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FINAL REPORT

Subject: A validated gas chromatographic method for the determination of total regulable residues of imazalil on bananas.

Research Facilities and Sponsor:

Janssen Research Foundation, Department of Plant Protection Research,
Turnhoutseweg 30, 2340 Beerse, Belgium.

ABSTRACT

Based on the EPA-approved GC-ECD method for the determination of total regulable residues of imazalil in citrus fruit and by-products, an adapted enforcement method for residues in bananas is presented. Validation of the method is described and a comparison is given between the ES (enforcement method) and an IS method.


Special attention is drawn to critical steps or chromatographic conditions to minimize the risk of unsuccessful application of the method in labs not familiar with residue analysis of imazalil and its major metabolite.

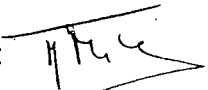
Protocol/Study number reference : AGR 16.


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Date: August 10, 1992

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I. INTRODUCTION

A. Scope

- A GC-ECD validated method is described for the determination of imazalil (Janssen compound R23979) and its major metabolite (Janssen compound R14821) in different banana-matrices (green bananas, yellow bananas and edible pulp).
The method is requested by EPA for the re-registration of imazalil.
- The method is based on the EPA-approved validated gas chromatographic method :
"A gas-liquid chromatographic method for determining total regulable residues of imazalil in citrus fruit and citrus by-products"
(Janssen Preclinical Research Report R23979/30, March 1982)
- Significant modifications to the basic method :
 1. use of commercial available WCOT fused silica megabore column CP-Sil 8CB instead of a home-made packed glass column.
 2. alkalinization during the extraction step with ammonium hydroxide instead of sodium hydroxide (better separation between organic and water layer).
 3. Whereas originally a 15 column was used for the determination of the total regulable residues in banana matrices (Study number AGR 3, Report No. 54), the use of a 50 m column is presented in this enforcement method due to improved separation between imazalil and its metabolite.

B. Principles

A representative sample of bananas or edible pulp is mixed thoroughly. Imazalil and its metabolite R14821 are released from the matrix by diluted hydrochloric acid. After alkalinization with concentrated ammonia, both compounds are extracted with a heptane-isoamyl alcohol mixture.

Prior to injection, R14821 is derivatized. Concentrations of imazalil and its metabolite are determined by GC-ECD.

Quantification is done by the external standard method with multilevel calibration over a linear dynamic range equivalent to 0.05 - 5 ppm (R23979) and 0.05 - 0.5 ppm (R14821) in the matrix.

The extraction recovery of the method exceeds 90 % for imazalil and 86 % for the metabolite. Limit of detection is found to be 0.02 ppm for R23979 and 0.03 ppm for R14821.

The described method is validated according to the protocol AGR 16, completed with a comparison between the external and internal standard method.

Note : To minimize the volume of the report, items specifically related to the internal standard method are given in italics throughout the text.

II. MATERIALS / METHODS

A. Equipment

1. Gas chromatograph :
Varian model 3600 gas chromatograph, equipped with a pulse-modulated constant-current Ni⁶³ electron capture detector and connected to a Chromjet integrator (Spectra Physics)
2. Homogenizer :
 - 2.1. Tecator 1094 homogenizer (Tecator AB, Höganäs, Sweden)
 - 2.2. Ultra-Turrax Model T 45 (Janke & Kunkel GmbH & Co. KG, Staufen, Germany)
3. Test tube mixer :
 - 3.1. IKA vibro-fix VF1 (Janke & Kunkel GmbH & Co. KG, Staufen, Germany)
 - 3.2. Labinco "Model 526" rotary-mixer (Labinco BV, Breda, The Netherlands)
4. Centrifuge :
Heraeus Omnifuge 2.0 RS (Vander Heyden, Brussels, Belgium)
5. Heating block / Sample concentrator :
Tecam Dri Block DB-3 & Sample Concentrator SC-3 (Techne, Cambridge, England)

B. Reagents and standards

1. Solvents :
Heptane Uvasol grade (Baker*)
Isoamyl alcohol PA (Baker*)
Methanol PA (Baker*)
Toluene pestanal grade (Riedel-de Haën**)
2. Inorganic reagents :
Hydrochloric acid 0.1 N solution PA (Merck***)
Ammonium Hydroxide 25 % solution PA (Baker*)

3. Silylation reagent :
N,O-Bis (trimethylsilyl) acetamide (BSA)
(Janssen Chimica, Beerse, Belgium)

4. pH-indicator strips :
Universal indicator pH 0-14 (Merck***)

Remarks :

* : J.T. Baker Chemicals BV, Deventer, Holland

** : Riedel-de Haën, Seelze, Germany

*** : E. Merck, Darmstadt, Germany

5. Reference materials

5.1. Analytical standards

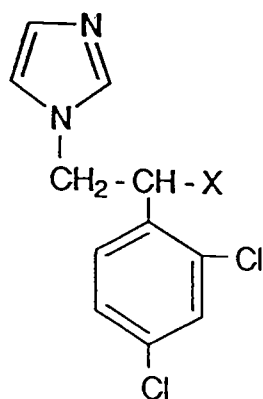
R23979 (imazalil) : batch no. V890-275 (98.7 %)

R14821 (metabolite) : batch no. V840-12 (> 99.9 %)

R30617 (internal standard) : batch no. V900-185 (> 99.9 %)

All obtained from Janssen Research Foundation,
Janssen Pharmaceutica N.V., B-2340 Beerse, Belgium

They can all be obtained commercially from Janssen Biotech
(Olen, Belgium).



- X	R-nr.	Compound
- O-CH ₂ -CH = CH ₂	R23979	imazalil
- OH	R14821	metabolite
- CH ₂ -(CH ₂) ₃ -CH ₃	R30617	<i>internal standard</i>

Figure 1 : chemical structures

5.2. Standard solutions

Separate stock solutions of imazalil, R14821 and R30617 are prepared at 1 mg/ml in methanol. As needed, these stock solutions are diluted further with methanol at such a rate that for each derived concentration, 100 µl must be spiked to the matrix.

6. Study samples

Control samples (unripened and ripened bananas) are obtained from a commercial plantation and ripening chamber.

After homogenization, samples are stored at $\leq -20^{\circ}\text{C}$ until use.

C. Analytical procedure

1. Homogenization of bananas

- 1.1. Take a representative sample of at least 10 bananas
- 1.2. Cut the bananas (or edible pulp after peeling) into pieces and bring them into a clean blender (e.g. "Tecator 1094 homogenizer").
- 1.3. Grind at high speed for at least 1 minute until a thoroughly blended pulp is obtained.
- 1.4. Transfer the homogenized sample in a clean storage container.

2. Extraction procedure

- 2.1. Weigh accurately about 1 g (± 0.005 g) of the homogenate into a glass test tube (content at least 10 ml).
- 2.2. Add 2.5 ml of 0.1 N hydrochloric acid and mix for 1 hour on a rotary mixer.
- 2.3. Alkalinize by adding 0.5 ml of concentrated ammonia
CHECK FOR pH ! (if pH < 9, adjust with concentrated ammonia to pH ≥ 9).
- 2.4. Add 5 ml of heptane-isoamyl alcohol (95 : 5 v/v) and mix for 15 minutes in a rotary mixer.
- 2.5. Centrifuge for 15 minutes at 2500 ppm.
- 2.6. Pipette off carefully the organic layer and bring into a graduated test tube (content at least 10 ml).
- 2.7. CHECK for pH the remaining aqueous layer (if pH < 9, adjust with concentrated ammonia to pH ≥ 9).
- 2.8. Add 5 ml of heptane-isomyl alcohol (95 : 5 v/v) to the aqueous layer and proceed as in 2.4., 2.5. and 2.6. .
- 2.9. Combine the organic layers and bring to 10 ml by adding heptane-isoamyl alcohol (95 : 5 v/v)

3. Derivatization procedure

- 3.1. Take 3 ml of the extract and evaporate until dryness in a sample concentrator at 50°C (DO NOT EXCEED THIS TEMPERATURE ; see comment H.2 page 11) under moderate nitrogen flush.

- 3.2. Add 40 µl of BSA to the residue, mix for 1 minute e.g. on an vibro-fix and dilute with 1.5 ml of toluene.
 - 3.3. Bring into an autosampler vial, close well and put the vial into a heating block for 30 minutes at 80° C. The sample is ready for injection. It can be stored for at least 24 hours at ambient temperature before analysis.
4. Fortification
 - 4.1. Reference solutions

Spike, after addition of the hydrochloric acid (see C. 2.2.), blank control homogenates with imazalil (range 0.05 - 5.0 ppm) and metabolite (range 0.05 - 0.5 ppm) using 100 µl of the respective diluted standard solutions. Proceed as described (see C. 2. and C. 3.).
 - 4.2. *Internal standard method*

If internal standard is used, spike at 1 ppm (100 µl of a 10 µg/ml solution in methanol) just after addition of the hydrochloric acid (see C. 2.2.).

D. Instrumentation

1. Apparatus
 - 1.1. Gas chromatograph : Varian GC-3600 equipped with a Ni⁶³ electron capture detector, a 1040 megabore™ injector and an autosampler Model 8035.
 - 1.2. Integrator and output device : Spectra Physics SP4400.
2. Operating conditions
 - 2.1. Gas chromatograph
 - Column : 50 m WCOT fused silica megabore column with an ID of 0.53 mm, coated with a 95 % dimethyl - 5 % phenyl-siloxane phase (CP-Sil 8 CB) of 2.00 µm film thickness.
 - Carrier gas : helium 20 ml/min
 - Injector : temperature : 250° C
 - Detector : ECD
 - sensitivity : range 10
 - attenuation 64
 - temperature : 350° C
 - make-up gas : nitrogen 50 ml/min

- Oven temperature : 250° C
- Autosampler : injection volume 1.5 µl
fast injection rate
- Retention times (after silylation)
 - R23979 : about 5.7 minutes
 - R14821 : about 4.9 minutes
 - R30617 : *about 8.7 minutes*
- Duration of one run : 10 minutes

2.2. Integrator and output device

- sensitivity : 1 mV full scale
- attenuation : 2
- chart speed : 0.5 cm/min.

3. Calibration procedure :

3.1. Reference solutions, see C.4.1. [*C.4.2. for internal standard method*], are extracted and analysed as described.

3.2. Calibration curves are constructed by plotting the peak heights [*peak height ratios R23979 or R14821/IS*] against the imazalil and R14821 concentration of each reference solution. Transform, if necessary, to log/log to obtain linearity.

3.3. Linear regression analysis from these curves is used to determine residue concentrations in real samples (sample solutions).

E. Interferences

1. Sample matrices -specificity

Interference of matrix components is checked by extracting blank samples of green (whole) and yellow (whole and edible) bananas. Resulting chromatograms are compared with those obtained from a spiked sample containing 0.05 µg R23979 + 0.05 µg R14821 [*1.0 µg internal standard*] per g of bananas.

2. Other pesticides : no data available

If bananas treated with other pesticides are used as blank control material, we recommend to check for interference, using the procedure described in E.1.

3. Solvents-reagents

All solvents and reagents are checked on their purity.

Heptane, isoamyl alcohol, methanol, toluene, heptane-isoamyl alcohol extracts of the inorganics, and the BSA-toluene mixture are injected into the chromatograph.

The obtained chromatograms are checked for the selectivity against imazalil - metabolite [*and internal standard*].

4. Glassware

Before starting analysis of samples, all glassware (test tubes, pipettes, ...) have to be carefully cleaned, especially when used previously for determination of imazalil (see "potential problems" H.4. page 11).

F. Confirmatory techniques

The identity of imazalil and its metabolite is confirmed when, on two consecutive chromatograms, the retention time recorded for a peak of the sample solution deviates by no more than 5 % from the retention time recorded for the reference substance in a reference solution.

For the metabolite R14821, an additional confirmation test is advised. Compare a chromatogram obtained from an unsilylated reference solution with that obtained from an unsilylated sample solution. Note that the retention time for R14821 differs between silylated and unsilylated compound and that the peak height is markedly increased after silylation. Moreover, this additional confirmation test enables the analyst to check the completeness of the derivatization step.

G. Time required for analysis

About 3.30 h is required to carry out the whole determination for one sample, i.e. from the rac-sampling to the end of the chromatographic analysis.

H. Potential problems

1. pH-control is essential.

- To release imazalil and the metabolite from the matrix, a pH ≤ 2 is required.

- Completeness of the extraction from the aqueous to the organic layer is determined by pH ($\text{pH} \geq 9$). pH can change during the extraction ; check pH of the aqueous layer after each extraction.

2. Evaporation step

- temperature is critical : do not exceed 50°C to prevent evaporation of the components to be determined once the solvent is fully evaporated.
- use a moderate nitrogen flush in the sample concentrator (max 2 psi for the SC-3 concentrator model). Target duration of evaporation is 30 minutes.
- at the end of the evaporation step, the residue may no longer be a liquid as remaining solvent can lead to an uncompleted derivatization.

3. Requirement of a blank control

Although the effect of a blank control is limited, it has been proven that extractives of the bananas matrices have a positive result on the response of imazalil and the metabolite. As a consequence, we advise the use of a blank control whenever it is available.

4. Glassware

Glass test tubes and pipettes are very sensitive to adsorption of imazalil and metabolite. We strongly recommend to rinse (in addition to normal good laboratory cleaning procedures) used glassware with acidic water or an acidic detergent dilution (e.g. Extran^(R) AP21 from Merck, Darmstadt, Germany) to avoid contamination.

5. Injector septa

To prevent adsorption/desorption phenomena of imazalil and/or metabolite at the septum surface and appearing of ghost peak(s) resulting from septum bleeding, a good choice of this injector part is necessary. Under the described chromatographic conditions, good results are obtained with the microsep^(R) F-174 from Supelco Inc. .

6. Solvent injections

Imazalil can cause strong cross contaminating effects. Therefore, it is recommended to perform two solvent (i.e. toluene) injections between sample or reference solutions, especially when the concentrations of two consecutive solutions differ greatly. When an autosampler is

used, each sample or reference solution containing vial is followed by a solvent containing vial to allow solvent rinsing of the connection tube and injection syringe.

