variation of 35% (96 observations) for contamination levels of 3 and 6 μg B_1/kg was calculated. Around 80–90% of the complete results were correct for 0 and 12 μg B_1/kg levels. The results of the visual estimations for aflatoxin B_1 obtained in this study together with the findings published by several authors involving different analytical procedures are summarized in **Table 2.**

It was concluded, that with the "anti-diagonal" procedure small amounts of aflatoxin B_1 ($\leqslant 10$ μg B_1/kg) may be detected for both visual- and fluorodensitometric techniques.

3.2.2. Quinine hydrochloride in soft drinks

Recovery experiments were performed with spiked soft drinks such as tonic water and fruit juice containing quinine. The recoveries varied from 97 to 101% while coefficients of variation of 2.0–2.5% (20–25 observations) were calculated for the densitometric procedure, representing the combined errors of sampling, spot application, TLC development and scanning procedure (Beljaars et al., 1973 b). A linear relationship was established between recorded peak-area and a series of standard spots (0.1–1.0 μg quinine hydrochloride $2H_20$/spot). The results of the densitometric method were compared with the UV-spectrophotometric procedures of the AOAC (1970), van Gils et al. (1972) and Hey (1972). Variation values of 0.74 – 0.85% (10 observations) were found for direct UV-spectrophotometric procedures (AOAC; van Gils et al.) for tonic waters, whereas a coefficient of variation of 1.38% (13 observations) was calculated for the indirect UV procedure (Hey) for lemonades or fruit juices containing quinine.

It was concluded, that the proposed densitometric procedure was suitable for routine determinations due to its simplicity and its general applicability to all types of soft drinks containing quinine (e.g. tonic waters, lemonades, fruit juices etc).

3.2.3. L(+)-Ascorbic acid (vitamin C) in buttermilk

The L(+)-ascorbic acid content in spiked buttermilk samples gave 98% recoveries of vitamin C from samples analyzed according to the proposed procedure with a coefficient of variation of 7–8% (7 samples containing 3–7 mg L(+)-ascorbic acid/100 ml). The densitometric transmittance measurements of the separated red-brown 2,4-dinitrophenylhydrazone spots from dehydroascorbic acid proved the existence of a linear relationship between integrated peak areas and standard spots up to a concentration level

of 1.0 μg L(+)-ascorbic acid/spot. At higher concentration levels a non linear relationship was established.

It was concluded, that although the results obtained using transmittance measurements appear satisfactory, the absence of a linear relationship for a large concentration range means that it cannot be considered as a suitable method for routine analysis. On the other hand the fluorodensitometric method does not have such a draw-back. The results obtained with the densitometric procedure for buttermilk samples were compared with those found by previous investigators using titrimetric-(Pelletier., 1967), potentiometric-(Hardesty., 1964) òr spectrophotometric techniques (Roe et al., 1944, Jaselskis et al., 1972) yielding coefficients of variation of 1–4%.

4. General conclusions

During the last decade much attention has been paid to the development of in situ densitometric procedures for the quantitative evaluation of thin-layer chromatograms. The increasing use of appropriate ready-made TLC plates, together with the development and improvement of the scanning devices promoted the application of densitometric procedures as an analytical method in various branches of science.

The application of this technique for the quantitative analysis of substances present in food and foodstuffs was investigated in this study. It was found that in situ fluoro-densitometric procedures for aflatoxin B_1 and quinine hydrochloride were appropriate for the quantitative analysis of these compounds in food and foodstuffs. Improvements of the extraction- and TLC procedures increased the precision of the analytical procedure. The results of the in situ transmittance measurements for L(+)-ascorbic acid were less suitable for routine analysis due to the absence of a linear relationship for a large concentration of L(+)-ascorbic acid.

The application of quantitative TLC in food control work has been shown to be suitable for solving difficult problems of food analysis as demonstrated in this study. Advances in spot application techniques, TLC conditions, scanning procedures etc can improve the accuracy of the densitometric procedures. Standardisation of the operating conditions, together with the development of electronic devices to process optical data, will improve quantitive TLC as a promising analytical procedure in the next future.

Summary

This thesis is dealing with the application of in situ spectrodensitometry for the quantitative evaluation of thin-layer chromatograms for compounds present in food and foodstuffs which are subjected to legal control. The validity of this procedure was investigated for the quantitative determination of the following compounds:

1. aflatoxin B_1 in peanuts and peaunut products,
2. quinine hydrochloride in soft drinks,
3. L(+)-ascorbic acid (vitamin C) in buttermilk.

The results of these individual determinations were obtained with a flying-spot densitometer.

