US EPA ARCHIVE DOCUMENT

Attachment 4

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TM-402 (KBR 2738) VOLUME NUMBER

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72	72	

DATA REQUIREMENT(S)

EPA AND CALIFORNIA	CANADA
860.1340: Residue analytical method: Crops	07.2.2: Enforcement analytical methodology: Crops

TITLE

METHOD FOR THE DETERMINATION OF KBR 2738 RESIDUES IN PLANT MATERIAL BY HPLC

OBJECTIVE

Determination of the recovery and blank values of control samples and of detector linearity with the objective of validating residue analysis method 00362 for the determination of KBR 2738 in plant material

AUTHOR(S)

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REPORT COMPLETION DATE

JUNE 16, 1995

LABORATORY

BAYER AG
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BAYER REPORT NUMBER	TOMEN REPORT NUMBER	LABORATORY STUDY NUMBER
MR-144/94; METHOD 00362	TMN-019B	P 612 4 1008

SUBMITTER

TM-402 (KBR 2738) Fungicide Task Force
Comprised of Tomen Agro, Inc. and Bayer Corporation
EPA Consortium No. 69436

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METHOD FOR THE DETERMINATION OF KBR 2738 RESIDUES IN PLANT MATERIAL BY HPLC

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Company Agent: Desmond Byrne 30 Jun '97

Dr. Desmond Byrne
Manager, Registrations and Regulatory Affairs
Tomen Agro, Inc.

Date

For:

TM-402 (KBR 2738) Fungicide Task Force

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GLP Declaration (Statement of compliance)

Study number: **P 612 4 1008**

Test substance: KBR 2738

Study Director: Dr. J. Bachmann
PF-E/MR, Geb. 6610
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Tel. 02173/38-3886

Testing facility: Bayer AG
PF-E/MR, Geb. 6610
D-51368 Leverkusen

Study title: Method for the determination of KBR 2738 residues in plant material by HPLC

The present method validation is not a so-called *non-clinical experimental study* in the sense of § 19a (1) of the Chemicals Law (ChemG). Despite this, the study was carried out in accordance with the principles of Good Laboratory Practice (GLP) as set out in Appendix 1 ChemG together with § 19a (1) ChemG of 25.07.1994.

Study Director's signature:

_____ 21.06.95

(Dr. J. Bachmann) (Date)

Submitter:

Desmond Byrne 13/6/95

Dr. Desmond Byrne
Manager, Registrations and Regulatory Affairs
Tomen Agro, Inc.
For the TM-402 (KBR 2738) Fungicide Task Force

Date

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for original signature.

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Ministry of the Environment, Environmental Planning, and Agriculture of the State of North Rhine-Westphalia

Postal address: 40190 Düsseldorf

Reference: VI C 4 - 31.11.59/62

GLP Certificate

Bescheinigung

Hiermit wird bestätigt, daß die Prüfeinrichtung

Institut für Metabolismus-forschung und Rückstands-analytik (PF-E/MR)

in 51368 Leverkusen
(Ort, Anschrift)

der Bayer AG
(Firma)

am 24./25.6., 13./14.7., 23.8., 25.8., 30.11. - 3.12.1993
(Datum)

von der für die Überwachung zuständigen Behörde über die Einhaltung der Grundsätze der Guten Laborpraxis inspiziert worden ist.

Es wird hiermit bestätigt, daß folgende Prüfungen in dieser Prüfeinrichtung nach den Grundsätzen der Guten Labor-praxis durchgeführt wurden:

Certificate

It is hereby certified that the test facility

Institut für Metabolismus-forschung und Rückstands-analytik (PF-E/MR)

in 51368 Leverkusen
(location, address)

of Bayer AG
(company name)

on 24./25.6., 13./14.7., 23.8., 25.8.,30.11. - 3.12.1993
(date)

was inspected by the commpetent authority regarding compliance with the Principles of Good Laboratory Practice.

It is hereby certified that studies in this test facility are conducted in compliance with the Principles of Good Laboratory Practice:

Kategorie 1

Physikalisch-chemische Eigenschaften in Bezug auf Anwenderexposition

Gehaltsbestimmungen

Kategorie 5

Prüfungen zum Verhalten in Boden, Wasser und Luft; Bioakkumulation, Metabolimus

Kategorie 6

Rückstände von Pflanzenschutzmitteln, Veterinärprodukten und verwandten Chemikalien

Kategorie 8

Analytische Untersuchungen zu den Prüfkategorien 5, 6 und 9

Kategorie 9

Gewinnung von Rückstandsproben

Prüfungen auf pysikalisch-chemische Eigenschaften, soweit sie mit den Prüfungen nach Kategorie 5 in Zusammenhang stehen (z.B. Quantenausbeute, Hydrolysen-Konstante, Kd-Werte),

Prüfungen auf toxikologische Eigenschaften, soweit Radiotracer-Techniken zum Einsatz kommen (z.B. Pharmakokinetische Parameter, Schilddrüsen-Funktions-Studie,

Verhalten und Metabolismus in Tieren und Pflanzen.

Category 1

Physical-chemical properties with respect to user exposure

Determination of contents

Category 5

Studies regarding the behaviour of substances in soil, water, air; bioaccumulation and metabolism

Category 6

Residues of plant protection products, veterinary products and related chemicals

Category 8

Analytical studies on test categories 5, 6 and 9

Category 9

Collection of residue samples

Examination of physical and chemical properties with respect to studies listed in category 5 (e.g quantum yield, hydrolysis, Kd-values)

Examination of toxicological properties for the evaluation of which radio-tracer techniques are used (e.g. pharmacokinetic parameters, studies on thyroid-function),

Behaviour and metabolism in animals an plants

Düsseldorf, 18 October 1994

(Dr. Pietrzeniuk)

GLP REPORT	
Quality Assurance Declaration	
Report No.: **MR-144/94**	**Study No.:** **P 61241008**
Report title: Method for the determination of KBR 2738 residues in/on strawberries, cherries, peaches, plums, and grapes and in products manufactured from grapes by HPLC	
The study procedure was inspected and the report was checked by the Quality Assurance Unit of Bayer AG, PF-S. The dates of inspections are shown below:	
Date of Protocol Inspection:	Date of Report to Management:
July 28, 1994	July 29, 1994
Date of Study Inspection:	Date of Report to Management:
August 8, 1994 September 6, 1994	August 9, 1994 September 6, 1994
Date of Final Report Audit:	Date of Report to Management:
June 21, 1995	June 21, 1995

The results reported in this study accurately reflect the raw data.

L.P. Herrmann

Quality Assurance Unit, PF-S **Date: 21.06.95**

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for original signature.

Study schedule:

Start of the study:	August 1994
End of the experimental part:	December 1994
Preparation of the report:	June 1995

Acknowledgement:

I wish to thank Frau Kuppels for her friendly cooperation in the development of the present method.

Summary

The purpose of the present method is the determination of residues of the fungicidal active ingredient KBR 2738 in plant material.

After extraction with acetone and liquid-liquid partition on a ChemElut column using a cyclohexane-ethyl acetate mixture, KBR 2738 was determined by HPLC with electrochemical detection. It has been shown from the hydrodynamic voltammogram while keeping the other measurement parameters constant that optimal sensitivity for KBR 2738 is in the potential range from 0.8 to 0.85 V (vs. Ag/AgCl).

After swelling in water and extraction on a ChemElut column, material having a low water content — raisins, raisin waste, and dried grape marc — was further purified on a silica gel column with cyclohexane-ethyl acetate as the mobile phase.

The electrochemical detector has a linear response to KBR 2738 in terms of peak area versus quantity of substance for injected quantities in the range 1.25-50 ng.

After spiking with 0.02-2.0 mg/kg or 0.05-5.0 mg/kg, the recovery (individual values) for KBR 2738 in each of the sample materials was in the range 81-110%, with a mean of 95% (n = 108). The relative standard deviation over all recovery rates and sample materials was 5.7%.

The limit of quantitation LOQ (corresponding to the smallest amount of material added with which the recovery study was carried out) was 0.02 mg/kg for grapes and 0.05 mg/kg for the other materials.

The blank values in control samples were below 30% of the limit of quantitation. In the case of dried grape marc a blank value of about 0.08 mg/kg was found in the controls at spiking levels of 0.5 mg/kg (i.e. 16.5% of the spiking level). Corrected recovery rates were used in the evaluation in this case.

High-pressure liquid-chromatography with UV detection is suitable for confirmation of the HPLC method with ELCD detection, as described in Section 7.5.

Zusammenfassung

Ziel der vorliegenden Methode ist die Bestimmung von Rückständen des fungiziden Wirkstoffs KBR 2738 in pflanzlichen Probenmaterialien.
Nach Extraktion mit Aceton und Flüssig-flüssig-Verteilung auf einer ChemElut-Säule mit einem Cyclohexan-Essigsäureethylester-Gemisch erfolgt die Bestimmung von KBR 2738 durch HPLC mit elektrochemischer Detektion. Aus dem hydrodynamischen Voltammogramm ergibt sich, daß im Potentialbereich von + 0,8 - 0,85 V (vs. Ag/AgCl) für KBR 2738 unter Beibehaltung der sonstigen Meßbedingungen das Optimum an Empfindlichkeit erreicht wird.
Die wasserarmen Probenmaterialien Rosine, Rosinenabfall und Traubentrester-trocken werden nach Vorquellen in Wasser und Extraktion mit einer ChemElut-Säule zusätzlich an einer Kieselgelsäule aufgereinigt mit Cyclohexan-Essigsäureethylester als Elutionsgemisch.
Der elektrochemische Detektor zeigt für KBR 2738 ein lineares Verhalten bezüglich Peakfläche versus Substanzmenge bei injizierten Mengen im Bereich von 1,25 bis 50 ng.

Die Wiederfindungsraten (Einzelwerte) für KBR 2738 lagen bei Zusätzen zwischen 0,02 und 2,0 mg/kg bzw. 0,05 und 5,0 mg/kg für sämtliche Probenmaterialien im Bereich zwischen 81% und 110% bei einem Mittelwert von 95% (n=108). Die relative Standardabweichung über alle Wiederfindungsraten und Probenmaterialien beträgt 5,7%.

Die Bestimmungsgrenze (entspricht dem niedrigsten Zusatz, mit dem Recoveryversuche durchgeführt wurden) lag im Falle von Traube bei 0,02 mg/kg und für die anderen Probenmaterialien bei 0,05 mg/kg.
Die Blindwerte der verwendeten Kontrollproben lagen unterhalb von 30 % der Bestimmungsgrenze. Nur im Fall von getrocknetem Traubentrester wurden beim Zusatzniveau von 0,5 mg/kg Blindwerte in der Kontrolle von ca. 0,08 mg/kg (d.h. 16,5 % bezogen auf das Zusatzniveau) gefunden. In diesem Fall gingen die korrigierten Wiederfindungsraten in die Auswertung ein.

Als Bestätigungsmethode für die HPLC-Methode mit ELCD-Detektion eignet sich die hochdruckflüssigchromatographische Trennung mit UV-Detektion, wie in 7.5 beschrieben.

See page 10-11 of 83
for the English translation.

1. Introduction

KBR 2738 is a new fungicide from the class of hydroxyanilides, active against Botrytis cinerea in grapes, strawberries, vegetables, and ornamental plants and against Monilia diseases in stone fruit.

In studies on its metabolism in apple, grape, and tomato after spray application it was found that unchanged active substance was the main constituent of the overall residue, and that it thus represents the relevant residue.