7. Inject samples or reference solutions always in ascending order of concentration to avoid misleading results.

I. Methods of calculation

1. ES-method

- 1.1. Construct calibration curves (R23979 and R14821) by plotting peak-heights on the y-axis against the concentrations (in ppm) in the reference solutions on the x-axis.
- 1.2. Apply linear regression analysis and calculate the correlation coefficients as well as the slopes and y-intercepts of both calibration curves (R23979 and R14821).
- 1.3. From the calibration curve slope and intercept, and peak-height of R23979 (respectively R14821) in a sample solution, calculate the concentration C_S (in ppm) using the following equation :

$$C_S \text{ (ppm)} = \frac{\text{peak height in sample solution} - \text{intercept (calib.)}}{\text{slope (calib.)}}$$

2. IS-method

- 2.1. Construct calibration curves (R23979 and R14821) by plotting peak-heights ratios (R23979/IS, respectively R14821/IS) on the y-axis against the concentrations (in ppm) on the x-axis.
- 2.2. Apply linear regression analysis and calculate the correlation coefficient as well as the slopes and y-intercepts of both calibration curves (R23979 and R14821).
- 2.3. From the calibration curve slope and intercept, and the peak-height ratio R23979/IS (respectively R14821/IS) in a sample solution, calculate the concentration C_S (in ppm) using the following equation :

$$C_S \text{ (ppm)} = \frac{\text{peak height ratio in sample solution} - \text{intercept (calib.)}}{\text{slope (calib.)}}$$

III. METHOD VALIDATION (ES-method)

A. Specificity/selectivity

1. Definition - Specificity of the method is a measure of the degree of interference (or absence thereof) from components that may be expected to be present in the sample solutions (e.g. impurities, degradation products, related chemical compounds etc.). Selectivity is a measure of the degree of interference (or absence thereof) in the analysis of complex sample mixtures.
2. Determination - Specificity of the method is determined by a check of resolution between imazalil and its metabolite R14821 (silylated or unsilylated) in the chromatogram of spiked samples (for the three commodities). Selectivity is checked by extracting blank samples of green (whole) and yellow (whole and edible pulp) bananas and comparing chromatograms with these obtained from spiked samples.
3. Results - Specimen chromatograms demonstrating the specificity/selectivity of the ES-method are shown in figures 2-3-5-7 pages 28, 29, 32, 35.

B. Linearity (and range)

1. Definition - Linearity is the ability to derive test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range.
The range is the interval between the upper and lower levels of analyte (including these levels) that have been demonstrated to be determined with analytical validation parameters using the method as written.
2. Determination - Using standard solutions of imazalil and its metabolite, samples of blank control materials (whole green, whole yellow and edible pulp) are spiked with imazalil and its metabolite. Spiking levels for whole bananas (green and yellow) are between 0.05 - 5 μg R23979, 0.05 - 0.5 μg R14821 and for edible pulp between 0.05 - 1.0 μg R23979, 0.05 - 0.2 μg R14821 per gram bananas. After extraction and analysis of these spiked samples calibration curves are constructed (transformation to log/log can be necessary to obtain linearity by plotting peak heights on the y-axis

against the concentration (in ppm) of imazalil/metabolite). Slope, y-intercept and correlation coefficient of each curve is determined.

3. Results :

Calibration curves of R23979 and R14821 for three commodities (whole green, whole yellow and edible pulp) are shown in figures 10-11, pages 40, 41, whilst chromatograms of the different calibration samples for the three commodities are given in figures 4-6-8, pages 30, 31, 33, 34, 36, 37.

C. Extraction recovery

1. Definition - The recovery of the method is a measure of the efficiency of the extraction of the analyte from the sample matrix.
2. Determination - Blank control materials are run through the extraction procedure. Final analyte solutions (before derivatization) are spiked at the same levels as for the calibration curves (see III. B. 2.). Analysis and construction of the curves is carried out in the same way as for the linearity. Slopes and intercepts of these curves are compared statistically with those from the corresponding linearity curves (II.B.2.). If the t-test for significance of difference between the slopes gives a positive result, one can conclude that recovery is constant over the whole concentration range tested, whereas differences in y-intercept correlate to the percentage of recovery. If so, a mean recovery can be calculated for the concentration range tested. Otherwise, recovery has to be given for each concentration level. Recovery is expressed as the ratio (in percent) of the peak area of imazalil (or metabolite) from the linearity curve over the peak area of the component from the recovery curve.
3. Results :
 - t-test slopes :

All calculated t-values are lower than the critical values, i.e. recovery is constant over the whole tested concentration range.
 - t-test y-intercepts :

All calculated t-values (except one, which differs only by 1.5 % from the critical value) are lower than the critical values, i.e. statistically recovery is 100 % over the whole tested concentration range and for

all commodities.

Detailed data are summarized in tables 1a and b, page 44.

D. Requirement of an untreated commodity (blank control)

1. Definition - Blank control is a measure of the degree of influence of blank ingredients on the chromatographic response.
2. Determination - Dilutions of standard solutions of imazalil and metabolite in the analyte solvent are made at the same levels as for the linearity. After derivatization, samples are injected and calibration curves constructed. Slopes and y-intercepts of these curves are compared statistically with those of the corresponding recovery curves (III.C.2.).

A t-test for significance of differences for the two slopes and the two y-intercepts is carried out and validated.

3. Results

The t-tests on the results for the three commodities show that the use of a blank control is to be recommended, although not really necessary.

Detailed information is given in table 3a page 46.

Chromatograms, after derivatization, of standard solutions in the analyte solvent are shown in figure 9, page 38, 39.

E. Precision (repeatability)

1. Definition - The precision of the method is the degree of agreement among individual test results when the procedure is applied repeatedly on multiple sub-samples from one homogeneous sample.
2. Determination - Blank banana samples (10) are spiked with imazalil and its metabolite at one level. After extraction and analysis, outliers tests (Dixon, Grubbs) are carried out on the results and the relative standard deviations calculated.

3. Results

The following RSD's are found :

- whole green bananas : 4.00 % (R23979) - 1.73 % (R14821)
- whole yellow bananas : 4.85 % (R23979) - 13.30 % (R14821)

- edible pulp : 4.21 % (R23979) - 15.65 % (R14821)
Detailed data can be found in table 4 page 48.

F. Limit of quantitation (LOQ)

1. Definition - The LOQ is the lowest concentration of analyte in the sample that can be determined with acceptable precision and accuracy under the experimental conditions. The following equation is used to determine the LOQ for imazalil and its metabolite :

$$\text{LOQ} = \frac{C_c (10 \times \text{PH}_n)}{\text{PH}_c} \text{ ppm } (\mu\text{g/g})$$

with C_c : concentration (in $\mu\text{g/g}$ of imazalil/metabolite in a blank control spiked after extraction with an exactly known amount of standard material.

PH_c : peak height (in mm) of imazalil/metabolite in the chromatogram

PH_n : peak height (in mm) of analytical background response in a peakfree area of the chromatogram.

2. Determination - From a suitable chromatogram PH_c and PH_n of both compounds (concentration C_c) are measured and the LOQ is calculated.
3. Result - The LOQ for R23979 is 0.05 ppm, and for R14821 0.08 ppm, for the three commodities.

G. Limit of detection (LOD)

1. Definition - The LOD is the lowest concentration of analyte in a sample that can be detected under the experimental conditions.
2. Determination - The LOD will be derived from the LOQ. A peak height equal to three times the magnitude of the noise shall be considered as the LOD.
3. Result :
The LOD is 0.02 ppm for R23979 and 0.03 ppm for R14821.

H. Stability of analyte solutions

1. Definition - Stability of analyte solutions is a measure of bias in the assay results within a preselected time interval (e.g. 24 hours).
2. Determination - Three replicate analyte samples (in the extraction solvent as well as after derivatization) containing three levels (low - medium - high) of imazalil and metabolite are analysed at time zero and after a storage time of 24 hours at ambient lab temperature. Stability is expressed as percentage of imazalil/metabolite concentrations found after the 24 hours storage period against those found at time zero.
3. Results :
Both imazalil and metabolite may be stored for at least 24 hours both in the extraction solvent mixture and in the analyte solvent (after derivatization).
Detailed results are given in table 6 page 50.

IV. METHOD VALIDATION (IS-method)

In addition to the method validation of the ES-method, the IS-method is validated too. For parameters, definitions and determinations we refer to the ES-method.

RESULTS :

A. Specificity/selectivity

Specimen chromatograms, showing the specificity and the selectivity are given in figures 2-3-5-7 pages 28, 29, 32, 35.

B. Linearity (range)

Calibration curves of R23979 and R14821 for the three commodities are shown in figures 12-13, pages 42, 43, whilst chromatograms of the different reference solutions for the three commodities can be found in figures 4-6-8 page 30, 31, 33, 34, 36, 37.

C. Recovery

t-test slopes :

All calculated t-values are lower than the critical values, i.e. recovery is constant over the whole tested concentration range.

t-test y-intercepts :

All calculated t-values (except one, YB-R14821) are lower than the critical values, i.e. statistically recovery is 100 % over the whole tested concentration range. Range of recovery for R14821-YB is between 91-151 %.

Detailed data are summarized in table 2 page 45.

D. Blank control

The t-tests on the results of the three commodities show that a blank control is used by preference.

Data are given in table 3b, page 47. Chromatograms, after derivatization of standard solutions in the analyte solvent, are given in figures 9-10, pages 38, 39, 40.

E. Precision (repeatability)

The following RSD are found for the three commodities.

whole green - R23979 : 3.26 %, R14821 : 4.39 %

whole yellow - R23979 : 1.65 %, R14821 11.56 %

edible pulp - R23979 : 3.27 %, R14821 : 11.29 %

Detailed information about the determination of the precision can be found in table 5, page 49.

F & G. Limit of Quantitation/Detection

Not measured as the LOQ and LOD are the same as for the ES-method.

H. Stability of analyte solutions

Both imazalil and metabolite, as well as the internal standard may be stored for at least 24 hours both in the extraction solvent and in the analyte solvent (after derivatization).

Detailed results are given in table 7, p. 51.

V. STATISTICAL COMPARISON BETWEEN ES-AND IS-METHOD

A. Wilcoxon T-test (related samples)

With the Wilcoxon matched-pairs signed-ranks test for related samples, the results of the precision tests (III.E and IV.E) of both methods are compared.

If the calculated T-value is greater than the critical T-value, the null hypothesis is accepted, i.e. the two methods give equal results.

Detailed data (tables 8-9-10, pages 52, 53 and 54) show that for all described commodities the null hypothesis is accepted.

B. Comparison of the two methods by least-squares fittings (Regression Analysis).

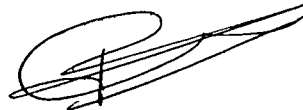
Linearity and recovery data, obtained with both methods, were compared by linear regression (after conversion to log/log) with results of the ES-method on the x-axis and results of the IS-method on the y-axis. Resulting graphs for the different commodities are shown in figures 14 and 15 page 55 and 56. Again, for all commodities, both methods perform equally (slopes very close to 1, intercepts negligible).

VI. CERTIFICATION

QA-ATTEST No: 306 a

Good Laboratory Practices Compliance Statement

The data and the report: "A validated gas chromatographic method for the determination of total regulable residues of imazalil on bananas." with the report number: "56" were produced and compiled in accordance with all pertinent Good Laboratory Practices regulations as described by inter alia OECD, FDA (21 CFR part 58) or EPA (40 CFR part 160).



Theo Ligtvoet

Study Director

date: August 13, 1992

Testing Facility Management Attestation

The undersigned certifies hereby that the GLP regulations as described by inter alia OECD, FDA (21 CFR part 58) or EPA (40 CFR part 160) are implemented within the testing facility.



J. Van Gestel

Management

Plant Protection Research

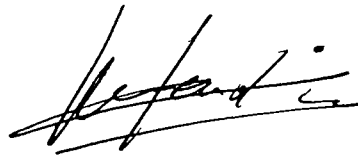
date: August 13, 1992

Quality Assurance Statement

The raw data and the final report: "A validated gas chromatographic method for the determination of total regulable residues of imazalil on bananas." with the report number: "56" were inspected by the Department of Quality Assurance-Research, Janssen Research Foundation, to assure compliance with the standard operation procedures, the pertinent Good Laboratory Practices Regulations as described by inter alia OECD, FDA (21 CFR part 58) or EPA (40 CFR part 160) and the protocol: "Validation of a GC-ES method for determining imazalil-related residues in bananas" with the protocol number: "AGR16".

Inspection date	Reporting date to Study Director and/or Management
January 6, 1992	
February 25, 1992	March 2, 1992
August 12-13, 1992	August 13, 1992

It is the opinion of the QAU that this report accurately reflects the raw data collected during this study.



R. Hendriks
Quality Assurance Manager
date:

August 13, 1992

VII. REFERENCES

1. Laboratory notebook Agroform Anal Res 01/023979/009
2. Janssen Preclinical Research Report R23979/29, March 1982.
"A gas-liquid chromatographic method for determining total regulable residues of imazalil in citrus fruit and citrus by-products: validation and application to a residue study of imazalil in citrus fruit,
by Woestenborghs R., Michielsen L., Meuldermans W., and Heykants J.
3. Janssen Preclinical Research Report R23979/30, June 1982.
"A gas-liquid chromatographic method for determining total regulable residues of imazalil in citrus fruit and citrus by-products".
4. Protocol/study number AGR 16
Amendment 1 to Protocol AGR 16
5. Definition of method validation parameters: USP XXII (United States Pharmacopeial Convention, Inc.)
- 6 Statistics
 - T-test (slopes, intercepts):
"Fundamentals of Clinical Pharmacokinetics", by John G. Wagner, First Edition 1975. (ISBN 0-914678-20-4).
 - Wilcoxon t-test and Regression Analysis:
"Chemometrics: a textbook (datahandling in Science and Technology)" by D.L. Massart et al. (ISBN 0-444-42660-4)
 - Dixon and Grubbs test:
"Statistical Techniques for Data Analysis" by John Keenan Taylor (1990) (ISBN 0-87371-250-1)

VIII. TABLES / FIGURES

A. List of abbreviations used in the following figures and tables

- GB : green whole bananas
- YB : yellow whole bananas
- EP : yellow bananas edible pulp

- 1 : R23979 (peak 1 in chroma's)
- 2 : R14821 silylated (peak 2 in chroma's)
- 3 : R30617 = IS (peak 3 in chroma's)
- 4 : R14821 unsilylated (peak 4 in chroma's)

- lin. : linearity
- rec. : recovery
- bc. : blank control

- calc. : calculated
- critic. : critical

- D : outlier Dixon test
- G : outlier Grubbs test
- CI : Confidence Interval
- SD : Standard Deviation
- RSD : Relative Standard Deviation (%)