1. Aflatoxin B_1 in peanuts and peanut products

A simple and rapid isolation procedure was developed for aflatoxin B_1 in peanuts and peanut products using methanol as the main extracting solvent (Methanol-method). The efficiency and precision of the Methanol-, Official Dutch (KB)- and IUPAC methods were examined in this study, employing both visual as well as objective densitometric techniques.

Densitometric procedures were used to investigate the behaviour and the properties of solid aflatoxin B_1 spots on different types of silica gel. Coefficient of variation values of ± 5–7% were calculated for single measurements of standard B_1 spots (1–4 ng/spot) on one plate. Spiked peanut butter extracts- and samples (5–40 μg B_1/kg) together with naturally contaminated peanut products (10–80 μg B_1/kg) were assayed by the 3 procedures. The average recoveries for spiked extracts were 99–105% (variation values of 11–12%; 3 procedures); recoveries of 69% (KB method) and 84% (Methanol- and IUPAC methods) were obtained for spiked samples (variation values of 13–15%; 3 procedures). The coefficient of variation calculated for the fluorodensitometric analysis of B_1 in naturally contamined samples was 13.6% (3 procedures).

The best results were obtained with the Methanol- and IUPAC methods for the quantitative assay of aflatoxin B_1 in peanuts and peanut products.

A coefficient of variation value of 36% was calculated for naturally contaminated peanut products (levels $\geqslant$ 10 μg B_1/kg) using semi-quantitative visual estimations. A special spotting procedure (called the "anti-diagonal" technique) was introduced to improve the precision for visual B_1 estimations and to facilitate location and comparison of B_1 spots from sample and standards after two-dimensional TLC development. With this technique a coefficient of variation of ± 35% was established for spiked extracts, representing low contamination levels (3–6 μg B_1/kg).

2. Quinine hydrochloride in soft drinks

An in situ fluorodensitometric method was developed for the quantitative determination of quinine hydrochloride in soft drinks containing quinine. The recoveries varied from

96–101% for spiked samples (levels 60 mg quinine hydrochloride/*l*; coefficient of variation 2-0–2.5%), which were compared with the results of direct- òr indirect spectrophotometric procedures for same samples (variation values of 0.7–1.4%). The proposed method proved to be simple and applicable as a routine method to all types of soft drinks containing quinine (e.g. tonic waters, lemonades and fruit juices).

3. L(+)-ascorbic acid (vitamin C) in buttermilk

A densitometric method was developed for the quantitative analysis of L(+)-ascorbic acid in buttermilk. L(+)-Ascorbic acid was determined by means of in situ transmittance measurements of the red-brown 2,4-DNPH spots of dehydroascorbic acid separated on TLC plates. Recoveries of 98% were obtained for spiked buttermilk samples containing 3–7 mg L(+)-ascorbic acid/100 ml (coefficient of variation values 7–8%). These results appeared to be satisfactory, although the absence of a linear relationship for a large concentration range (> 1.0 μg L(+)-ascorbic acid/spot) indicated that the procedure cannot be considered as a suitable method for routine analysis.

Generally, it can be concluded, that the application of quantitative in situ TLC analysis has been shown to be suitable in solving difficult problems of food analysis as demonstrated in this study. Advances in instrumentation, standardisation of the operating conditions, refinements of extraction and TLC techniques, will improve quantitative TLC as a promising analytical procedure.

Samenvatting

Dit proefschrift behandelt de toepassing van de in situ spectrodensitometrie voor de kwantitatieve analyse van dunnelaag chromatogrammen van bestanddelen in levensmiddelen, die onderworpen zijn aan wettelijke contrôle. De bruikbaarheid van deze methode werd onderzocht aan de hand van de kwantitatieve bepaling van de volgende verbindingen:

1. aflatoxine B_1 in pinda's en pinda produkten,
2. kinine hydrochloride in frisdranken,
3. L(+)-ascorbinezuur (vitamine C) in karnemelk.

De resultaten van deze afzonderlijke onderzoekingen werden verkregen m.b.v. een flying-spot densitometer.

1. Aflatoxine B_1 in pinda's en pinda produkten

Een eenvoudige en snelle extractie procedure werd ontwikkeld voor aflatoxine B_1 in pinda's en pindaprodukten, waarbij gebruik werd gemaakt van methanol als de belangrijkste extractievloeistof (Methanol-methode). De efficientie en nauwkeurigheid van de Methanol-, de Warenwet (KB) - en de IUPAC-methoden werden in dit onderzoek vergeleken onder gebruik making van zowel visuele- als densitometrische technieken.