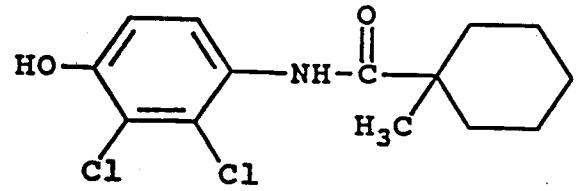
The objective of the present method is to determine KBR 2738 residues in various plant materials. To this end the active substance recovery was determined in each of the materials cited in this method.

The chemical and physical properties of KBR 2738 are summarized below.

Physicochemical properties of KBR 2738

Chemical name: (C.A.)	N-(2,3-dichloro-4-hydroxyphenyl)-1-methyl-cyclohexanecarboxamide
CAS-Reg. No.:	13141-86-1
Chemical code:	KBR 2738

Structural formula:



Empirical formula:	$C_{14}H_{17}Cl_2NO_2$
Molecular weight:	302.0 g/mol
Appearance:	pale beige powder
Melting point:	152.8°C
Vapour pressure:	2.29×10^{-10} mbar (20°C) 6.43×10^{-10} mbar (25°C)
Solubility:	water (pH 3): 0.022 g/l (20°C) water (pH 7): 0.038 g/l (20°C) water (pH 9): 0.022 g/l (20°C) acetone: 160 g/l (20°C) acetonitrile: 15 g/l (20°C) n-hexane: < 0.1 g/l (20°C) toluene: 5.7 g/l (20°C)
Partition coefficient: (n-octanol/water)	log (P_{OW}): 3.53

Hydrolytic stability: pH 5 (40°C): $t_{1/2}$ > 500 h
pH 7 (40°C): $t_{1/2}$ > 500 h
pH 9 (40°C): $t_{1/2}$ > 500 h
medium: acetonitrile/buffer (1:1)

2. Description of the method

KBR 2738 is extracted from the plant material with acetone. Materials of low water content are swelled with water before the extraction. An aliquot of the extract is taken and evaporated down to an aqueous residue.

The aqueous residue is placed on a disposable ChemElut column and subjected to liquid-liquid partition with a cyclohexane-ethyl acetate mixture. The residue obtained after evaporation is dissolved in a mixture of methanol and sodium acetate buffer (pH 4.8). In the case of sample materials of low water content — *raisins, raisin waste, and dried grape marc* — after separation on the ChemElut column further purification is carried out on a silica gel column using cyclohexane-ethyl acetate as the mobile phase.

Determination of KBR 2738 is carried out by high-performance liquid chromatography with electrochemical detection. The limit of quantitation of this method was 0.02 or 0.05 mg/kg.

3. Equipment

High-speed pulverizer (e.g. Ika Labortechnik Ultra Turrax T50)
Glass bottle, 1 l, wide-neck with ground-glass joint
Volumetric flasks, 500 ml, with glass stoppers
Glass funnel, 120 mm, with fluted filter

Porcelain Büchner funnel, 110 mm i.d., with black-band filters
Filter flask, 1 l
Measuring cylinder, 250 ml
Round-bottom flasks, 250 and 500 ml
Chromatography column, 10 mm i.d. with sinter
Dispensettes, 10, 50, and 100 ml (e.g. Brand, 6980 Wertheim)
Pasteur pipettes
Bulb pipettes, 0.5, 1, 2, 3, 4, and 6 ml
Test-tubes with ground-glass joints, 10 ml, graduated
Beaker, 150 ml
Vacuum rotary evaporator with water bath, bath temperature 40°C
High-pressure liquid chromatograph with autosampler and thermostated column oven (e.g. Spectra System P 4000 and AS 3000)
Electrochemical detector (e.g. BAS LC-4B amperometric detector) with
dual glassy carbon working electrode No. MF-1000, Ag/AgCl reference electrode RE-4 No. MF-2021, KCl electrolyte (3 M), flow-cell with 0.005" thick Teflon cell gasket No. MF-1047
Integrator (e.g. HP 3357 on LAS HP 1000)
Ultrasound bath
pH meter

4. Reagents

Acetone for residue analysis
Acetonitrile for residue analysis
Cyclohexane for residue analysis
Ethyl acetate for residue analysis

Methanol for residue analysis
Toluene for residue analysis
Water, Milli-Q quality
Concentrated acetic acid for analysis
Sodium acetate trihydrate for analysis
Ammonium acetate for analysis
Filtration aid, e.g. Celite 545
Disposable extraction columns, ChemElut 2050 (Varian)
Sodium sulfate, AR, anhydrous (e.g. Riedel de-Haen)
Silica gel 60, 0.063-0.200 mm, 70-230 mesh (Merck, water content 4%, Cat. No. 07734)
Glass wool
Millipore filter, Millex SR 0.5 µm filter unit
125 x 4 mm Lichrospher 100 RP-18, particle size 5 µm
250 x 4 mm Lichrospher 100 RP-18, particle size 5 µm
Pre-column: 4 x 4 mm Lichrospher 100 RP-18, particle size 5 µm
Mobile phase 1: cyclohexane/ethyl acetate 85/15 (v/v)
Mobile phase 2: cyclohexane/ethyl acetate 80/20 (v/v)

Acetate buffer: (aqueous solution)	0.1 mol/l sodium acetate and 0.001 mol/l ammonium acetate. The mixture is subsequently adjusted to pH 4.8 with conc. acetic acid.

Methanol/acetate buffer mixture: 60% methanol,
40% 0.1 mol/l sodium acetate and 0.001 mol/l ammonium acetate.
The mixture is subsequently adjusted to pH 4.8 with conc. acetic acid.

4.1 Reference substance/test substance

Substance name:	KBR 2738
Certificate of Analysis:	931216ELB01 of 11 January 1994

Empirical formula:	$C_{14}H_{17}Cl_2NO_2$
Molecular weight:	302.3 g/mol
Assay:	99.0%
Expiry date:	December 1995

4.2 Stock solution and dilutions

Dilutions of 100, 50, 12.5, 10, 5, 2.5, 2.0, 1.5, 1.25, 1.0, 0.75, 0.5, 0.25, and 0.125 mg/l were prepared from a methanolic stock solution of 1000 mg KBR 2738/l. The solvent used for dilution was the HPLC eluent (mobile phase). The dilutions, used for spiking, as standards, and for the determination of detector linearity, were stored in a refrigerator at an average temperature of 4°C.

5. Sampling

The samples for analysis were collected and prepared in accordance with Section VIII of *DFG-Methodensammlung zur Rückstandsanalytik von Pflanzenschutzmitteln* (DFG Collection of Methods for Crop Protection Agent Residue Analysis, see Deutsche Forschungsgemeinschaft, 1987). If immediate analysis was not possible, the samples were stored in a freezer at a mean temperature of -18°C.

6. Safety measures

The German professional laboratory association guidelines [Richtlinien für Laboratorien der Berufsgenossenschaft] (e.g. Notice M006) or comparable guidelines in other countries must be followed in the implementation of this method. The following chemicals classified as dangerous according to the Dangerous Substances Decree are used (this classification is based on the German guidelines, and must be adapted when the method is used in other countries):

- acetonitrile,
- methanol.

Appropriate safety regulations (e.g. Sections R and S) must be followed when working with these substances.

7. Analytical procedure

7.1 Extraction and liquid-liquid partition

7.1.1 Samples from strawberry, cherry, plum, peach, and grape (all as *fruit*)

Treat 50 g of deep-frozen analytical sample with 250 ml acetone and macerate in a high-speed pulverizer for about 3 min. Add about 15 g of filtration aid (Celite) and filter into a 500 ml volumetric flask through a fluted filter. Rinse through the maceration beaker and filter residue with 3 portions of 50 ml acetone, then make up to 500 ml with acetone. Stopper the volumetric flask and shake several times. Next measure out 250 ml into a measuring cylinder, transfer into a 500 ml round-bottom flask, and evaporate down to an aqueous residue on a rotary evaporator with a water bath temperature of 40°C. Rinse the inside of the round-bottom flask with about 20 ml water. Apply the entire aqueous phase (about 40 ml) to a dry disposable extraction column (ChemElut CE 2050) and allow it to soak in for about 10 min. Next elute with 4 portions of 50 ml of 85:15 v/v cyclohexane-ethyl acetate mixture (mobile phase 1) (flow time 45 min), using the first 50 ml to rinse out the flask before application on the column. Collect the eluate in a 250 ml round-bottom flask. Now evaporate the eluate under vacuum on the rotary evaporator at a water bath temperature of 40°C. Continue treatment of the residue as described in Section 7.3.1.

7.1.2 Raisins

Allow 50 g of the analytical sample to swell in 100 ml water for 30 min, add 250 ml acetone, and macerate for about 3 min in a high-speed pulverizer. Next add about 15 g of filtration aid and filter through a porcelain Büchner funnel into a 1 l filtration flask under slight underpressure. Rinse through the maceration beaker and filter residue with 3 portions of

50 ml acetone, transfer the filtrate to a 500 ml volumetric flask, and make up to 500 ml with acetone. Stopper the volumetric flask and shake several times. Next measure out 250 ml into a measuring cylinder, transfer into a 500 ml round-bottom flask, and evaporate down to an aqueous residue on a rotary evaporator with a water bath temperature of 40°C. Transfer the aqueous phase (about 40 ml) to a dry disposable extraction column (ChemElut CE 2050) and allow to soak in for about 10 min. Next elute with 4 portions of 50 ml of 85:15 v/v cyclohexane-ethyl acetate mixture (mobile phase 1), using the first 50 ml to rinse out the flask before application on the column. Collect the eluate in a 250 ml round-bottom flask. A flow time of 45 min should be observed after the last 50 ml of mobile phase 1 has soaked in. Now evaporate the eluate under vacuum on the rotary evaporator at a water bath temperature of 40°C. Dissolve the residue in 5 ml toluene and continue treatment as described in Section 7.2.

7.1.3 Raisin waste

Allow 12.5 g of the analytical sample to swell in 50 ml water for 30 min, add 200 ml acetone, and macerate for about 3 min in a high-speed pulverizer. Next add about 15 g of filtration aid and filter through a porcelain Büchner funnel into a 1 l filter flask under slight underpressure. Rinse through the maceration beaker and filter residue with 3 portions of 50 ml acetone, transfer the filtrate to a 500 ml round-bottom flask, and evaporate down to an aqueous residue on a rotary evaporator with a water bath temperature of 40°C. Transfer the aqueous phase (about 40 ml) to a dry disposable extraction column (ChemElut CE 2050) and allow to soak in for about 10 min. Next elute with 4 portions of 50 ml of 85:15 v/v cyclohexane-ethyl acetate mixture (mobile phase 1), using the first 50 ml to rinse out the flask before application on the column. Collect the eluate in a 250 ml round-bottom flask. A flow time of 45 min should be observed after the last 50 ml of mobile phase 1 has soaked in. Now evaporate the eluate under vacuum on the rotary evaporator at a water bath temperature of 40°C. Dissolve the residue in 5 ml toluene and continue treatment as described in Section 7.2.

7.1.4 Grape marc (dried)

Allow 25 g of the analytical sample to swell in 100 ml water for 30 min. Further working up is as described in Section 7.1.2.

7.1.5 Grape marc (moist)

Treat 50 g of the deep-frozen material with 250 ml acetone and macerate in a high-speed pulverizer for about 3 min. Further working up is as described in Section 7.1.2, except that purification over silica gel (Section 7.2) is not carried out. The final residue is dissolved as described in Section 7.3.2.