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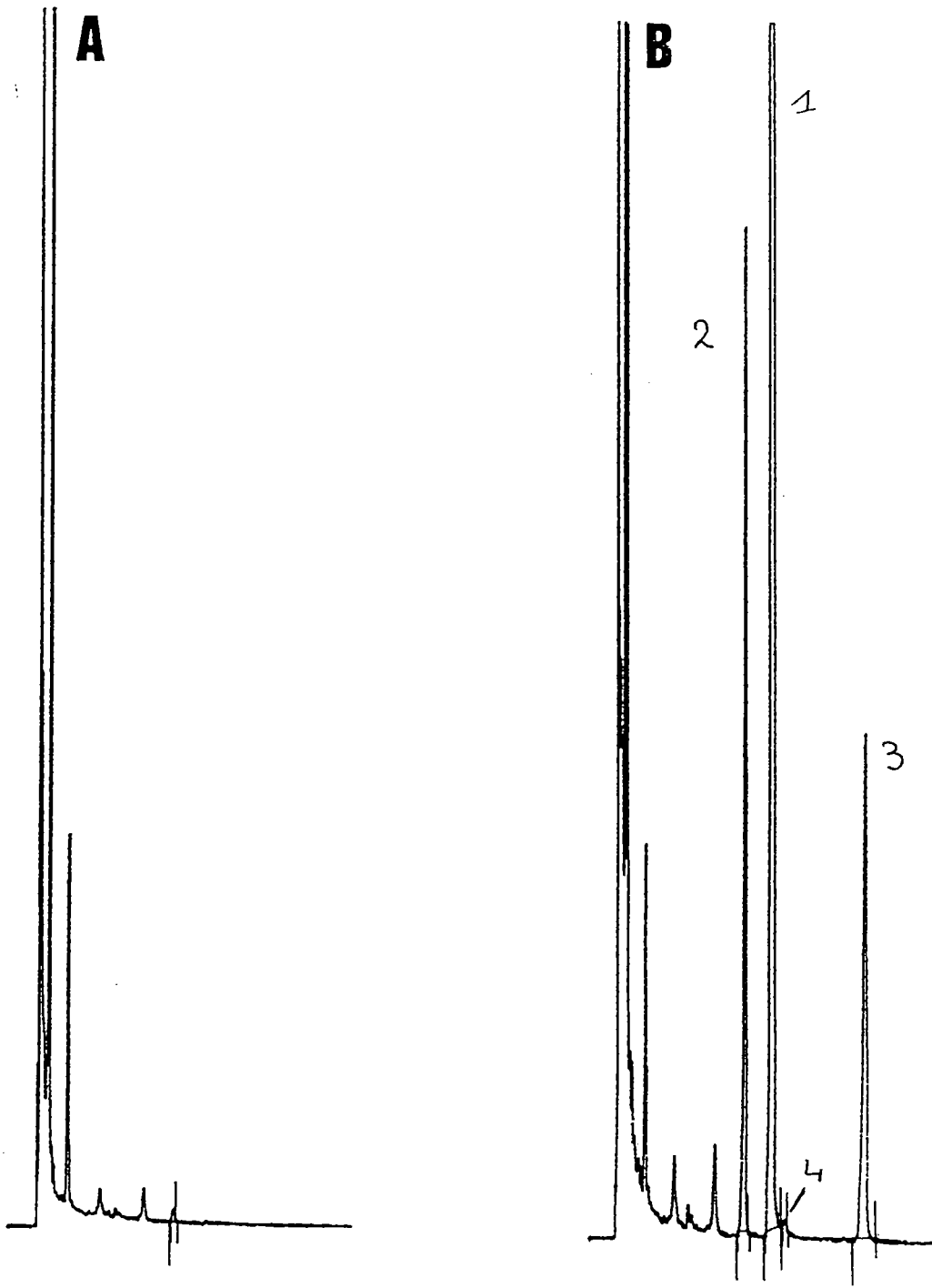


Figure 2 : a. Toluene / BSA-mixture
b. Specimen chromatogram demonstrating the specificity of the method

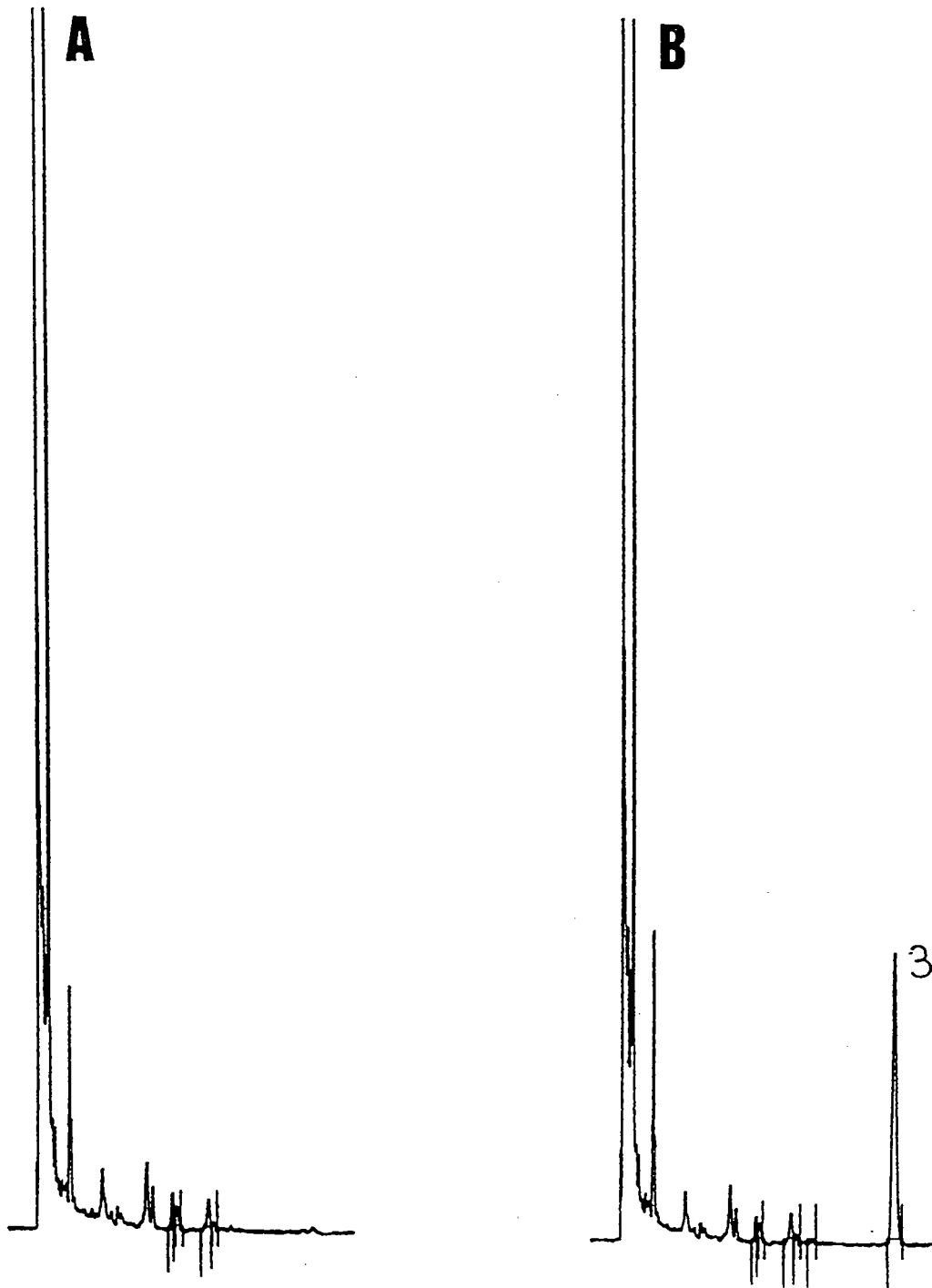


Figure 3 : GB-specimen chromatograms of blank material demonstrating the selectivity of the method
a. without IS-addition
b. with IS-addition

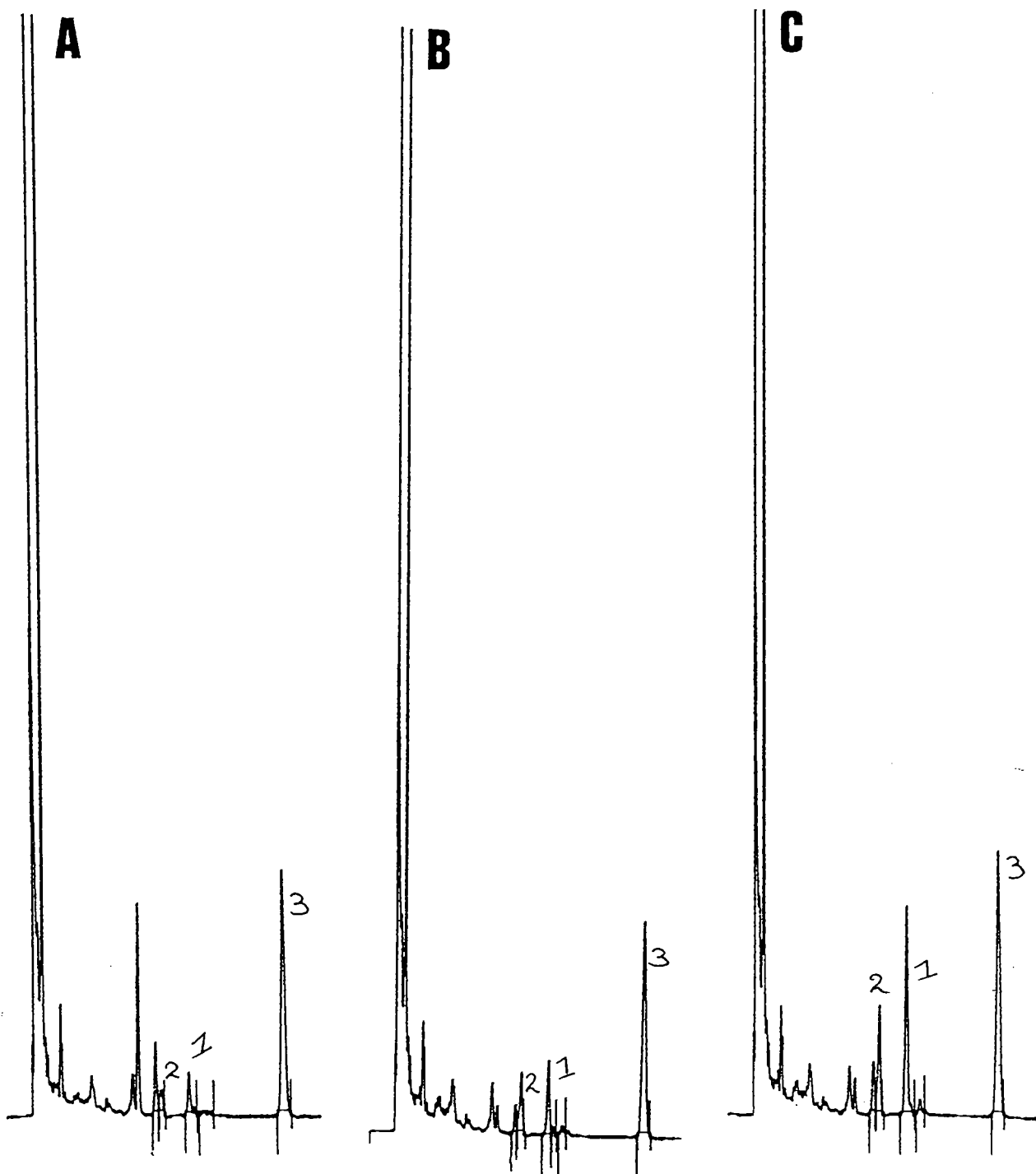


Figure 4 : GB-specimen chromatograms of the reference solutions with concentrations (in $\mu\text{g/g}$ banana)

a.	R23979 : 0.05	R14821 : 0.04	(R30617 : 1)
b.	R23979 : 0.10	R14821 : 0.09	(R30617 : 1)
c.	R23979 : 0.50	R14821 : 0.18	(R30617 : 1)

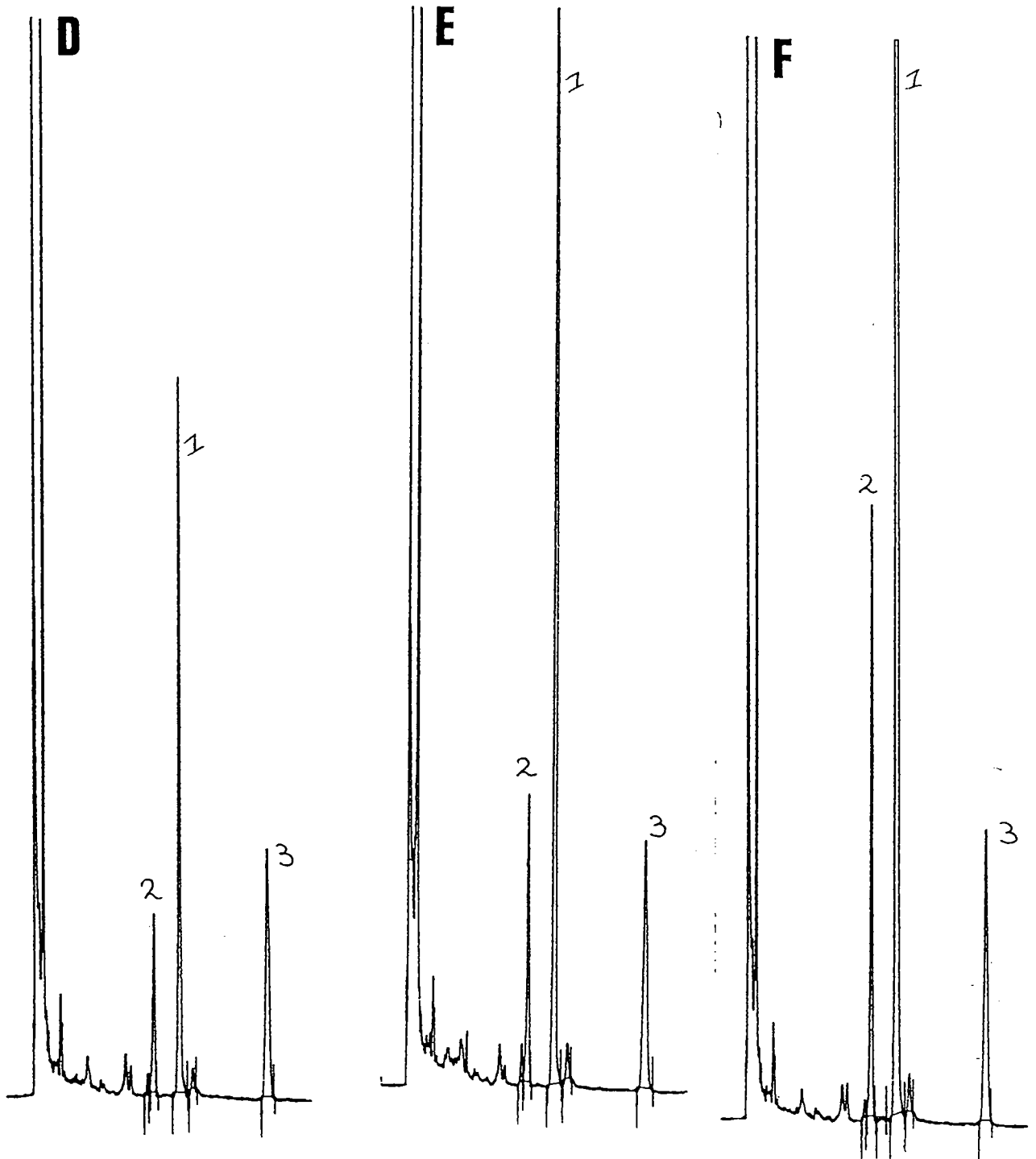


Figure 4 continued : GB-specimen chromatograms of the reference solutions with concentrations (in $\mu\text{g/g}$ banana)

d.	R23979 : 1.01	R14821 : 0.27	R30617 : 1
e.	R23979 : 2.01	R14821 : 0.36	R30617 : 1
f.	R23979 : 6.04	R14821 : 0.53	R30617 : 1