Densitometrische methoden werden gebruikt om het gedrag en de eigenschappen van aflatoxine B_1 vlekken op verschillende soorten silica gel te bestuderen. Variatie coëfficienten van ± 5–7% werden berekend voor enkelvoudige metingen van standaard B_1 vlekken (1–4 ng/vlek) per plaat. Pindakaas-extracten en monsters pindakaas, waaraan bekende hoeveelheden aflatoxine B_1 toegevoegd waren (5–40 μg B_1/kg), alsmede van nature besmette pinda produkten (10–80 μg B_1/kg)werden volgens de hierboven genoemde 3 methoden van onderzoek geanalyseerd. De gemiddelde opbrengsten voor pindakaas-extracten waren 99–105% (variatie coëfficienten van 11–12%; 3 methoden); opbrengsten van 69% (KB-methode) en 84% (Methanol- en IUPAC methoden) werden gevonden voor de monsters pindakaas (variatie coëfficienten van 13–15%; 3 methoden). Een variatie coëfficient van 13,6% werd berekend voor de fluorodensitometrische bepaling van aflatoxine B_1 in pinda en pinda produkten (3 methoden).

De beste resultaten werden verkregen met de Methanol- en de IUPAC methoden voor de kwantitatieve analyse van aflatoxine B_1 in pinda en pinda produkten.

Een variatie coëfficient van 36% werd berekend voor van nature gecontamineerde pinda produkten, die geanalyseerd waren volgens de visuele semi-kwantitatieve analyse methode (gehalten $\geqslant$ 10 μg B_1/kg).

Een speciale opbrengtechniek (de z.g.n. "anti-diagonale" techniek) werd ontwikkeld en toegepast teneinde de nauwkeurigheid van het visuele schatten te verbeteren alsmede de opsporing van en het onderlinge vergelijk tussen B_1 vlekken van monster en standaarden te vergemakkelijken na twee-dimensionale dunnelaag chromatografische ontwik-

keling. Met deze techniek werd een variatie coefficiënt van ± 35% gevonden voor pindakaas-extracten, die gecontamineerd waren met geringe hoeveelheden aflatoxine B_1 (3–6 μg/kg).

2. Kinine hydrochloride in frisdranken

Een fluorodensitometrische methode werd ontwikkeld voor de kwantitatieve bepaling van kinine hydrochloride in kininehoudende frisdranken. De opbrengsten varieerden van 96–101% voor monters met bekende concentraties (gehalten van 60 mg kinine hydrochloride/*l*; variatie coëfficienten van 2,0–2,5%). Vergelijkbare uitkomsten werden verkregen met de direkte- en indirekte spektrofotometrische methoden voor dezelfde monsters (variatie coëfficienten van 0,7–1,4%). De voorgestelde methode was eenvoudig en toepasbaar als routine methode voor alle soorten kininehoudende frisdranken (b.v. tonics, limonades en vruchtensappen).

3. L(+)-Ascorbinezuur (vitamine C) in karnemelk

Een densitometrische methode werd ontwikkeld voor de kwantitatieve bepaling van L(+)-ascorbinezuur in karnemelk. L(+)-Ascorbinezuur werd bepaald d.m.v. transmissie metingen van de rood-bruin gekleurde 2,4-DNPH vlekken van dehydroascorbinezuur na scheiding op dunnelaag platen. Opbrengsten van 98% werden verkregen voor karnemelk monsters met bekende concentraties van 3–7 mg L(+)-ascorbinezuur/100 ml (variatie coëfficienten van 7–8%). Deze resultaten leken bevredigend, hoewel de afwezigheid van een lineair verband voor een groot concentratiegebied (> 1,0 μg L(+)-ascorbinezuur/vlek) aantoonde, dat de methode niet beschouwd kan worden als een geschikte methode voor het routine onderzoek.

In het algemeen, kan de konklusie getrokken worden, dat de toepassing van de kwantitatieve dunnelaag chromatografische analyse, zoals in deze studie beschreven, geschikt blijkt voor het oplossen van moeilijkheden bij de analyse van levensmiddelen. Instrumentele ontwikkelingen, standaardisatie van de omstandigheden, verfijningen van de extractie- en chromatografie technieken, zullen de kwantitatieve dunnelaag chromatografie maken tot een veelbelovende analytische methode.

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Appendix – legislation

Aflatoxin B_1
The toxic effects of aflatoxin B_1 to man and animals together with the occurance of this compound in peanuts and peanut products directed much effort towards achieving an effective control for this active and powerful hepatocarcinogenic substance. In order to protect public health the following section was added in 1966 to the Dutch Food Law. It is stated in Art. 3.q.q. paragraph 1 of the General Decree that:

Official text:
"In grondnoten (Arachis hypogaea), bestemd of geschikt voor directe consumptie, alsmede in uit of met grondnoten bereide eet- en drinkwaren, mag aflatoxine niet aantoonbaar zijn."