7.1.6 Drinks (must, juice, wine)

Apply 50 g of the analytical sample directly on a dry disposable extraction column (ChemElut CE 2050) and allow to soak in for 10 min. Next elute with 4 portions of 50 ml of 85:15 v/v cyclohexane-ethyl acetate mixture (flow time: 45 min). Evaporate the eluate to dryness on a vacuum rotary evaporator. Continue treatment of the residue as described in Section 7.3.1.

7.2 Purification by column chromatography on silica gel

Fill the chromatography column in the following order: 5 ml toluene, 5 g silica gel suspended in toluene, about a 10 mm layer of sodium sulfate, and a glass wool plug. Drain out the toluene to the level of the glass wool plug.

Add the solution from Section 7.1.2 or 7.1.3 to the column using a pipette. Rinse the flask with 5 ml toluene and add the solution to the column. After allowing the solution to flow

down to the glass wool plug, rinse the flask with 7 ml of 80:20 v/v cyclohexane/ethyl acetate (mobile phase 2) and add the solution to the column. Repeat this procedure with a further 7 ml of mobile phase 2. Next elute the active substance into a 250 ml round-bottom flask with 40 ml of mobile phase 2. Evaporate the eluate to dryness on a vacuum rotary evaporator. Continue treatment of the residue as described in Section 7.3.2.

7.3 Preparation of the final volume (V_{END})

7.3.1 Variant 1

Dissolve the residue in a defined volume (e.g. 2 ml) of the HPLC mobile phase and sonify in an ultrasonic bath for about 20 sec. Filter the sample through a Millipore filter (5 µm) before filling it into sample bottles for the autosampler.

7.3.2 Variant 2

Dissolve the residue in a defined volume (3 ml) of methanol using an ultrasonic bath and dilute to a defined total volume (5 ml) with pure acetate buffer mixture (2 ml). This difference from variant 1 is necessary to obtain reproducible and satisfactory recovery for the extracts from raisins, raisin waste, moist marc, and dried marc. Filter the sample through a Millipore filter (5 µm) before filling it into sample bottles.

7.4 High-pressure liquid-chromatographic determination with electrochemical detection (HPLC-ELCD)

HPLC instrument:	e.g. Spectra System with P 4000 pump and AS 3000 autosampler
Detector:	e.g. BAS LC-4B/CC-4 amperometric detector
- Working electrode:	glassy carbon
- Reference electrode:	Ag/AgCl (electrolyte 3 M KCl)
- Auxiliary electrode:	instrument housing
Detection:	direct current amperometry (DC-amperometry)
- Working potential:	+0.8 V (vs. Ag/AgCl)
- Range:	output: 1 V
- Filter:	1 Hz
Flow-cell volume:	2-5 µl
Column:	stainless steel column, 4 mm i.d., 125 mm long, packed with Merck LiChrospher 100 RP-18, particle size 5 µm, with 4 x 4 mm LiChrospher 100 RP-18 precolumn, particle size 5µm.
Oven temperature:	40°C
Mobile phase:	mixture consisting of (1) 60% methanol and (2) 40% 0.1 mol/l sodium acetate and 0.001 mol/l ammonium acetate; pH 4.8 (adjusted with acetic acid)

Elution:	isocratic, elution time 20 min
Flow rate:	1 ml/min
Injection volume:	10 µl
Retention time:	7.6-8.2 min for KBR 2738

7.5 Method of confirmation

To confirm the HPLC-ELCD determination, some samples were also measured with a UV detector. Only the samples with the highest spiking (2.0 or 5.0 mg/kg) were used for this purpose. For the various sample materials the measurements gave a scatter of 84-107% for the recovery (individual values), with a relative standard deviation of 3.2% over all sample materials.

The UV detector's linear range is between 1.0 and 100 ng at both wavelengths used, 246 and 289 nm. The HPLC conditions were appropriately optimized for the UV detector.

Instrumental parameters and measurement conditions

HPLC instrument:	e.g. Spectra System with P 2000 pump and AS 1000 autosampler
UV detector:	Spectra System UV 2000
Detection:	246 or 289 nm

Range:	output: 1 V
Flow-cell volume:	10 µl
Column:	stainless steel column, 4 mm i.d., 250 mm long, stationary phase LiChrospher 100 RP-18, particle size 5 µm, precolumn 4 x 4 mm LiChrospher 100 RP-18, 5 µm
Oven temperature:	40°C
Mobile phase (eluent):	A: acetonitrile; B: Milli-Q water

The active ingredient is determined under isocratic conditions. Because of the large proportion of matrix in the samples, a rinsing gradient is used to recondition the column.

Gradient:	0-7 min:	60% A, 40% B
	8 min:	80% A, 20% B
	8-28 min:	80% A, 20% B
	29 min:	60% A, 40% B
	29-45 min:	60% A, 40% B

Flow rate:	1 ml/min
Injection volume:	10 µl
Retention time (KBR):	6.6-6.8 min.

Determination of detector linearity for KBR 2738 by HPLV-UV

The characteristic of the UV detector used was determined by 3-fold injections of 1.0, 2.5, 5.0, 10, 25, 50, and 100 ng KBR 2738 in 10 µl injection volumes at two different wavelengths (absorption maxima).

Table 1: UV detection at 246 nm

Standard quantity (ng)	Peak areas				
	individual measured values			measured mean	calculated mean
1.0	7156	6889	6641	6895	6496
2.5	19171	19078	18969	19073	18112
5.0	37248	39350	38163	38254	37472
10	76954	76615	76700	76756	76192
25	190116	190540	191993	190883	192352
50	381940	383753	383409	383034	385952
100	774003	777081	773634	774906	773152

Regression parameters of straight-line equation $y = a + bx$:

Ordinate intercept: $a = -1248$

Slope: $b = 7744$

Standard deviation (calculated for b): 199.6

Table 2: UV detection at 289 nm

Standard quantity (ng)	Peak areas				
	individual measured values			measured mean	calculated mean
2.5	9125	9053	6773	8317	7641
5.0	15153	13152	15254	14520	14598
10	27034	30012	31116	29387	28513
25	68932	69132	69533	69199	70258
50	138144	139711	138254	138703	139833
100	282032	277655	279537	279741	278983

Regression parameters of straight-line equation $y = a + bx$:

Ordinate intercept: $a = 683$

Slope: $b = 2783$

Standard deviation (calculated for b): 121.8

The results of the recovery rate determination with the UV detector are summarized in the following table:

Table 3: Recovery rates for KBR 2738 (HPLC, UV detection)

Crop	Sample material	Spiking (mg/kg)	Recovery (%) individual values** 246 nm	289 nm	mean	RSD*
Grape	fruit	2.0	99, 96, 99	93, 96, 100	97	2.6
	must	2.0	101, 99, 98	90, 95, 91	96	4.5
	juice	2.0	102, 100, 104	93, 96, 98	99	4.0
	wine	2.0	104, 100, 107	93, 96, 98	100	5.2
	raisin	5.0	95, 97, 97	98, 101, 99	98	2.0
	raisin waste	5.0	95, 95, 93	94, 97, 93	95	1.5
	moist marc	5.0	97, 99, 91	97, 106, 92	97	5.4
	dried marc	5.0	88, 84, 84	94, 92, 92	89	4.3
Strawberry	fruit	5.0	96, 96, 95	97, 100, 102	98	2.7
Cherry	fruit	5.0	93, 89, 92	91, 94, 90	92	1.9
Plum	fruit	5.0	93, 88, 95	97, 91, 102	94	4.9
Peach	fruit	5.0	95, 95, 99	98, 99, 97	97	1.8

*RSD = relative standard deviation for all recovery rates for one sample material

**Each worked-up sample was measured at two wavelengths (246 and 289 nm)

8. Evaluation

8.1 Evaluation procedure

The quantitative evaluation was carried out by determination and comparison of peak areas of the analytical solutions (F_A) against peak areas of standard solutions (F_{St}), using a computer program. For KBR 2738 the electrochemical detector used shows a linear relationship between peak area and the quantity of substance injected in the range 1.25-50 ng. The UV detector used for the confirmation method (see Section 6.5) was linear in the ranges 1.0-100 ng (246 nm) and 2.5-100 ng (289 nm).

8.2 Calculation of residue levels

The residue levels, in mg/kg, are calculated as follows:

$$R = \frac{F_A}{F_{St}} \times \frac{V_{END}}{V_{INJ}} \times \frac{W_{St}}{G} \times \frac{A1}{A2}$$

where:

G = analytical sample weight in g

V_{END} = final volume of the 60:40 v/v methanol/acetate buffer mixture as per Section 6.3.1 or 6.3.2

V_{INJ} = volume injected into the liquid chromatograph in µl

F_A = peak area of the analytical solution obtained from V_{INJ}

F_{St} = peak area of the standard solution obtained from W_{St}

W_{St} = quantity of KBR 2738 injected in the standard solution in ng

A1 = volume of extract from the original sample (matrix), e.g. 500 ml

A2 = quantity of A1 used for the determination, e.g. 125 ml

9. Results and evaluation

The recovery rates were determined in additional tests, in which control samples had been spiked with defined quantities of KBR 2738 before extraction. The results of the recovery tests are summarized in Table 1.

The limit of quantitation (corresponding to the lowest spike used in the recovery tests) was 0.02 mg/kg for grapes (fruit, must, juice, and wine) and 0.05 mg/kg for all other sample materials described.

The blank values for all sample materials used are summarized in Table 2. With one exception (1 of 2 samples of dried grape marc) the blank values were less than 30% of the quantitation limits. The recovery rates were evaluated against pure standard.

The test or reference substance is stable in the standard and analytical solutions if these are treated as described in the method procedure. This ensures that falsification of the results as a result of decomposition does not occur during the normal time of processing of the analyses.

Since all the recovery rates determined were in the range 70-110%, with a relative standard deviation of < 20%, the general requirements of residue analyses are fulfilled. The present method is thus successfully validated.

Table 4: Recovery rates for KBR 2738 by HPLC-ELCD

Crop	Sample material	Spiking (mg/kg)	Recovery (%)		
			individual values	mean	RSD*
Grape	fruit	0.02	92, 89, 88	90	4.3
		0.2	86, 95, 93	91	
		2.0	97, 98, 99	98	
	must	0.02	110, 109, 105	108	6.2
		0.2	95, 94, 98	96	
		2.0	100, 98, 98	99	
	juice	0.02	104, 100, 103	102	2.1
		0.2	99, 99, 98	99	
		2.0	100, 100, 101	100	
	wine	0.02	89, 90, 83	87	5.8
		0.2	96, 95, 94	95	
		2.0	100, 100, 104	100	
	raisin	0.05	107, 107, 99	104	5.1
		0.5	98, 95, 98	97	
		5.0	94, 96, 100	97	
	raisin waste	0.05	98, 98, 96	97	2.7
		0.5	94, 99, 94	96	
		5.0	99, 102, 99	100	
	moist marc	0.05	93, 95, 98	95	2.7
		0.5	96, 100, 95	97	
		5.0	101, 97, 94	97	
	dried marc	0.05	95, 87, 92	91	6.9
		0.5	94, 99, 102	98	
		5.0	84, 83, 83	83	
Strawberry	fruit	0.05	92, 88, 92	91	3.5
		0.5	96, 95, 96	96	
		5.0	99, 99, 95	98	
Cherry	fruit	0.05	88, 83, 92	88	4.2
		0.5	87, 81, 91	86	
		5.0	93, 88, 92	91	
Plum	fruit	0.05	82, 79, 94	85	5.5
		0.5	90, 86, 92	89	
		5.0	92, 82, 83	86	
Peach	fruit	0.05	92, 87, 92	90	2.9
		0.5	92, 94, 95	94	
		5.0	94, 96, 89	93	

* RSD = relative standard deviation for all recovery rates for one sample material
** ELCD = electrochemical detector

Table 5: Blank values of KBR 2738 in control samples

Crop	Sample material	Quantitation limit (mg/kg)	Residues (mg/kg), individual values	Origin
Grape	fruit	0.02	< 0.02	supermarket
	juice	0.02	< 0.02	supermarket
	must A	0.02	< 0.02	Spain
	must B	0.02	< 0.02*	Müller-Thurgau grapes
	wine A	0.02	< 0.02	Spain
	wine B	0.02	< 0.02**	Müller-Thurgau grapes
	raisin	0.05	< 0.05	supermarket
	raisin waste	0.05	< 0.05	South Africa
	dried marc A	0.05	< 0.05	own processing
	dried marc B	0.05	0.083	own processing
	moist marc	0.05	< 0.05	own processing
Strawberry	fruit	0.05	< 0.05	-
Cherry	fruit	0.05	< 0.05	-
Peach	fruit	0.05	< 0.05	-
Plum	fruit	0.05	< 0.05	-

*found 0.007 mg/kg

**found 0.008 mg/kg

Determination of HPLC-ELCD detector linearity for KBR 2738

Electrochemical detector (ELCD):

The characteristic of the UV detector used was determined by 3-fold injections of 1.25, 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 20.0, 25.0, and 50.0 ng KBR 2738 in 10 µl injection volumes (see Fig. 1).