Figure 5 : YB-specimen chromatogram of blank material demonstrating the selectivity of the method
a. without IS-addition
b. with IS-addition

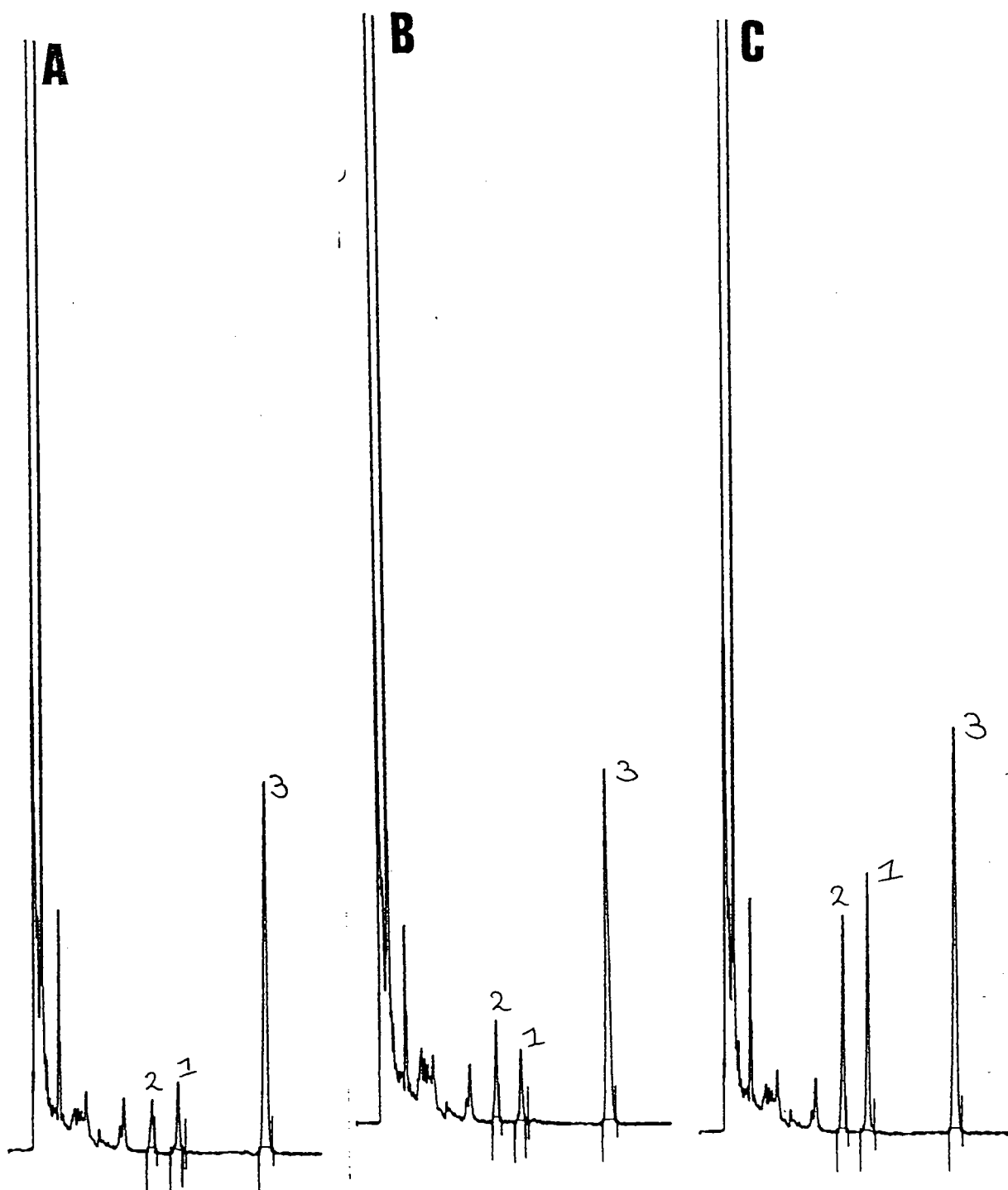


Figure 6 : YB-specimen chromatograms of the reference solutions with concentrations (in $\mu\text{g/g}$ banana)

a.	R23979 : 0.05	R14821 : 0.04	(R30617 : 1)
b.	R23979 : 0.10	R14821 : 0.09	(R30617 : 1)
c.	R23979 : 0.50	R14821 : 0.18	(R30617 : 1)

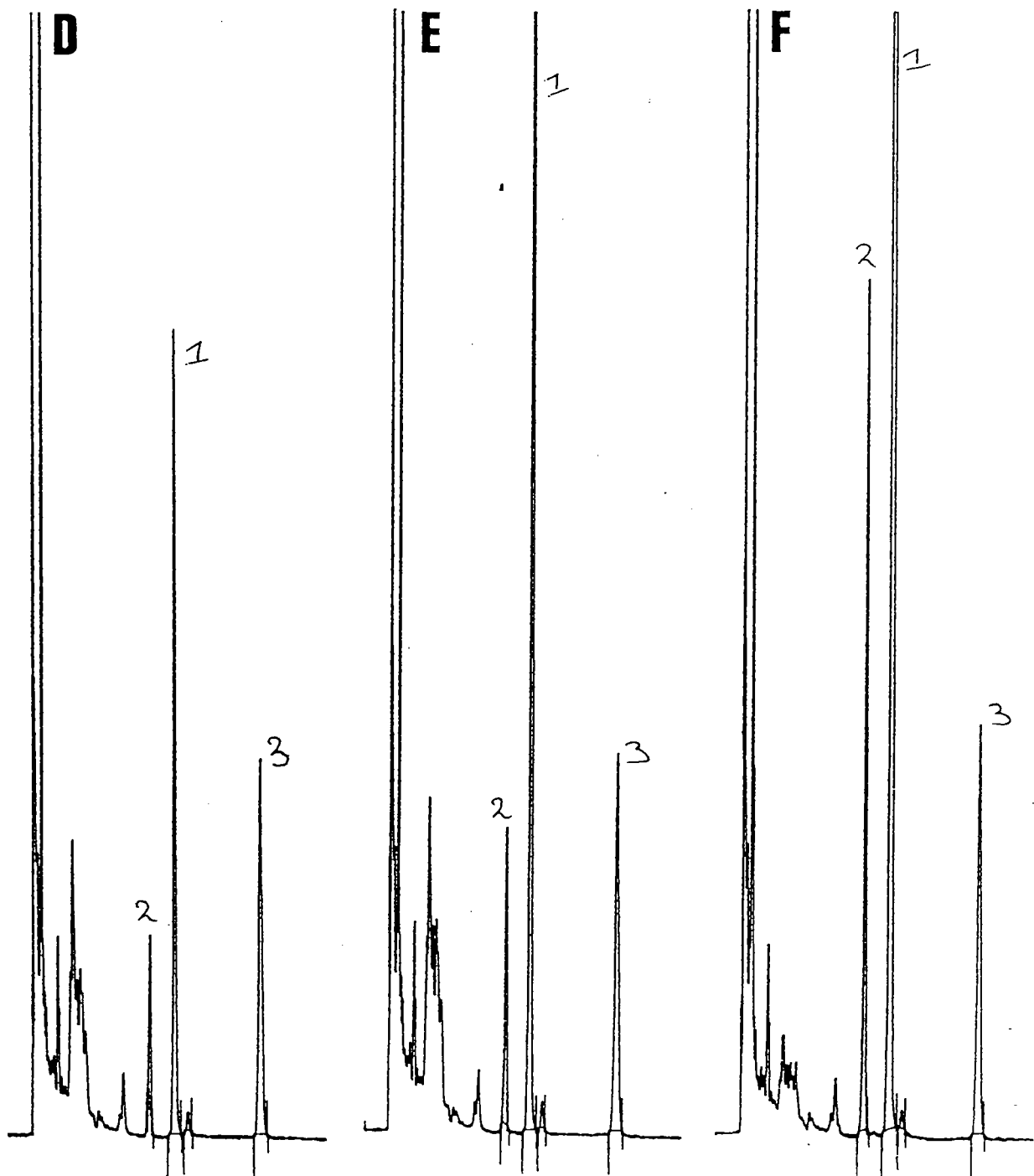


Figure 6 continued : YB-specimen chromatograms of the reference solutions with concentrations (in $\mu\text{g/g}$ banana)

d.	R23979 : 1.01	R14821 : 0.27	R30617 : 1
e.	R23979 : 2.01	R14821 : 0.36	R30617 : 1
f.	R23979 : 6.04	R14821 : 0.53	R30617 : 1

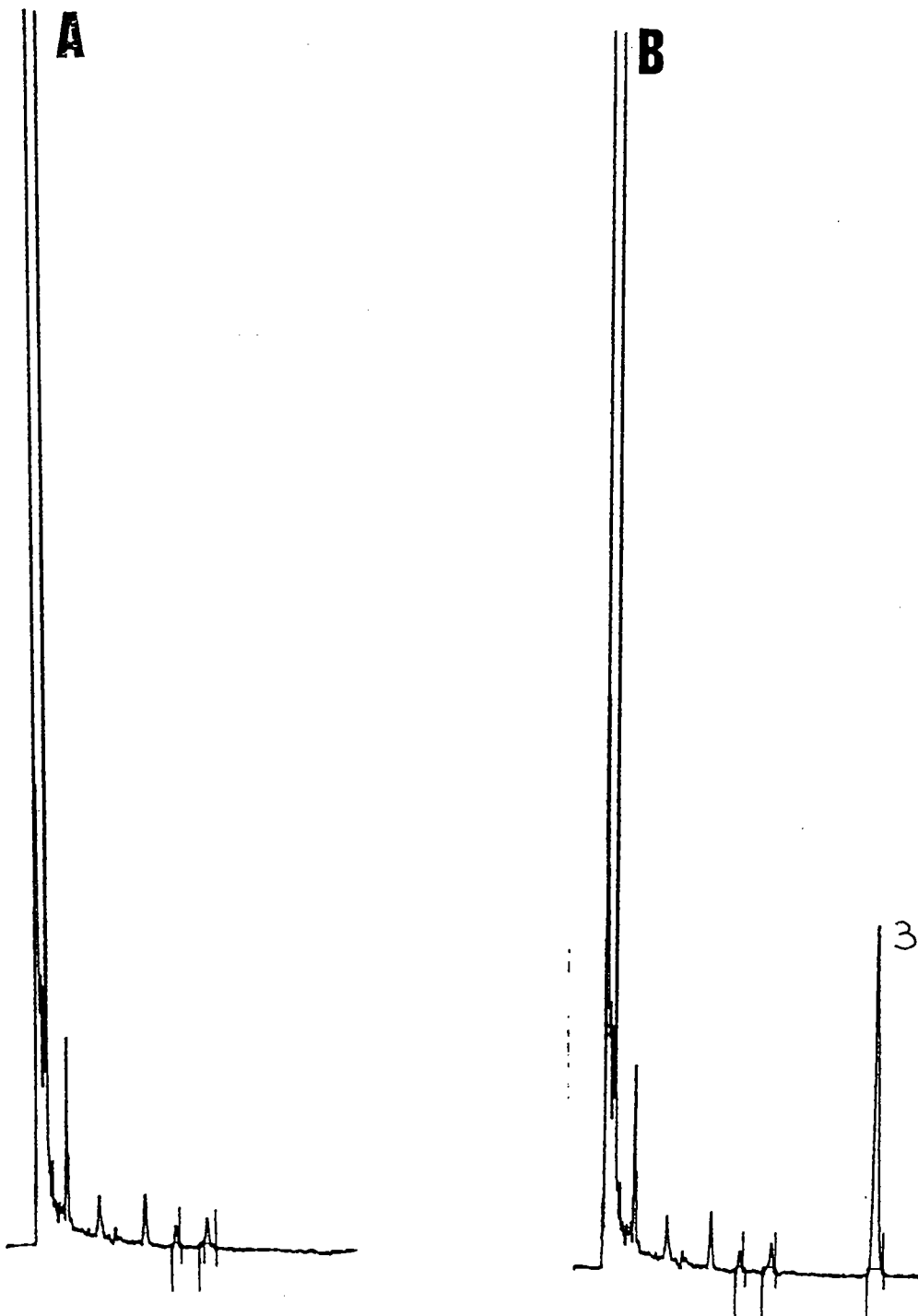


Figure 7 : EP-specimen chromatograms of blank material demonstrating the selectivity of the method
a. without IS-addition
b. with IS-addition



Figure 8 : EP-specimen chromatograms of the reference solutions with concentrations (in $\mu\text{g/g}$ banana)

a.	R23979 : 0.05	R14821 : 0.04	R30617 : 1
b.	R23979 : 0.08	R14821 : 0.07	R30617 : 1
c.	R23979 : 0.10	R14821 : 0.09	R30617 : 1