English translation:
"In groundnuts (Arachis hypogaea) intended to or suitable for direct consumption together with food and drinks prepared from or with groundnuts, aflatoxin may not be demonstrable."

An Official Dutch (KB) method of analysis (1966) was prescribed for the semi-quantitative analysis of aflatoxin B_1 in peanuts and peanut products. It is supposed that by this method contamination levels of 5 μg B_1/kg are detectable. Revisions of the Official Dutch method have been recommended known as the proposed KB method (1971) and the proposed KB method (1972).

Quinine hydrochloride
In most countries, use of quinine hydrochloride (òr sulphate) in soft drinks is determined by statutory regulations allowing its addition between fixed limits. In the Netherlands the addition of quinine hydrochloride to tonic water is governed by Art. 18 paragraph 8a of the Jam- and Lemonade Decree of Dutch Food Law. It is stated:

Official text:
"Tonic moet ten minste 40 mg kinine hydrochloride per liter bevatten."

English translation:
"Tonic water should contain not less than 40 mg quinine hydrochloride/*l*."

The upper limit is supposed te be set by the natural bitterness of quinine in these beverages. No statutory regulations are imposed for fruit juices òr lemonades containing quinine. In that case, manufacturars are obliged to indicate the minimum quinine hydrochloride content on these products.

No Official method of analysis is prescribed by Dutch Food Law for the quantitative analysis of quinine hydrochloride in tonic water and other soft drinks containing quinine.

L(+)-Ascorbic acid (vitamin C)
The addition of vitamins to food and drinks is not allowed by Dutch Food Law, as is stated in Art. 10 bis paragraph 1 of the General Decree.

Official text:
"Aan eet- en drinkwaren, alsmede aan kauwpreparaten, behalve die van tabak, mogen antibiotica, vitamines, jodiumverbindingen, fluorverbindingen, één of slechts enkele aminozuren en de zouten hiervan, met uitzondering van glutamine zuur en glutaminaten, niet worden toegevoegd, tenzij Onze Minister, met de uitvoering van dit besluit belast, hiertoe toestemming heeft verleend. Aan deze toestemming kunnen voorwaarden worden verbonden."

English translation:
"The addition of antibiotics, vitamins, compounds containing iodine òr fluorine, one òr some animo acids and their salts with the exceptance of glutamic acid and glutaminates to food and drinks as well as chewing commodities, except for tobacco, is not permitted unless Our Minister in charge of the enforcement of this Decree has consented for this purpose. To this consent conditions can be made."

An exception is made for the addition of L(+)-ascorbic acid as an anti-oxidant to minced meat, which is regulated in Art. 5 paragraph 2 of the Meat- and Meatproducts Decree. It is stated that:

Official text:
"In toebereid rauw gehakt mag het gehalte aan ascorbinezuur ten hoogste 0.05%, aan nicotinezuur ten hoogste 0.015% en aan citroenzuur ten hoogste 0.01% bedragen."

English translation:
"Minced meat is allowed to contain ascorbic acid (max. 0.05%), nicotinic acid (max. 0.015%) and citric acid (max. 0.01%)."

No Official Dutch method of analysis is prescribed for L(+)-ascorbic acid in food and foodproducts.

Curriculum vitae

De schrijver van dit proefschrift behaalde in 1959 het einddiploma HBS–B aan de gemeentelijke HBS te Nijmegen. In hetzelfde jaar werd met de studie in de scheikunde begonnen aan de Rijksuniversiteit te Utrecht. Het kandidaatsexamen, richting g, werd afgelegd in juni 1963, waarna het doctoraalexamen volgde in juli 1966 met als hoofdvak organische chemie (o.l.v. Prof. Dr. J. F. Arens) en als bijvak levensmiddelenchemie (o.l.v. Prof. Dr. J. F. Reith). Vanaf september 1966 tot en met mei 1968 was de auteur verbonden als post-doctoral fellow aan het Department of Chemistry of the University of Alberta, te Edmonton, Canada alwaar onderzoek werd verricht op het gebied van de organische chemie (o.l.v. Prof. Dr. R. K. Brown).

Sinds 17 juni 1968 is de auteur werkzaam als scheikundige 1^{e} klas bij de Keuringsdienst van Waren te Maastricht. Vanaf december 1968 werd een aanvang genomen met het hier beschreven onderzoek onder leiding van Prof. Dr. W. Pilnik, hoogleraar in de levensmiddelenchemie aan de Landbouwhogeschool te Wageningen.

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