Table 6: KBR 2738 linearity measurement data after HPLC, conditions in Section 7.4

Standard quantity [ng]	Peak areas			
	individual values			mean values
1.25	308440	290892	309896	303076
2.5	575446	603698	681342	620162
5.0	1293442	1298858	1314514	1302271
7.5	1757686	1960372	2011304	1909787
10	2668058	2692476	2727212	2695915
12.5	3417122	3434060	3456406	3435863
15	4026092	4041304	4128150	4065182
20	5414692	5476236	5456612	5449180
25	6846208	6097554	6453018	6465593
50	13566660	13656242	13641810	13621571

Regression parameters for straight-line equation $y = a + bx$:

Ordinate intercept: $a = -53491$

Slope: $b = 2704254$

Correlation coefficient: 0.99824

Standard deviation (calculated for b): 65593

Determination of the *hydrodynamic voltammogram (HDV)* of KBR 2738

All measurements of a standard quantity of 10 ng KBR 2738 (standard concentration: 1 mg/l KBR 2738 and 10 µl injection volume) were carried out under the HPLC conditions given in Section 6.4. The potential of the working electrode was increased from 0.5 to 1.0 V in steps of 0.05 V after each set of 3 injections. Peaks were plotted with an analogue plotter (Servogor 220 D) at a chart speed of 0.5 cm/min. The same measurement sequence was carried out with the mobile phase given in Section 6.4 (60:40 v/v methanol/acetate buffer mixture).

Table 7: Characteristics of the hydrodynamic voltammogram

Potential of working electrode (V)	Peak height of KBR standard solution (cm)	Peak height of mobile phase (cm)
+ 0.50	0	0
+ 0.55	0	0
+ 0.60	0	0
+ 0.65	0	0
+ 0.70	0.11	0
+ 0.75	0.70	0
+ 0.80	1.50	0
+ 0.85	1.70	0
+ 0.90	1.72	0
+ 0.95	1.80	0
+ 1.00	1.80	0

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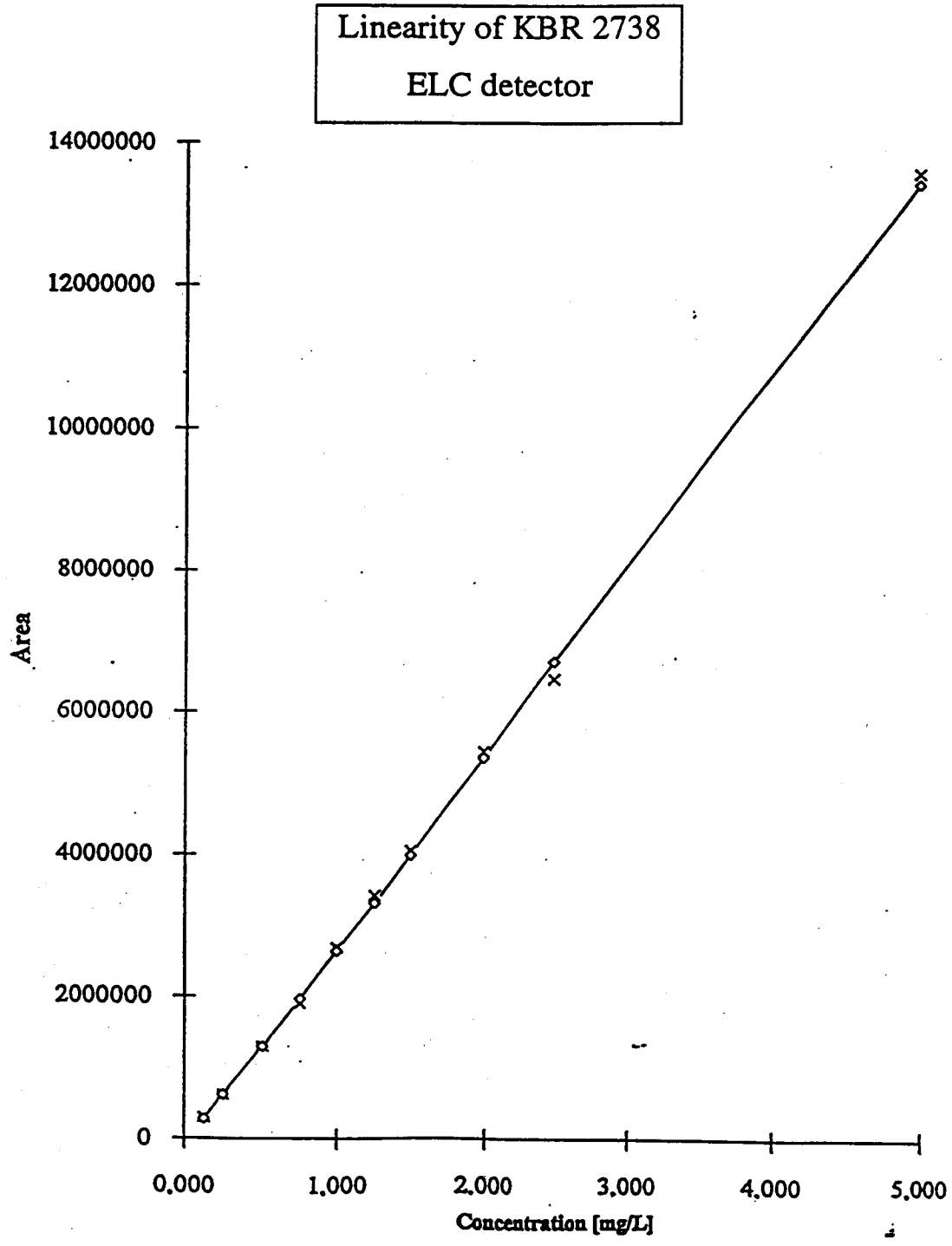

Fig. 1: Electrochemical detector linearity in the determination of KBR 2738

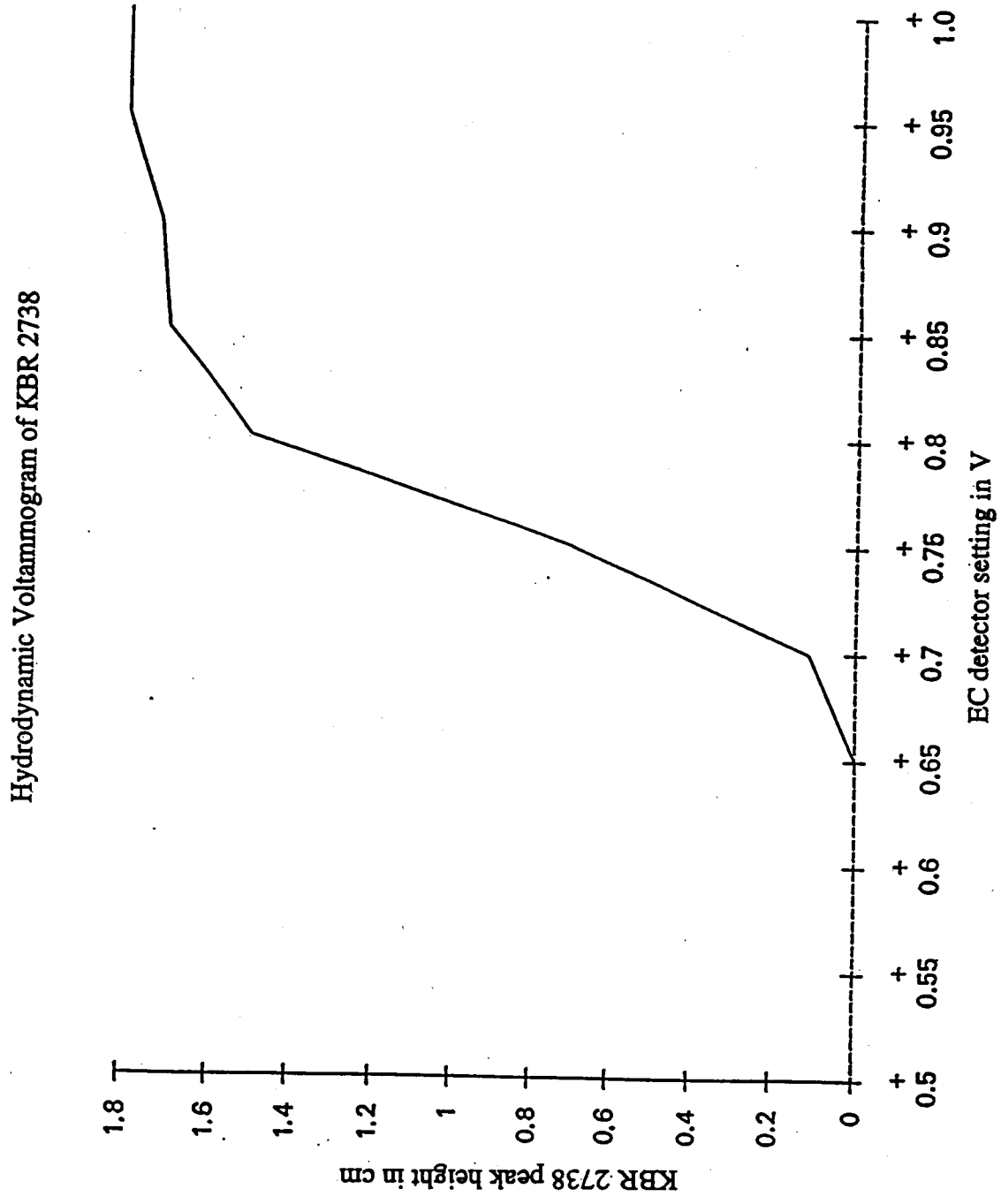

Fig. 2: Hydrodynamic voltammogram of KBR 2738 in the potential range from +0.5 V to +1.0 V (vs. Ag/AgCl)