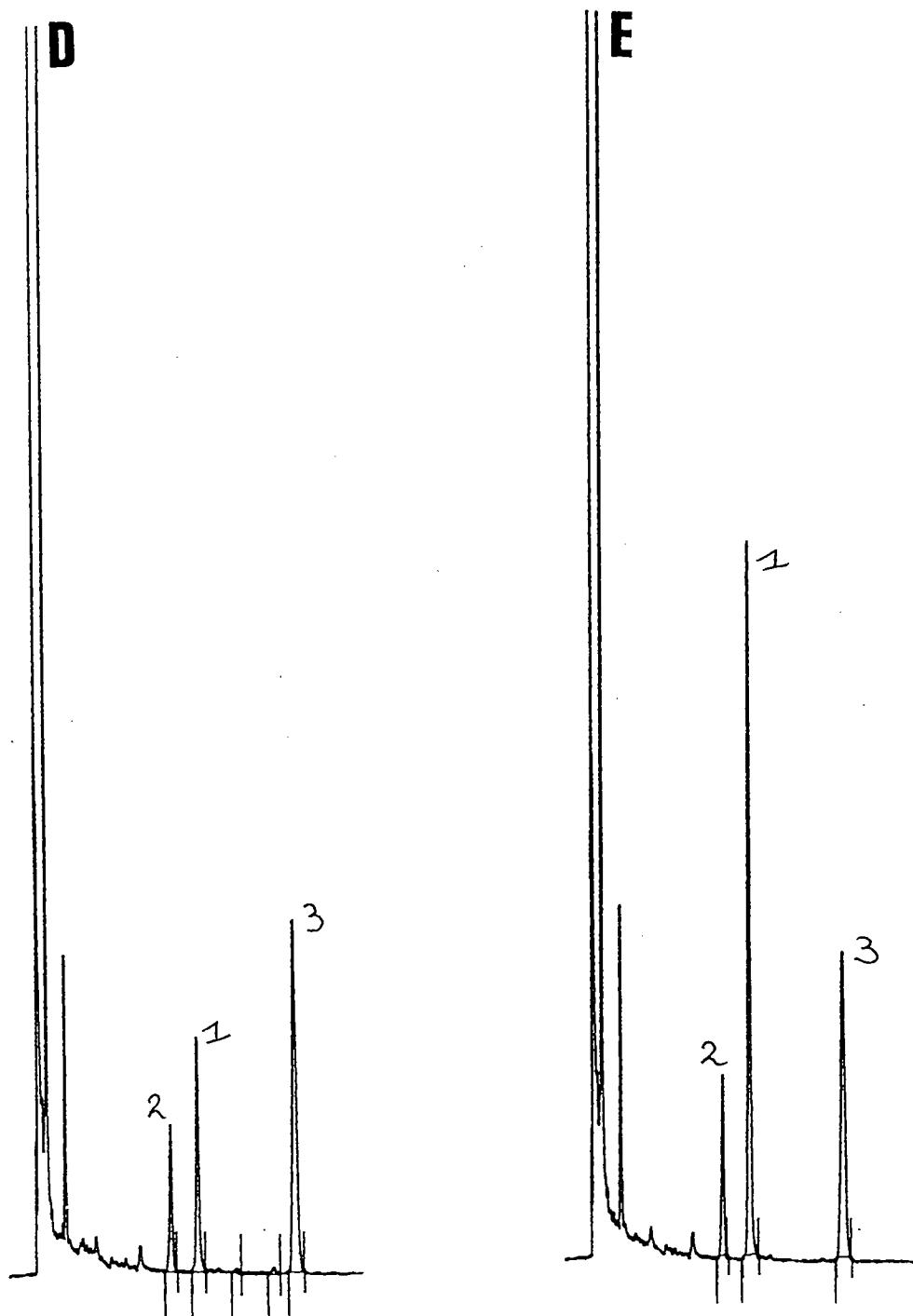


Figure 8 continued : EP-specimen chromatograms of the reference solutions with concentrations (in $\mu\text{g/g}$ banana)

d.	R23979 : 0.50	R14821 : 0.13	R30617 : 1
e.	R23979 : 1.01	R14821 : 0.18	R30617 : 1

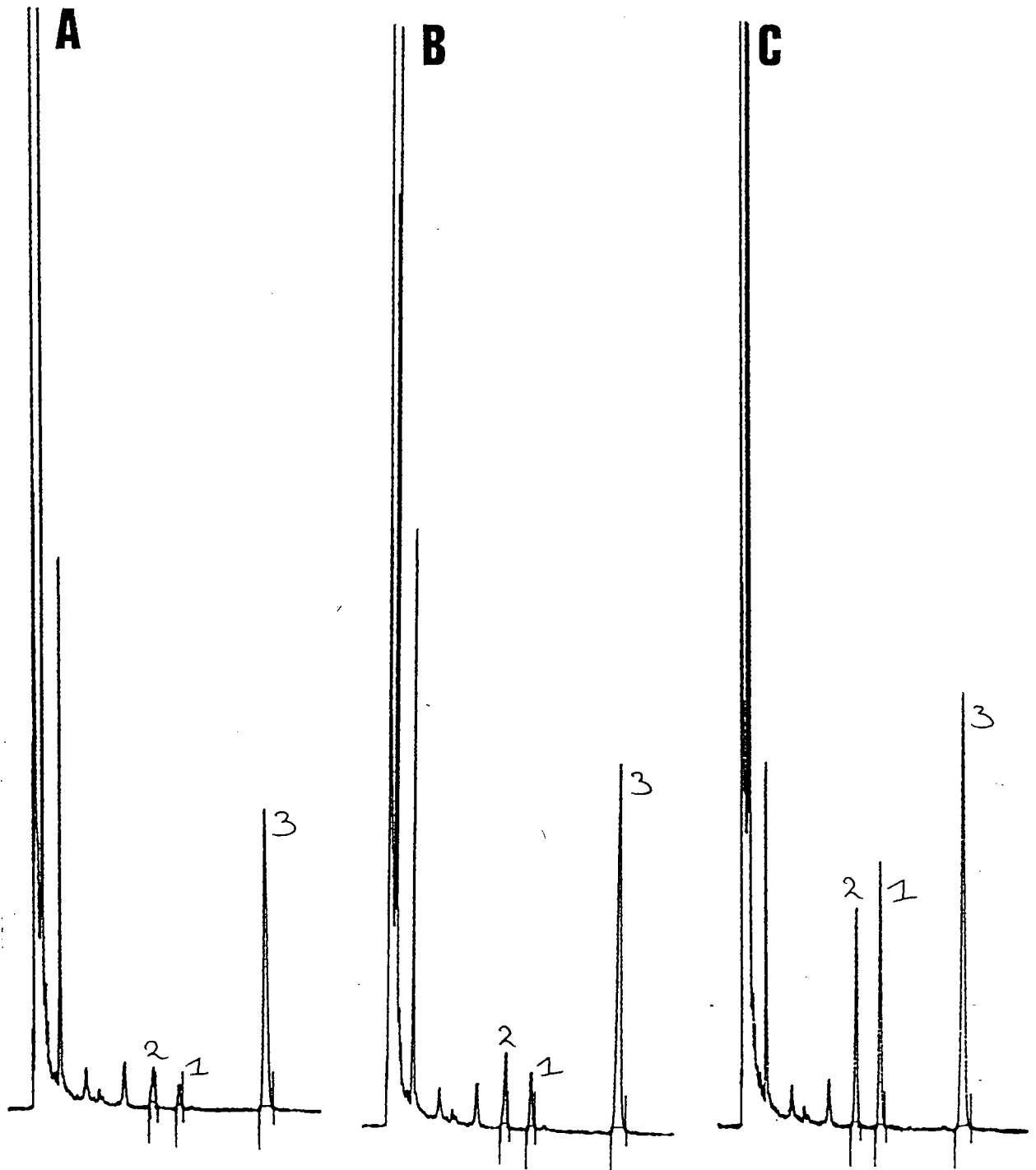


Figure 9 : Specimen chromatograms of standard dilutions of R23979 and R14821 in the analyte solvent with concentrations (expressed as $\mu\text{g/g}$ banana)

a.	R23979 : 0.05	R14821 : 0.04	R30617 : 1
b.	R23979 : 0.10	R14821 : 0.09	R30617 : 1
c.	R23979 : 0.50	R14821 : 0.18	R30617 : 1

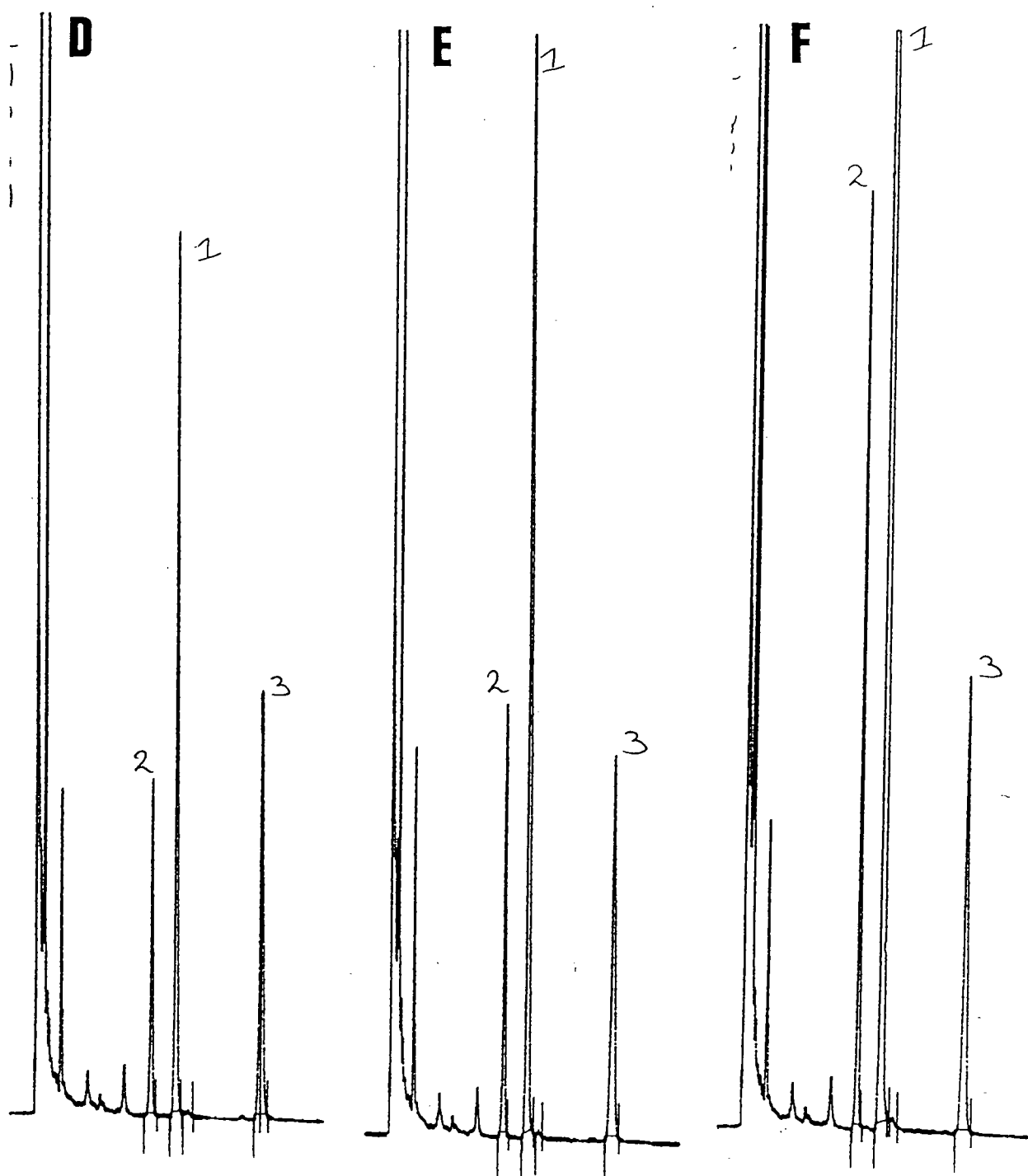


Figure 9 continued : Specimen chromatograms of standard dilutions of R23979 and R14821 in the analyte solvent with concentrations (expressed as $\mu\text{g/g}$ banana)

d.	R23979 : 1.01	R14821 : 0.27	R30617 : 1
e.	R23979 : 2.01	R14821 : 0.36	R30617 : 1
f.	R23979 : 6.04	R14821 : 0.53	R30617 : 1

Figure 10 : Linearity graphs, R23979-determination with ES-method
(x = log X, y = log Y)

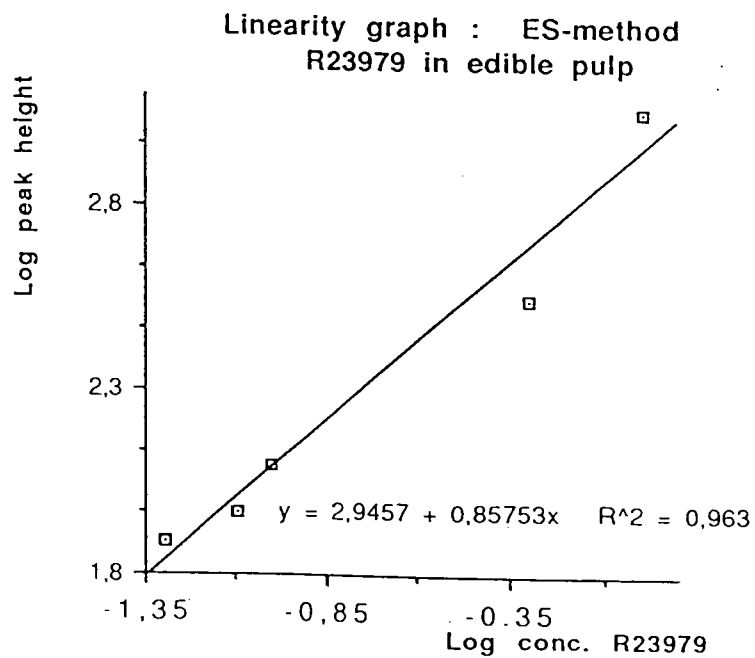
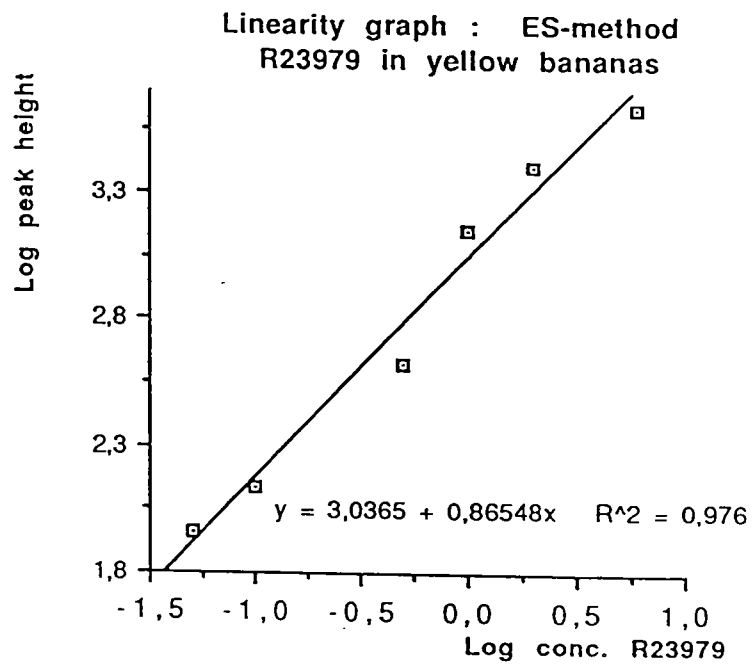
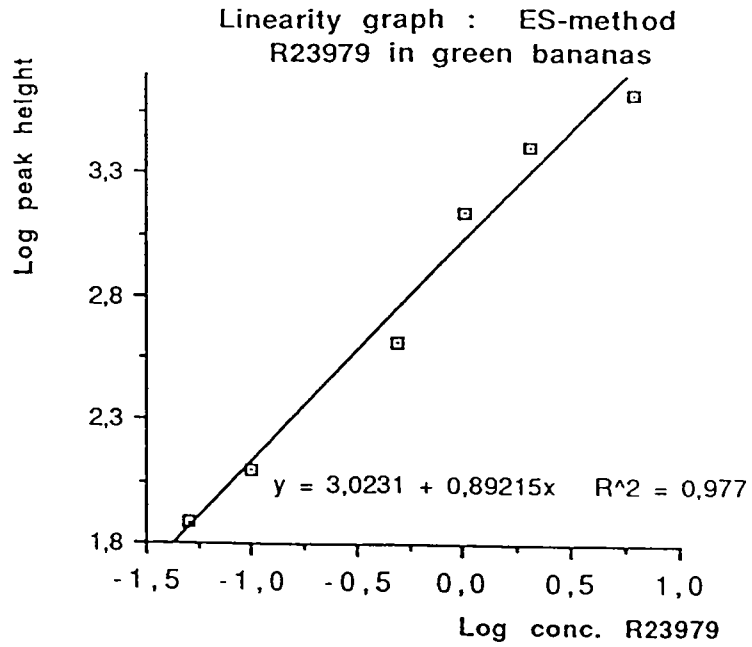