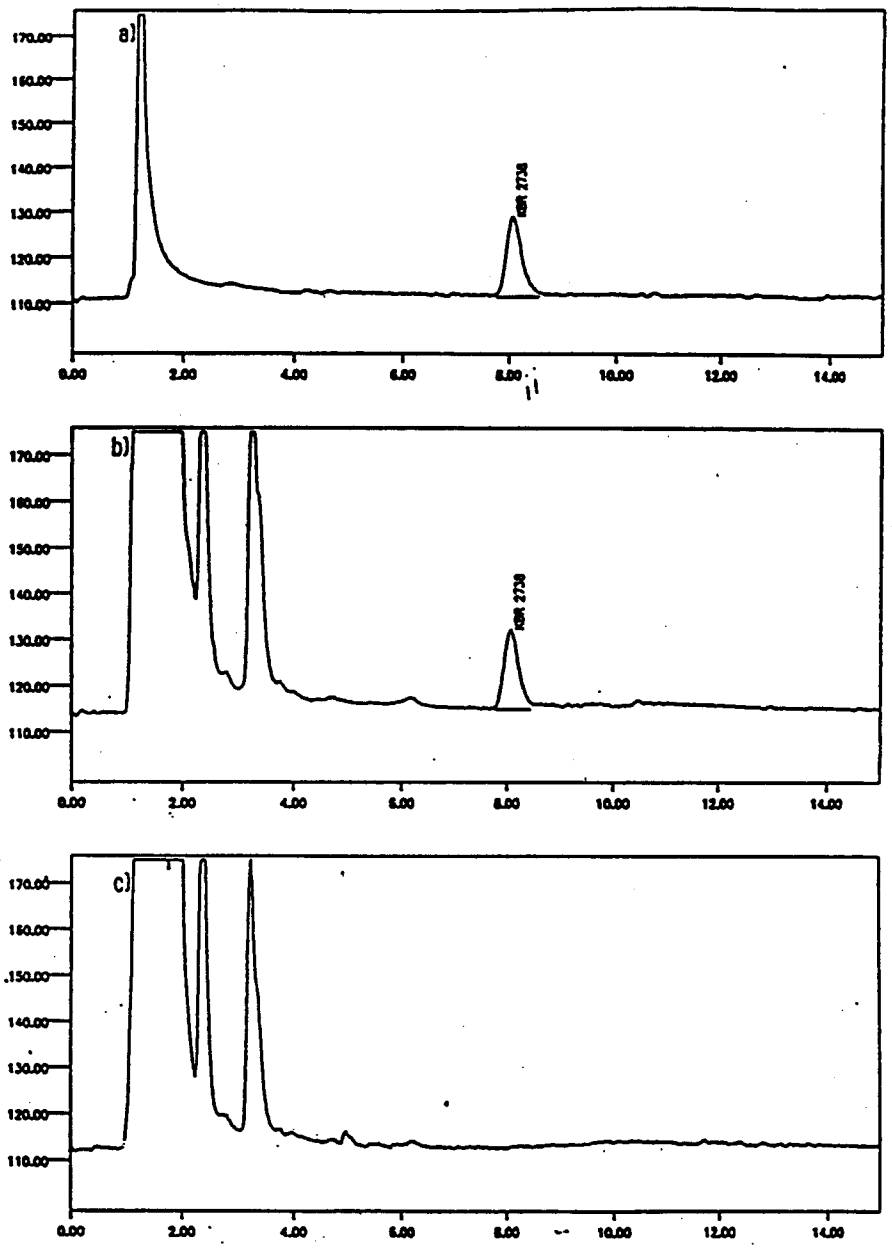

Fig. 3: Determination of the recovery of KBR 2738 in/on strawberries (fruit) by HPLC with an electrochemical detector

A) Standard concentration 0.25 ng/µl (corresponding to 0.05 mg/kg in the evaluation); 10 µl injection volume; solvent = mobile phase; results file KP_KBR_A4_EB#002.RES
B) Addition 0.05 mg/kg A: recovery 92% of standard at a spiking level of 0.05 mg KBR 2738/kg in strawberries (fruit); results file KP_KBR_A4_EB#038.RES
C) Control sample for strawberries (fruit); finding: no KBR 2738 detected; results file KP_KBR_A4_EB#034.RES

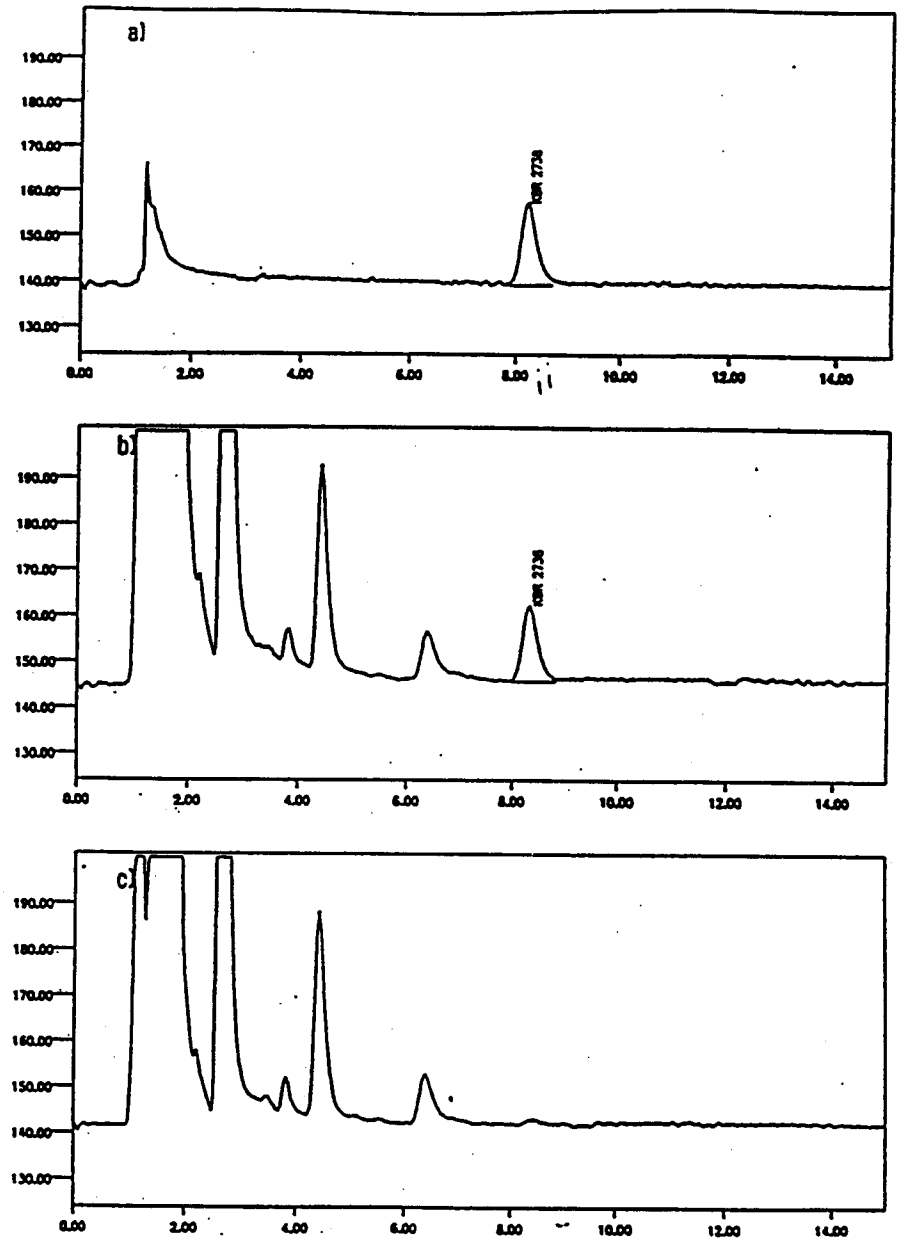

Fig. 4: Determination of the recovery of KBR 2738 in/on cherries (fruit) by HPLC with an electrochemical detector

A) Standard concentration 0.25 ng/µl (corresponding to 0.05 mg/kg in the evaluation); 10 µl injection volume; solvent = mobile phase; results file KP_KBR_A4_KS#002.RES
B) Addition 0.05 mg/kg A: recovery 88% of standard at a spiking level of 0.05 mg KBR 2738/kg in cherries (fruit); results file KP_KBR_A4_KS#006.RES
C) Control sample for cherries (fruit); finding: no KBR 2738 detected; results file KP_KBR_A4_KS#004.RES

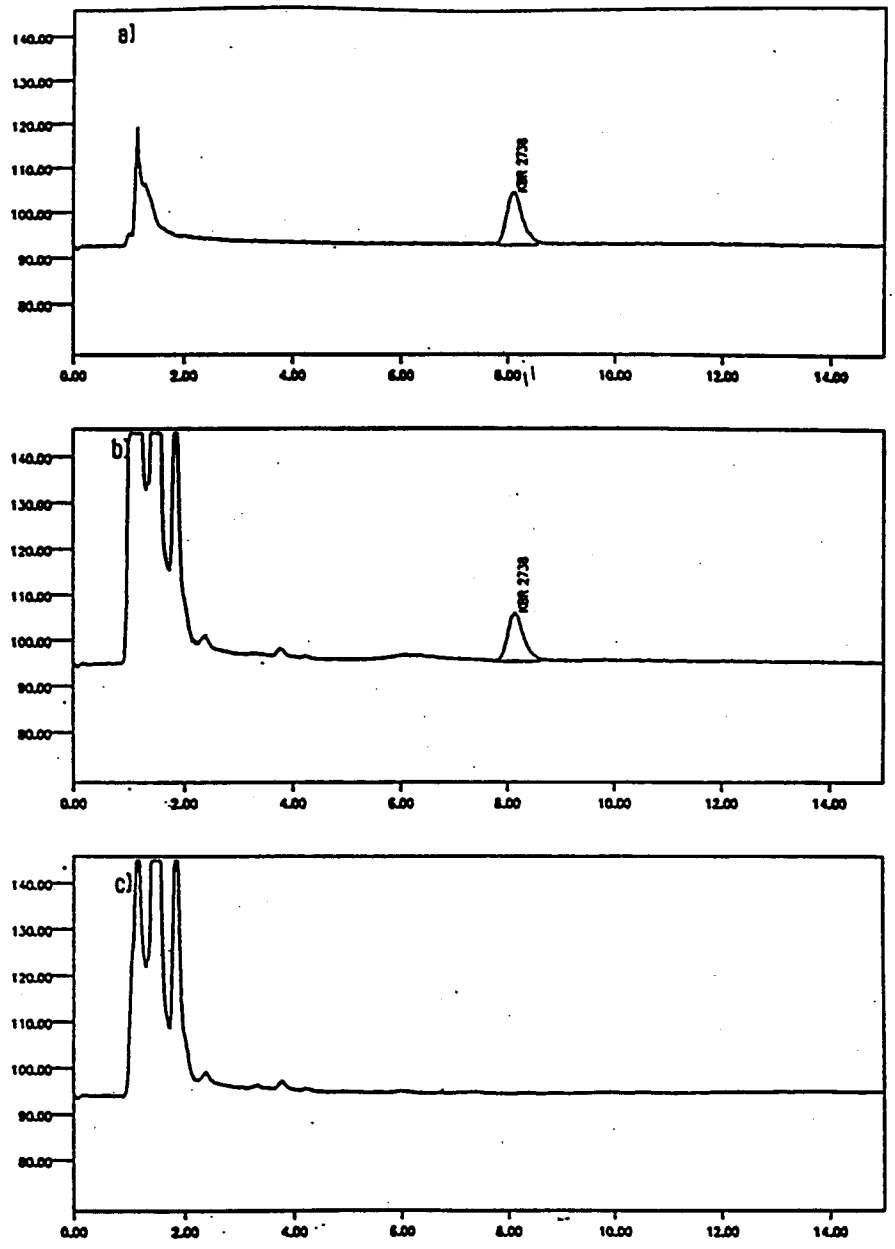

Fig. 5: Determination of the recovery of KBR 2738 in/on peaches (fruit) by HPLC with an electrochemical detector

A) Standard concentration 0.25 ng/µl (corresponding to 0.05 mg/kg in the evaluation); 10 µl injection volume; solvent = mobile phase; results file KP_KBR_A4_PI#032.RES

B) Addition 0.05 mg/kg A: recovery 92% of standard at a spiking level of 0.05 mg KBR 2738/kg in peaches (fruit); results file KP_KBR_A4_PF#035.RES

C) Control sample for peaches (fruit); finding: no KBR 2738 detected; results file KP_KBR_A4_PF#034.RES

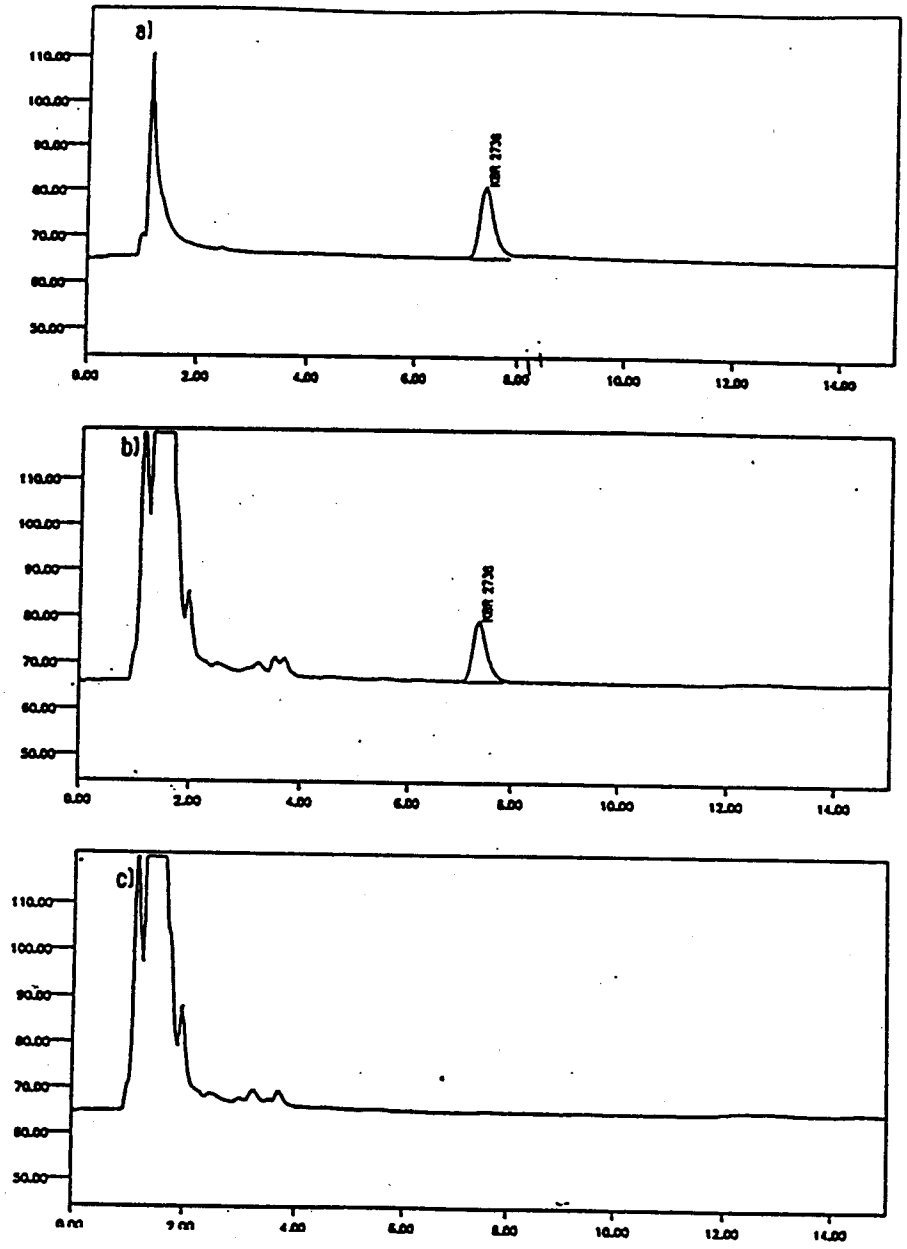

Fig. 6: Determination of the recovery of KBR 2738 in/on plums (fruit) by HPLC with an electrochemical detector

A) Standard concentration 0.25 ng/µl (corresponding to 0.05 mg/kg in the evaluation); 10 µl injection volume; solvent = mobile phase; results file KP_KBR_A4_PF#036.RES

B) Addition 0.05 mg/kg A: recovery 82% of standard at a spiking level of 0.05 mg KBR 2738/kg in plums (fruit); results file KP_KBR_A4_PF#038.RES

C) Control sample for plums (fruit); finding: no KBR 2738 detected; results file KP_KBR_A4_PF#034.RES

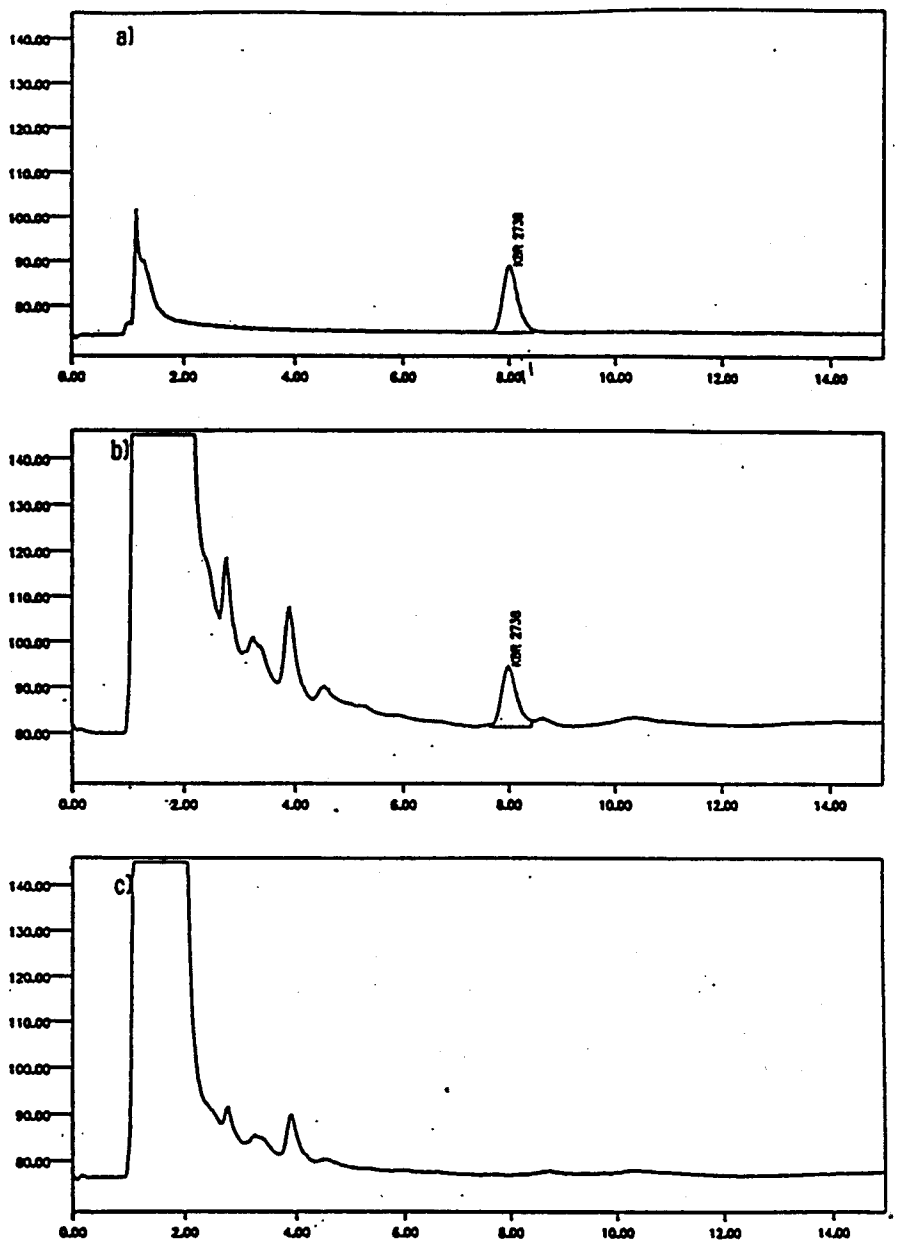

Fig. 7: Determination of the recovery of KBR 2738 in/on grapes (fruit) by HPLC with an electrochemical detector

A) Standard concentration 0.25 ng/µl (corresponding to 0.02 mg/kg in the evaluation); 10 µl injection volume; solvent = mobile phase; results file KP_KBR_A4_WT#002.RES
B) Addition 0.02 mg/kg A: recovery 92% of standard at a spiking level of 0.02 mg KBR 2738/kg in grapes (fruit); results file KP_KBR_A4_WT#006.RES
C) Control sample for grapes (fruit); finding: no KBR 2738 detected; results file KP_KBR_A4_WT#004.RES

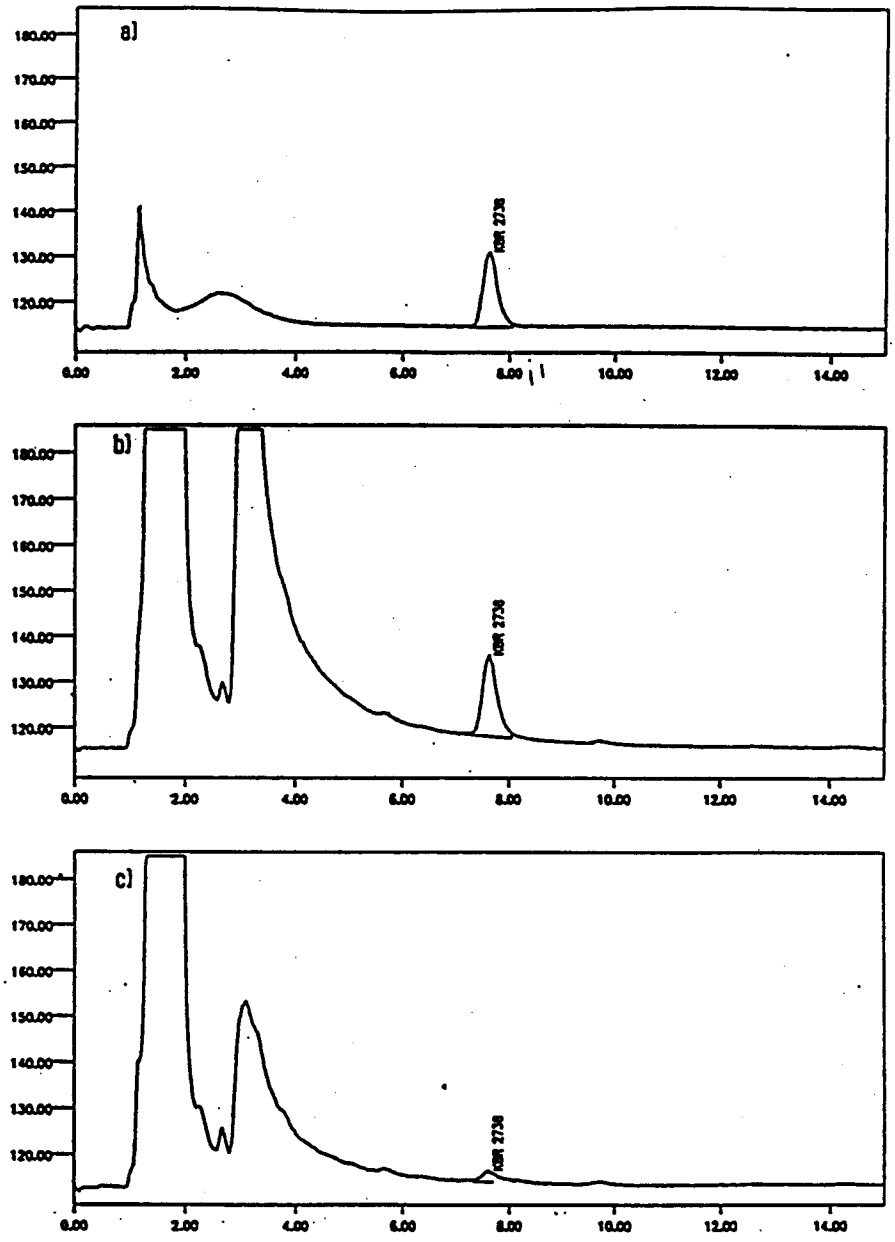

Fig. 8: Determination of the recovery of KBR 2738 in/on grapes (must) by HPLC with an electrochemical detector