Figure 11 : Linearity graphs, R14821-determination with ES-method
(x = logX, y = logY)

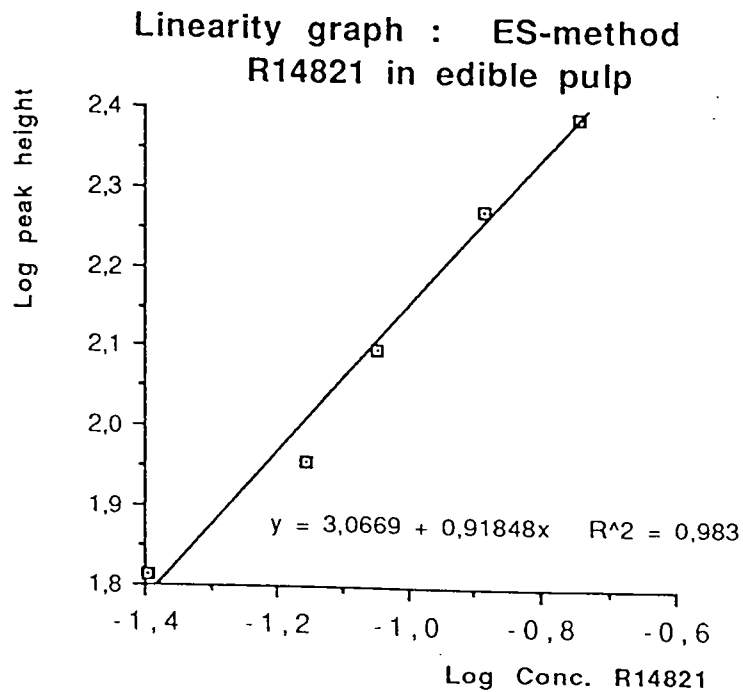
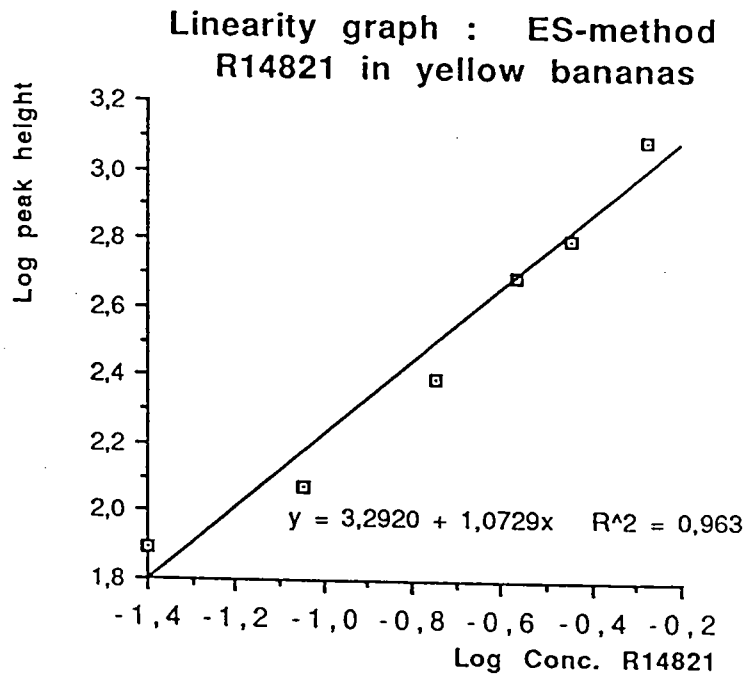
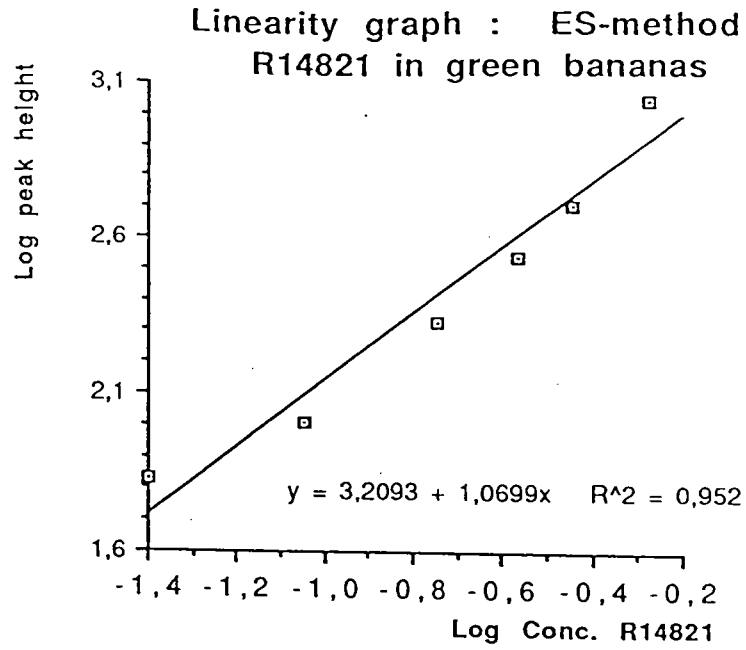


Figure 12 : Linearity graphs, R23979-determination with IS-method
(x = log X, y = log Y)

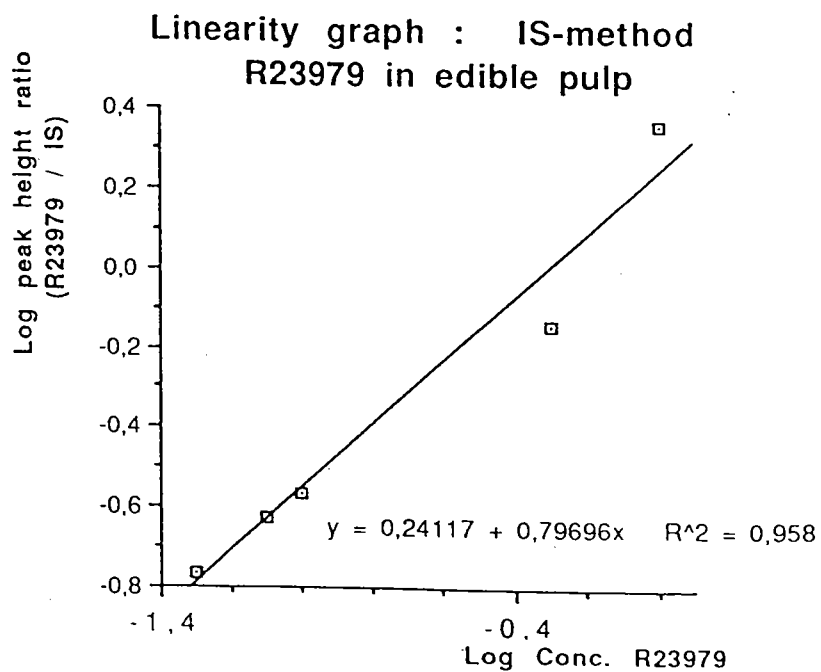
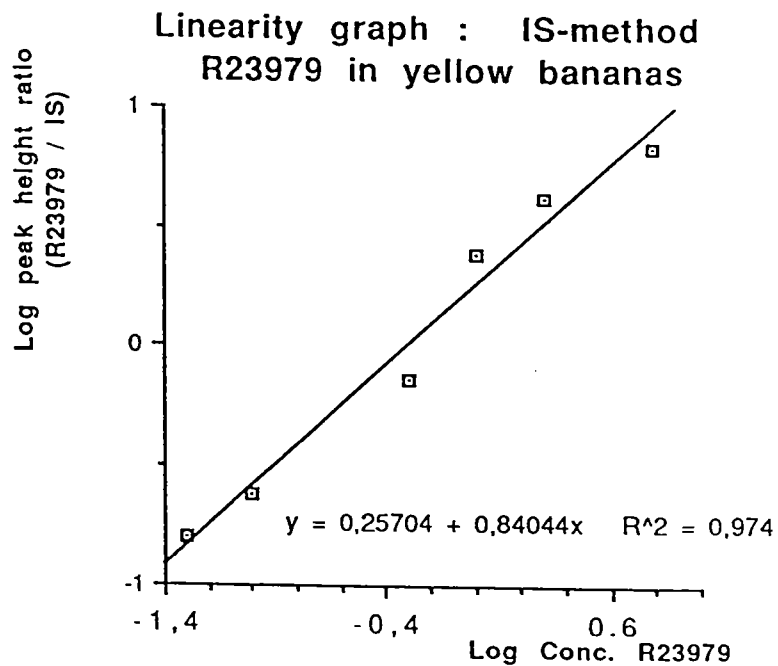
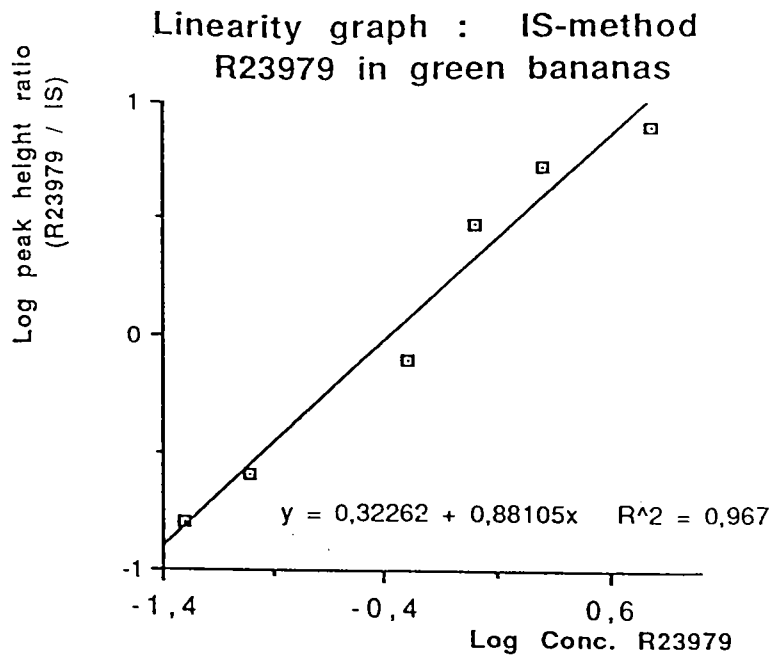


Figure 13 : Linearity graphs, R14821-determination with IS method
(x = log X, y = log Y)

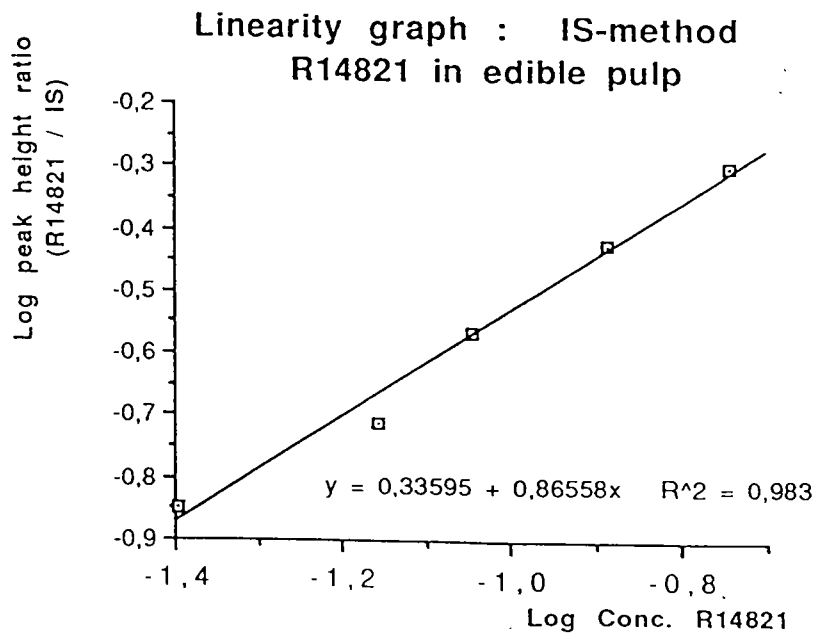
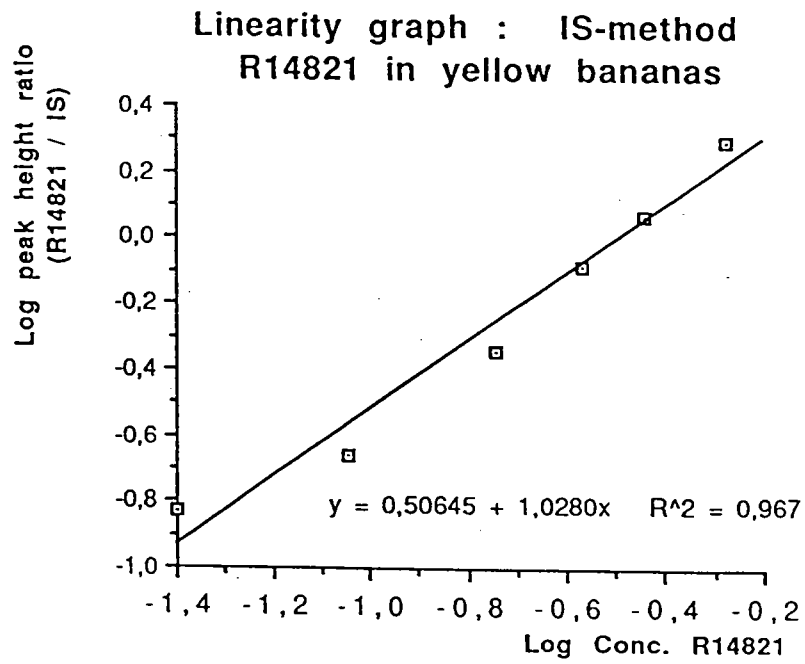
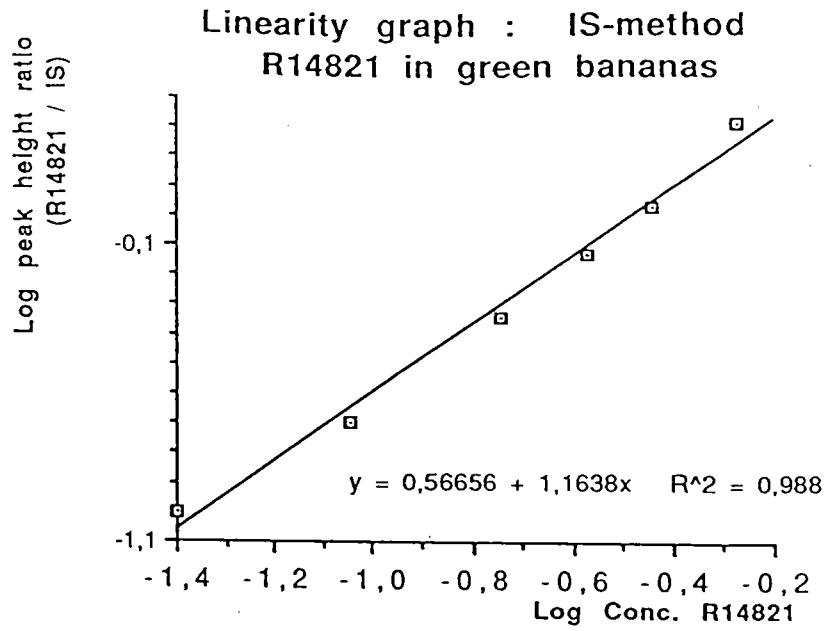


Table 1a : Recovery (ES-method), t-tests

	R23979				R14821			
t-test for significance of difference of two slopes (2-tail test with 95 % confidence level)								
	slopes		t-values		slopes		t-values	
	lin.	rec.	calc.	critic.	lin.	rec.	calc.	critic.
GB	0.8922	0.8839	0.083	2.228	1.0699	1.1485	0.490	2.228
YB	0.8655	0.8792	0.147	2.228	1.0729	0.9855	0.551	2.228
EP	0.8575	0.8141	0.307	2.306	0.9185	0.9103	0.061	2.306
t-test for significance of difference of two y-intercepts (2-tail test with 95 % confidence level)								
	y-intercept		t-values		y-intercept		t-values	
	lin.	rec.	calc.	critic.	lin.	rec.	calc.	critic.
GB	3.0231	3.0242	0.020	2.228	3.2093	3.2640	1.401	2.228
YB	3.0365	3.0463	0.193	2.228	3.2920	3.2281	1.655	2.228
EP	2.9457	2.9584	0.142	2.306	3.0669	3.1230	2.337	2.306

Table 1b : Practical recovery (ES-method) results

commodity	R23979		R14821	
	mean	range	mean	range
GB	100 %	93 - 108 %	101 %	93 - 122 %
YB	99 %	93 - 103 %	102 %	77 - 139 %
EP	90 %	84 - 96 %	86 %	81 - 92 %

Table 2a : Recovery (IS-method), t-tests

	R23979				R14821			
t-test for significance of difference of two slopes (2-tail test with 95 % confidence level)								
	slopes		t-values		slopes		t-values	
	lin.	rec.	calc.	critic.	lin.	rec.	calc.	critic.
GB	0.8811	0.8602	0.182	2.228	1.1638	1.1088	0.501	2.228
YB	0.8404	0.8447	0.041	2.228	1.0280	0.9417	0.559	2.228
EP	0.7970	0.7996	0.021	2.306	0.8656	0.8726	0.063	2.306
t-test for significance of difference of two y-intercepts (2-tail test with 95 % confidence level)								
	y-intercept		t-values		y-intercept		t-values	
	lin.	rec.	calc.	critic.	lin.	rec.	calc.	critic.
GB	0.3226	0.3357	0.309	2.228	0.5666	0.5482	0.688	2.228
YB	0.2570	0.2329	0.640	2.228	0.5065	0.3886	3.128	2.228
EP	0.2412	0.2070	0.995	2.306	0.3360	0.3471	0.466	2.306

Table 2b : Practical recovery (IS-method) results

commodity	R23979		R14821	
	mean	range	mean	range
GB	96 %	88 - 99 %	95 %	83 - 100 %
YB	107 %	100 - 113 %		91 - 151 %
EP	109 %	107 - 116 %	99 %	92 - 102 %

Table 3a : Blank control of the ES-method, t-tests

	R23979				R14821			
t-test for significance of difference of two slopes (2-tail test with 95 % confidence level)								
	slopes		t-values		slopes		t-values	
	rec.	bc.	calc.	critic.	rec.	bc.	calc.	critic.
GB	0.8839	0.9965	0.972	2.228	1.1485	1.1834	0.260	2.228
YB	0.8792	0.9080	0.272	2.228	0.9855	1.0766	0.658	2.228
EP	0.8141	1.1720	2.622	2.306	0.9103	0.9731	0.474	2.306
t-test for significance of difference of two y-intercepts (2-tail test with 95 % confidence level)								
	y-intercept		t-values		y-intercept		t-values	
	rec.	bc.	calc.	critic.	rec.	bc.	calc.	critic.
GB	3.0242	3.0206	0.084	2.228	3.2640	3.4871	6.807	2.228
YB	3.0463	3.0700	0.607	2.228	3.2281	3.4352	6.143	2.228
EP	2.9584	3.0970	3.286	2.306	3.1230	3.1484	1.237	2.306

Table 3b : Blank control of the IS-method, t-tests

	R23979				R14821			
t-test for significance of difference of two slopes (2-tail test with 95 % confidence level)								
	slopes		t-values		slopes		t-values	
	rec.	bc.	calc.	critic.	rec.	bc.	calc.	critic.
GB	0.8602	0.9414	0.698	2.228	1.1088	1.0887	0.170	2.228
YB	0.8447	0.8239	0.164	2.228	0.9417	1.0386	0.647	2.228
EP	0.7996	1.1371	3.746	2.306	0.8726	0.8439	0.152	2.306
t-test for significance of difference of two y-intercepts (2-tail test with 95 % confidence level)								
	y-intercept		t-values		y-intercept		t-values	
	rec.	bc.	calc.	critic.	rec.	bc.	calc.	critic.
GB	0.3357	0.1274	4.838	2.228	0.5482	0.5362	0.415	2.228
YB	0.2329	0.2093	0.505	2.228	0.3886	0.5310	3.903	2.228
EP	0.2070	0.3834	7.235	2.306	0.3471	0.3167	1.222	2.306

Table 4 : Precision (repeatability) for the ES-method

n	GB		YB		EP	
	Peak height	Peak height	Peak height	Peak height	Peak height	Peak height
	R23979	R14821	R23979	R14821	R23979	R14821
1	527	D/G	576	625	604	336
2	551	427	592	616	G	337
3	547	436	592	520	661	440
4	535	424	636	575	649	436
5	539	426	608	498	627	414
6	515	414	612	512	588	351
7	499	424	563	508	619	380
8	543	439	572	395	584	440
9	558	430	539	476	641	472
10	500	423	564	480	648	519
11	559	D/G			631	516
n	11	9	11	11	10	11
CI	534 ± 14	427 ± 6	585 ± 20	521 ± 50	625 ± 19	422 ± 44
Range	499 - 559	414 - 439	539 - 636	395 - 625	584 - 661	336 - 519
SD	21.35	7.40	28.38	69.22	26.30	65.63
RSD	4.00 %	1.73 %	4.85 %	13.30 %	4.21 %	15.56 %

with
$$CI = \bar{x} \pm t \cdot \frac{S}{\sqrt{n}}$$

\bar{x} = mean value of x (peak height)

t = critical value of two sided t-test for (n-1) degrees of freedom and a confidence level of 95 %

S = Standard Deviation (SD)

CI = Confidence Interval (peak height)

Table 5 : Precision (repeatability) for the IS-method

n	GB		YB		EP	
	Peak height ratio	Peak height ratio	Peak height ratio	Peak height ratio	Peak height ratio	Peak height ratio
	R23979	R14821	R23979	R14821	R23979	R14821
1	1.864	D/G	1.861	2.018	1.697	0.944
2	1.854	1.436	1.897	1.974	1.709	1.120
3	1.828	1.458	1.865	1.638	1.811	1.205
4	1.937	1.536	1.873	1.692	1.810	1.216
5	1.860	1.470	1.924	1.574	1.761	1.162
6	1.892	1.520	1.937	1.620	1.673	0.997
7	1.826	1.553	1.881	1.699	1.757	1.080
8	1.802	1.457	1.919	1.326	1.666	1.255
9	1.998	1.539	1.896	1.676	1.728	1.272
10	1.946	1.646	1.952	1.661	1.688	1.352
11	1.939	1.592			1.646	1.347
n	11	10	10	11	11	11
CI	1.886 ± 0.040	1.521 ± 0.048	1.901 ± 0.022	1.688 ± 0.143	1.722 ± 0.040	1.177 ± 0.087
Range	1.802 - 1.998	1.436 - 1.646	1.861 - 1.952	1.326 - 2.018	1.646 - 1.811	0.944 - 1.352
SD	0.06	0.07	0.03	0.20	0.06	0.13
RSD	3.26 %	4.39 %	1.65 %	11.57 %	3.27 %	11.29 %

with
$$CI = \bar{x} \pm t \cdot \frac{S}{\sqrt{n}}$$

\bar{x} = mean value of x (peak height)

t = critical value of two sided t-test for (n-1) degrees of freedom and a confidence level of 95 %

S = Standard Deviation (SD)

CI = Confidence Interval (peak height)

Table 6 : Stability of analyte solutions (ES-method) after a 24 hours storage period at ambient lab temperature.

Results are expressed as percentage of R23979/R14821 found after 24 hours against the concentrations at time zero.

	Low level		Medium level		High level	
	R23979 0.05 µg/g	R14821 0.04 µg/g	R23979 1.01 µg/g	R14821 0.27 µg/g	R23979 6.04 µg/g	R14821 0.53 µg/g
Stored in extraction solvent (heptane isoamyl alcohol)						
GB	107	109	99	92	101	100
YB	101	100	99	120	102	96
EP	117	137	98	146	104	122
Stored in the derivatization mixture (toluene/BSA)						
GB	92	133	94	122	101	132
YB	98	112	97	110	102	130
EP	95	125	89	124	102	113

Table 7 : Stability of analyte solutions (IS-method) after a 24 hours storage period at ambient temperature
 Results are expressed as percentage of R23979/R14821 found after 24 hours against the concentrations at time zero.

	Low level		Medium level		High level	
	R23979 0.05 µg/g	R14821 0.04 µg/g	R23979 1.01 µg/g	R14821 0.27 µg/g	R23979 6.04 µg/g	R14821 0.53 µg/g
Stored in extraction solvent (heptane isoamyl alcohol)						
GB	104	114	99	93	100	95
YB	100	103	103	108	104	104
EP	112	138	101	143	103	125
Stored in the derivatization mixture (toluene/BSA)						
GB	100	139	102	134	102	134
YB	101	117	95	108	98	125
EP	107	142	100	138	102	123

Protocol/Study number: AGR16 (December 17, 1991)

Method validation: comparison ES-method & IS-method

Analyst: Marc Nuyts

Reference: Laboratory Notebook III/65

Date: February 28, 1992

**Table 8 : Comparison of both methods with Wilcoxon T-test (two sided test with confidence level of 95 %).
Commodity : green bananas**

Non-parametric tests for the comparison of methods (Wilcoxon T-test)											
(Confidence level for Wilcoxon T-test = 95% Two-sided test)											
Chemometrics (p51)											
Green bananas											
N	Conc. R23979 ES-method (x)	Conc. R23979 IS-method (y)	di = X(ES)-X(IS)	Rank	Signed rank	N	Conc. R14821 ES-method (x)	Conc. R14821 IS-method (y)	di = X(ES)-X(IS)	Rank	Signed rank
1	0.467	0.478	-0.011	9.5	-9.5	1	outlier	outlier			
2	0.468	0.484	-0.016	11	-11	2	0.180	0.170	0.010	6	6
3	0.482	0.485	-0.003	4	-4	3	0.184	0.173	0.011	7	7
4	0.493	0.492	0.001	1	1	4	0.179	0.182	-0.003	2	-1
5	0.501	0.493	0.008	8	8	5	0.180	0.174	0.006	5	6
6	0.505	0.494	0.011	9.5	9.5	6	0.175	0.180	-0.005	3.5	-3.5
7	0.508	0.502	0.006	5	5	7	0.179	0.184	-0.005	3.5	-3.5
8	0.512	0.514	-0.002	2.5	-2.5	8	0.185	0.172	0.013	8	8
9	0.516	0.514	0.002	2.5	2.5	9	0.181	0.182	-0.001	1	-2
10	0.523	0.516	0.007	6.5	6.5	10	0.178	0.195	-0.017	9	-7
11	0.523	0.530	-0.007	6.5	-6.5	11	outlier	0.188			
				T(crit.)	11					T(crit.)	6
				T+	32.5					T+	27
				T-	33.5					T-	17
				T	32.5					T	17

Summary	R23979	T > T(crit.)	null hypothesis accepted
T-test		no difference between the two methods	
Summary	R14821	T > T(crit.)	null hypothesis accepted
T-test		no difference between the two methods	

Protocol/Study number: AGR16 (December 17, 1991)

**Table 9 : Comparison of both methods with Wilcoxon T-test (two sided test with confidence level of 95 %)
Commodity : yellow bananas**