A) Standard concentration 0.25 ng/µl (corresponding to 0.02 mg/kg in the evaluation); 10 µl injection volume; solvent = mobile phase; results file KP_KBRA4_WTMO066.RES
B) Addition 0.02 mg/kg A: recovery 110% of standard at a spiking level of 0.02 mg KBR 2738/kg in grapes (must); results file KP_KBRA4_WTMO072.RES
C) Control sample for grapes (must); finding: KBR 2738 << LOQ; results file KP_KBRA4_WTMO063.RES

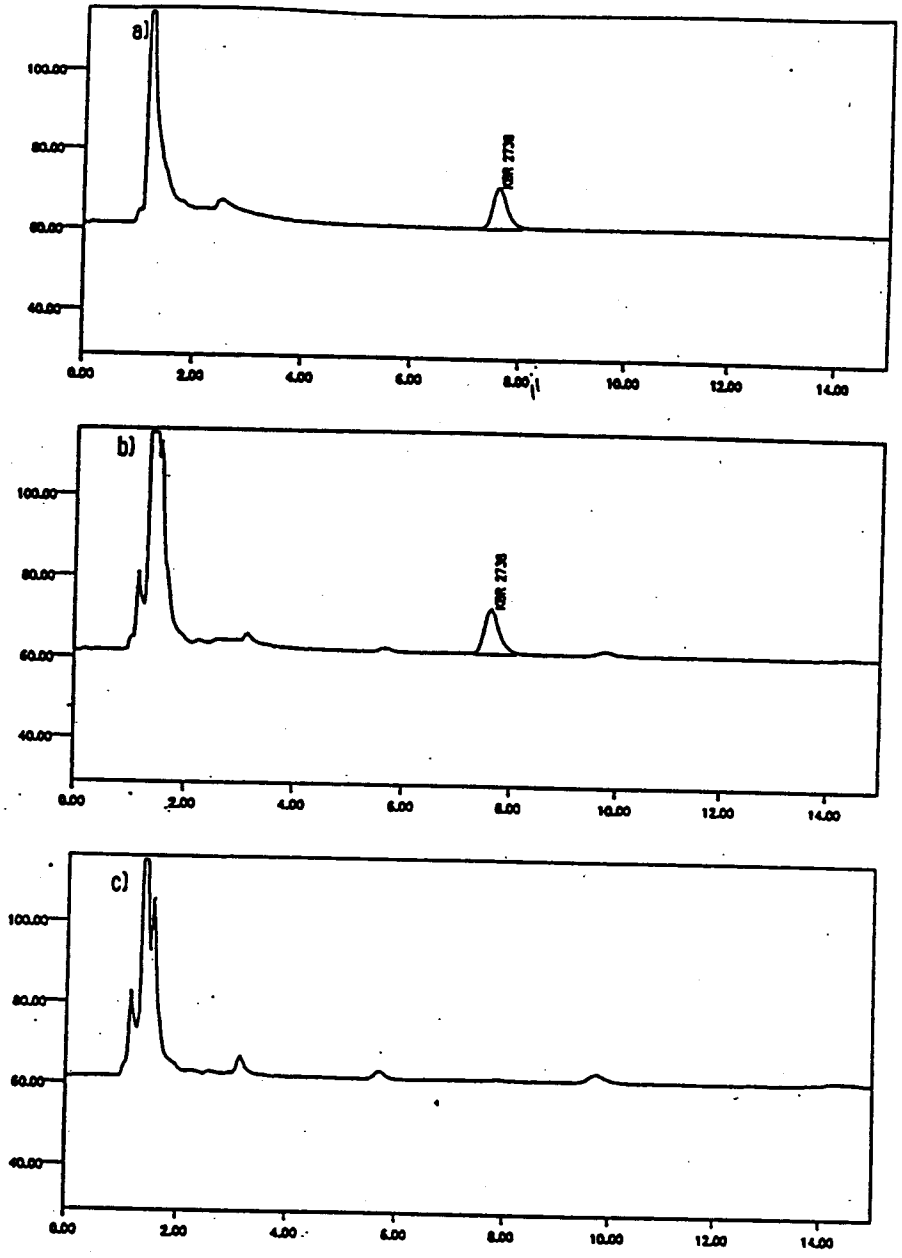

Fig. 9: Determination of the recovery of KBR 2738 in/on grapes (juice) by HPLC with an electrochemical detector

A) Standard concentration 0.25 ng/µl (corresponding to 0.02 mg/kg in the evaluation); 10 µl injection volume; solvent = mobile phase; results file KP_KBRA4_WTSA002.RES
B) Addition 0.02 mg/kg A: recovery 104% of standard at a spiking level of 0.02 mg KBR 2738/kg in grapes (juice); results file KP_KBRA4_WTSA006.RES
C) Control sample for grapes (juice); finding: no KBR 2738 detected; results file KP_KBRA4_WTSA004.RES

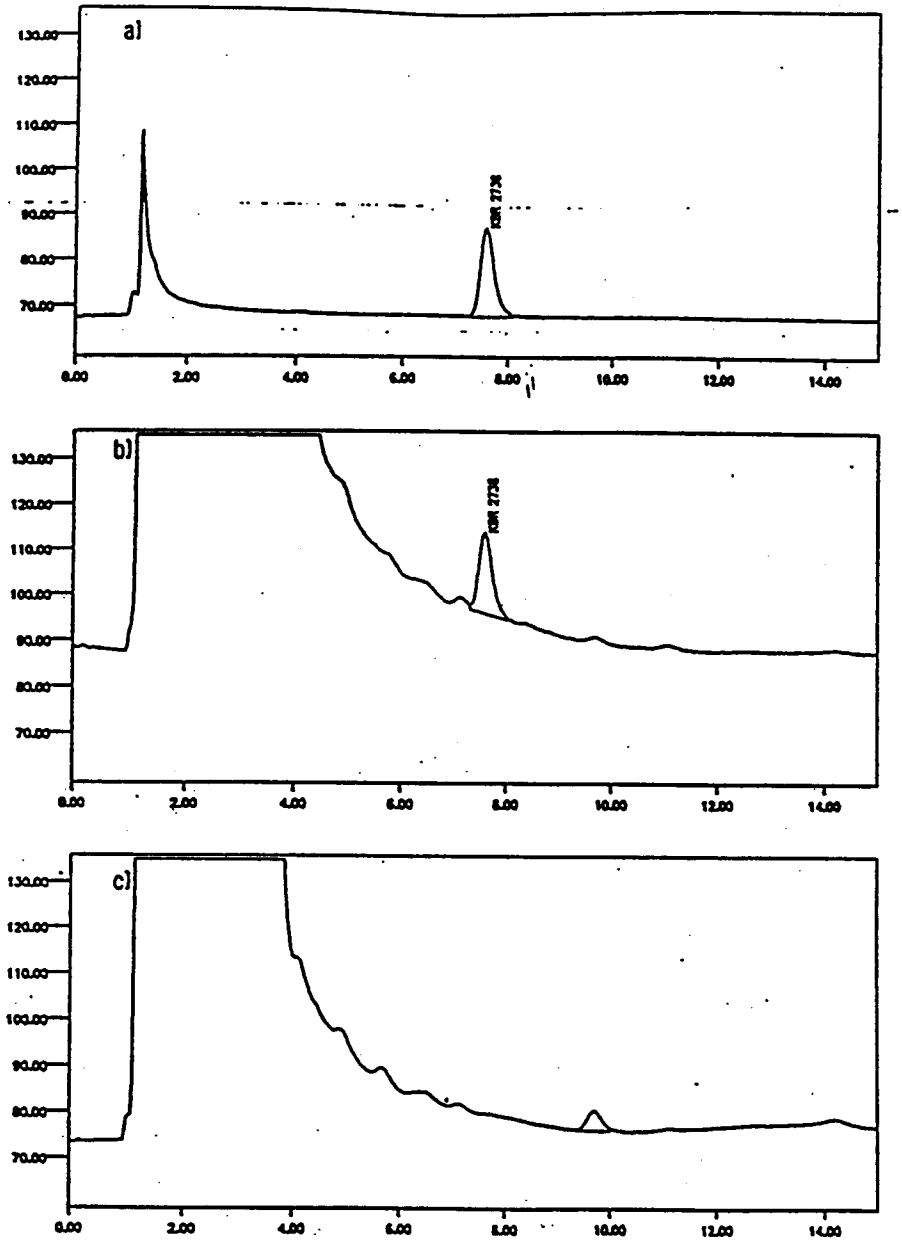

Fig. 10: Determination of the recovery of KBR 2738 in/on grapes (wine) by HPLC with an electrochemical detector

A) Standard concentration 0.25 ng/µl (corresponding to 0.02 mg/kg in the evaluation); 10 µl injection volume; solvent = mobile phase; results file KP_KBRA4_WTWE032.RES
B) Addition 0.02 mg/kg A: recovery 89% of standard at a spiking level of 0.02 mg KBR 2738/kg in grapes (wine); results file KP_KBRA4_WTWE036.RES
C) Control sample for grapes (wine); finding: no KBR 2738 detected; results file KP_KBRA4_WTWE034.RES