Method validation: comparison ES-method & IS-method

Analyst: Marc Nuyts

Reference: Laboratory Notebook III/65

Date: February 28, 1992

Non-parametric tests for the comparison of methods (Wilcoxon T-test)										
(Confidence level for Wilcoxon T-test = 95% Two-sided test)										
Yellow bananas										
Conc. R23979					Conc. R14821					
N	ES-method (x)	IS-method (y)	$d_i = X(ES)-X(IS)$	Signed rank	N	ES-method (x)	IS-method (y)	$d_i = X(ES)-X(IS)$	Signed rank	
1	0.492	0.490	0.002	1	1	0.216	0.215	0.001	1	
2	0.506	0.499	0.007	2	2	0.213	0.211	0.002	2	
3	0.506	0.491	0.015	6	3	0.180	0.175	0.005	6.5	
4	0.544	0.493	0.051	10	4	0.199	0.180	0.019	10	
5	0.520	0.506	0.014	4.5	5	0.172	0.168	0.004	4	
6	0.523	0.510	0.013	3	6	0.177	0.173	0.004	4	
7	0.481	0.495	-0.014	4.5	7	0.176	0.181	-0.005	6.5	
8	0.489	0.505	-0.016	7	8	0.137	0.141	-0.004	4	
9	0.461	0.499	-0.038	9	9	0.165	0.179	-0.014	9	
10	0.482	0.513	-0.031	8	10	0.166	0.177	-0.011	8	
				T(+)					T(+)	27.5
				T(-)					T(-)	27.5
				T					T	27.5
				T(crit.)					T(crit.)	8

Summary	R23979	T > T(crit.)	null hypothesis accepted
T-test		no difference between the two methods	
Summary	R14821	T > T(crit.)	null hypothesis accepted
T-test		no difference between the two methods	

Protocol/Study number: AGR16 (December 17, 1991)

Method validation: comparison ES-method & IS-method

Analyst: Marc Nuyts

Reference: Laboratory Notebook III/65

Date: February 28, 1992

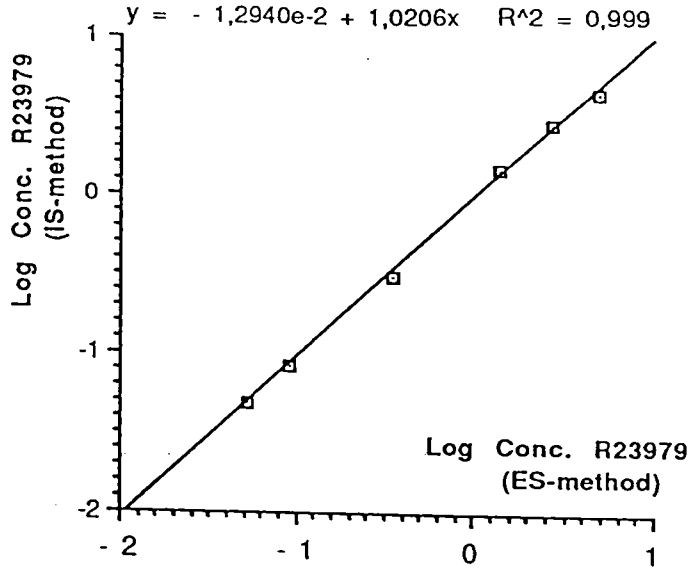
**Table 10 : Comparison of both methods with Wilcoxon T-test (two sided test with confidence level of 95 %)
Commodity : edible pulp**

Non-parametric tests for the comparison of methods (Wilcoxon T-test)											
(Confidence level for Wilcoxon T-test = 95% Two-sided test)											
Edible Pulp											
Chemometrics (p51)											
Conc. R23979					Conc. R14821						
N	ES-method (x)	IS-method (y)	di = X(ES)-X(IS)	Rank	Signed rank	N	ES-method (x)	IS-method (y)	di = X(ES)-X(IS)	Rank	Signed rank
1	0.483	0.493	-0.010	4	-4	1	0.143	0.144	-0.001	2.5	-2.5
2	outlier	0.496	outlier			2	0.144	0.171	-0.027	11	-11
3	0.529	0.526	0.003	1	1	3	0.188	0.184	0.004	6.5	6.5
4	0.519	0.526	-0.007	2	-2	4	0.186	0.186	0.000	1	1
5	0.502	0.511	-0.009	3	-3	5	0.177	0.178	-0.001	2.5	-2.5
6	0.470	0.486	-0.016	7	-7	6	0.150	0.153	-0.003	4.5	-4.5
7	0.495	0.510	-0.015	6	-6	7	0.162	0.165	-0.003	4.5	-4.5
8	0.467	0.484	-0.017	8	-8	8	0.188	0.192	-0.004	6.5	-6.5
9	0.513	0.502	0.011	5	5	9	0.201	0.195	0.006	8	8
10	0.518	0.490	0.028	10	10	10	0.221	0.207	0.014	9.5	9.5
11	0.505	0.478	0.027	9	9	11	0.220	0.206	0.014	9.5	9.5
				T(+)	25					T(+)	34.5
				T(-)	30					T(-)	31.5
				T	25					T	31.5
				T(crit.)	8					T(crit.)	11

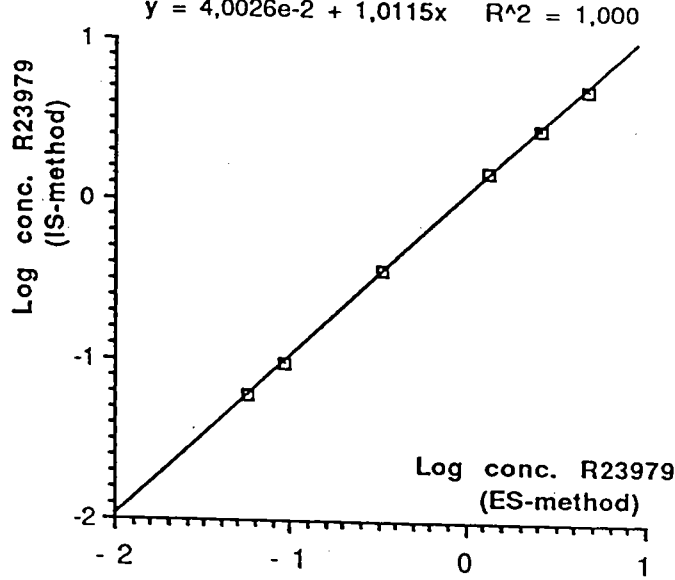
Summary	R23979	T > T(crit.)	null hypothesis accepted	Summary	R14821	T > T(crit.)	null hypothesis accepted
T-test		no difference between the two methods		T-test		no difference between the two methods	

Figure 14 : Comparison of both methods (R23979-determination) by linear regression (x = logX, y = logY)

Green Bananas : R23979-determination



Yellow bananas : R23979-determination



Edible Pulp : R23979-determination

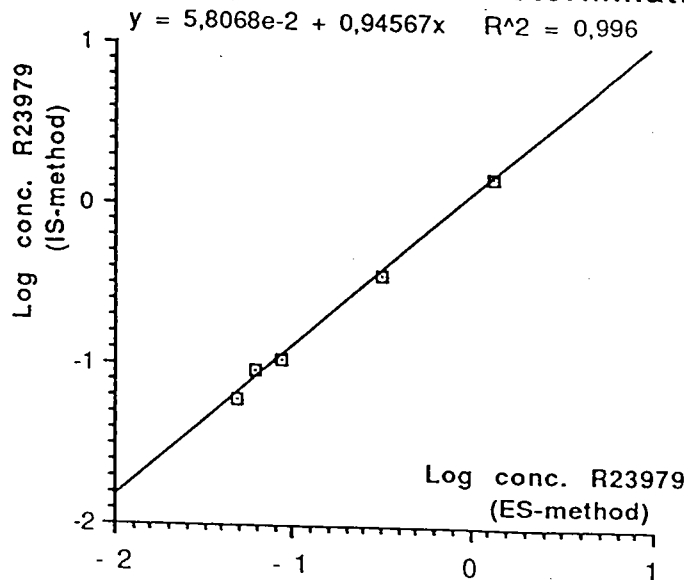
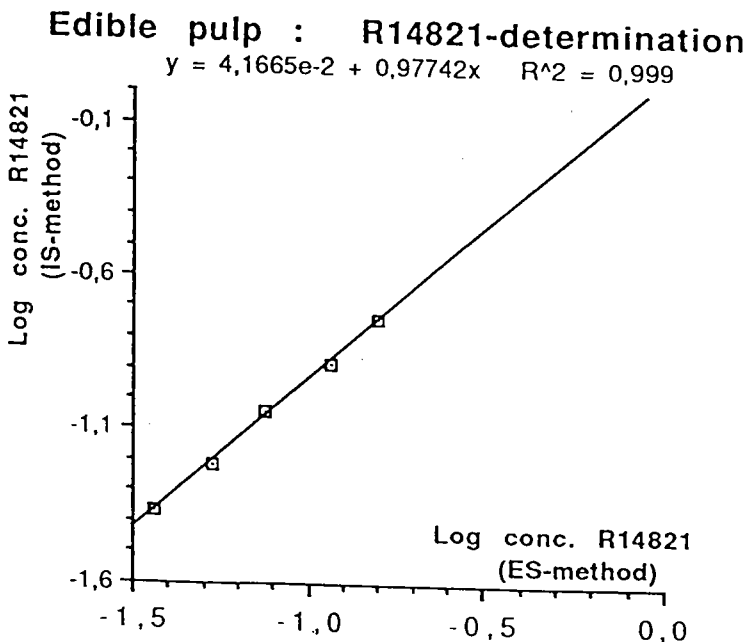
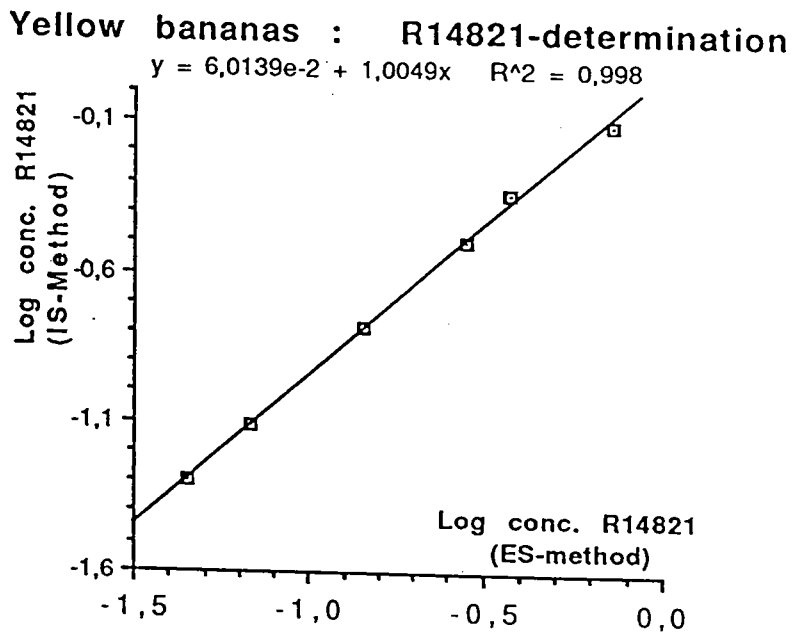
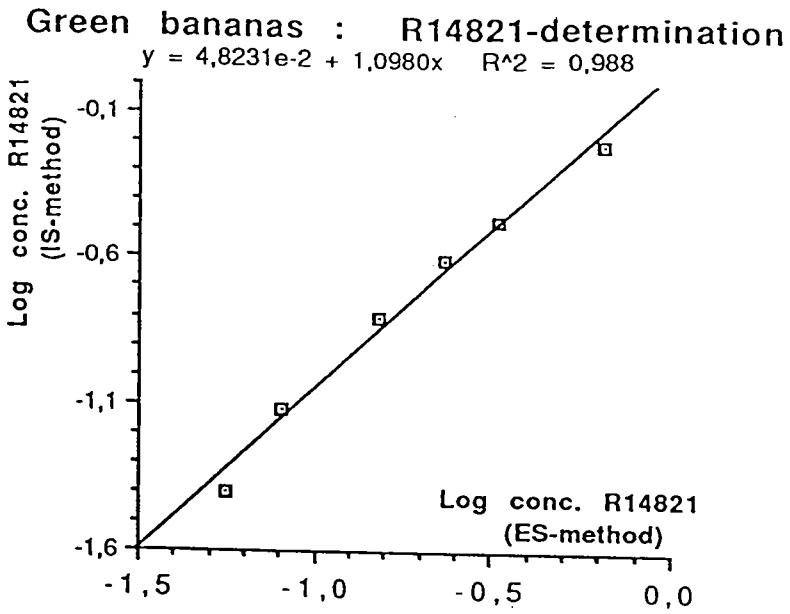


Figure 15 : Comparison of both methods (R14821-determination) by linear regression (x = logX, y = logY)



171-4(c) & (d) Residue Analytical Method

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?

1. List of equipment, reagents and standards provided ^{no} (along with U.S. sources/suppliers of same)
2. Instrumentation and operating conditions described
3. Detailed description of each step in procedure to enable use by competent analyst unfamiliar with method
4. Discrete response for analyte
5. Control values reasonably low compared to tolerance
6. Adequate recoveries (generally $\geq 70\%$) obtained for fortifications at the tolerance level
7. Recoveries don't vary significantly from sample to sample
8. Evidence that aged residues extracted by procedure (may reference work in metabolism studies)
9. Data showing that method releases and recovers bound residues (if latter of toxicological concern)
10. Requirements for regulatory methods (as opposed to those used only for collecting residue data)
 - Does not require untreated commodity as blank
 - Does not require internal or procedural standard to correct for recoveries (Addition of internal standard in final step just prior to injection is acceptable for calibration of retention times. However, use of internal standard throughout entire procedure to correct for recoveries is not acceptable unless data are available on numerous samples of each matrix to show analyte and internal standard behave identically in each step.)
 - Does not require exotic equipment or reagents
 - Reasonably rapid in execution
 - Specific to measure residue in presence of other reasonably expected pesticides
 - Sensitive in relation to tolerance
 - Confirmatory method available
 - Method not claimed to be Confidential Business Information
 - Does not use hazardous reagents (justification needed for use of benzene as solvent or diazomethane as methylating agent)

Remark 8. : Storage stability study running.


Criteria marked with a * are supplemental and may not be required for every study.

GLP STATEMENT

This study meets the requirements of 40 CFR Part 160

PHOTOCOPY

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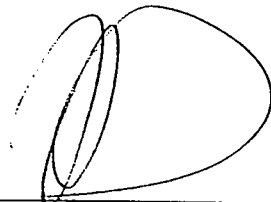
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Signature Date

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