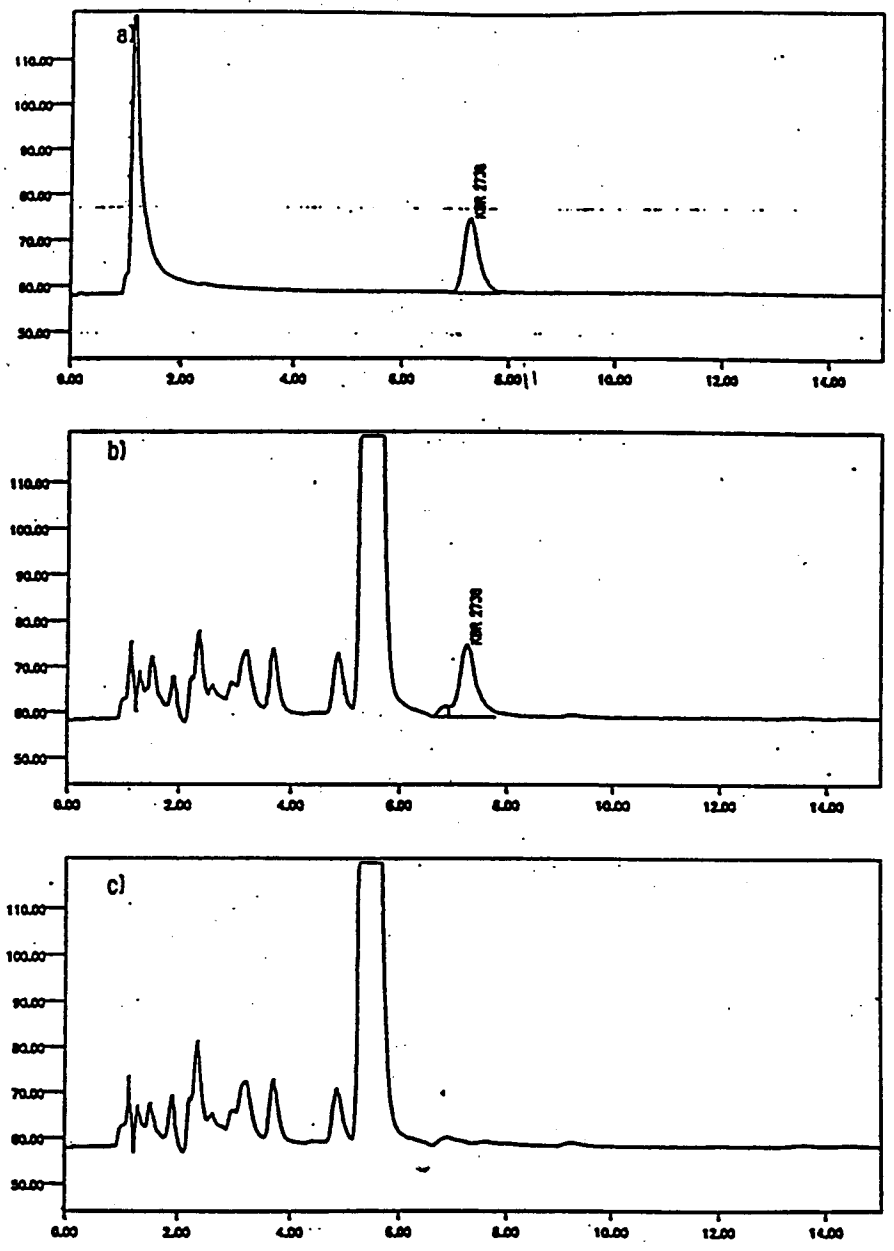

Fig. 11: Determination of the recovery of KBR 2738 in/on grapes (raisins) by HPLC with an electrochemical detector

A) Standard concentration 0.25 ng/µl (corresponding to 0.05 mg/kg in the evaluation); 10 µl injection volume; solvent = mobile phase; results file KP_KBRA4_WTRS#006.RES
B) Addition 0.05 mg/kg A: recovery 107% of standard at a spiking level of 0.05 mg KBR 2738/kg in grapes (raisins); results file KP_KBRA4_WTRS#008.RES
C) Control sample for grapes (raisins); finding: no KBR 2738 detected; results file KP_KBRA4_WTRS#004.RES

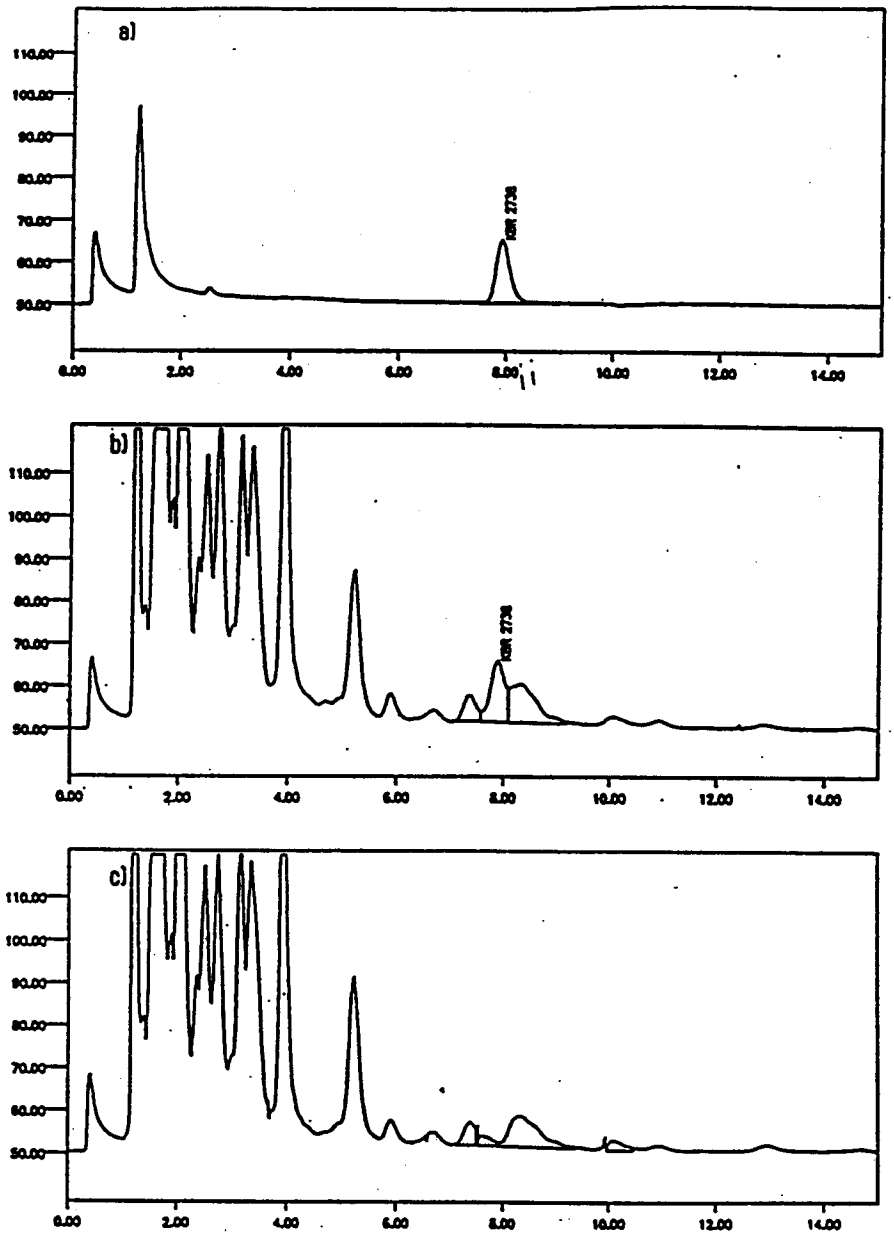

Fig. 12: Determination of the recovery of KBR 2738 in/on grapes (raisin waste) by HPLC with an electrochemical detector

A) Standard concentration 0.25 ng/µl (corresponding to 0.05 mg/kg in the evaluation); 10 µl injection volume; solvent = mobile phase; results file KP_KBRA4_RSAB#005.RES

B) Addition 0.05 mg/kg A: recovery 98% of standard at a spiking level of 0.05 mg KBR 2738/kg in grapes (raisin waste); results file KP_KBRA4_RSAB#008.RES

C) Control sample for grapes (raisin waste); finding: KBR 2738 << LOD; results file KP_KBRA4_RSAB#003.RES

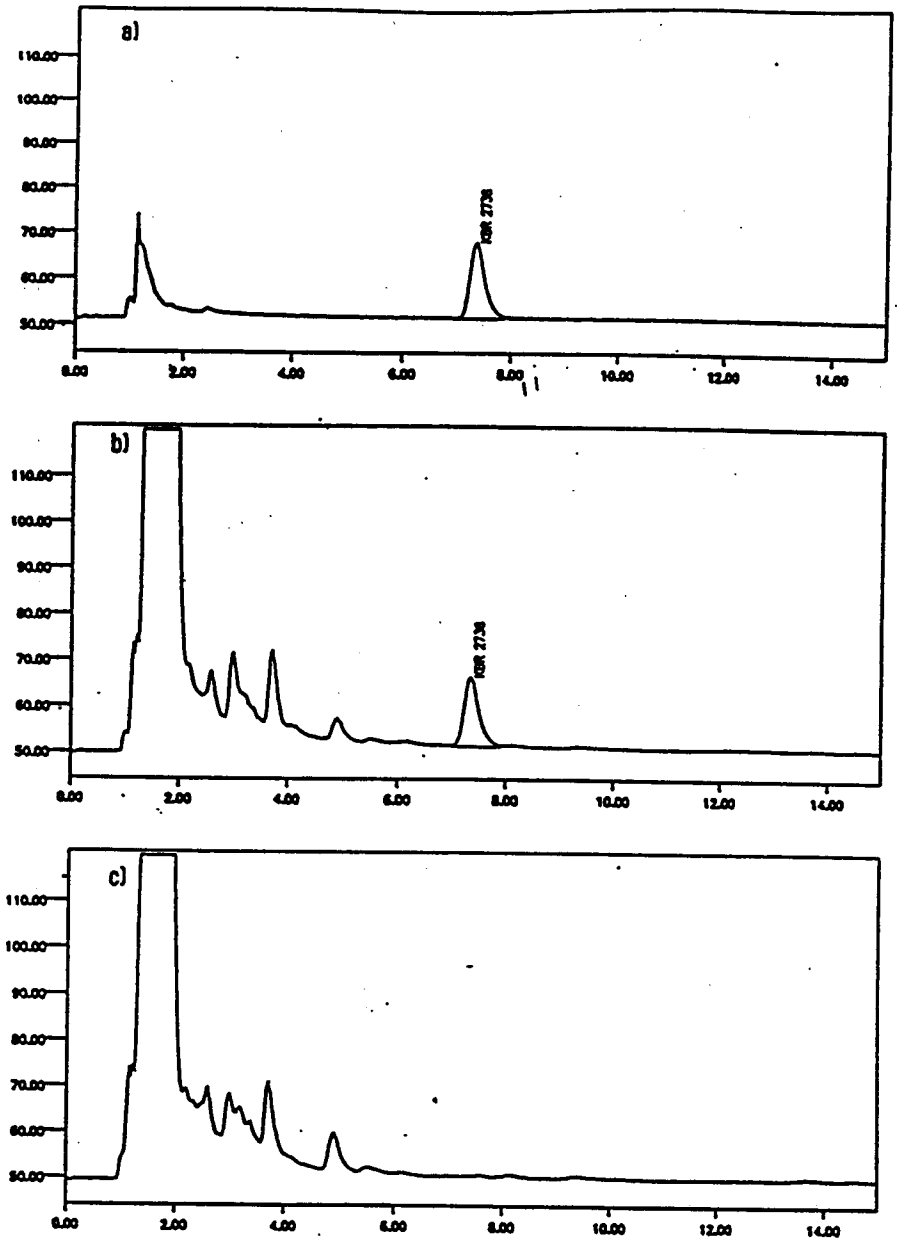

Fig. 13: Determination of the recovery of KBR 2738 in/on grapes (moist marc) by HPLC with an electrochemical detector

A) Standard concentration 0.25 ng/µl (corresponding to 0.05 mg/kg in the evaluation); 10 µl injection volume; solvent = mobile phase; results file KP_KBRA4_WTTR142.RES
B) Addition 0.05 mg/kg A: recovery 93% of standard at a spiking level of 0.05 mg KBR 2738/kg in grapes (moist marc); results file KP_KBRA4_WTTR136.RES
C) Control sample for grapes (moist marc); finding: no KBR 2738 detected; results file KP_KBRA4_WTTR134.RES

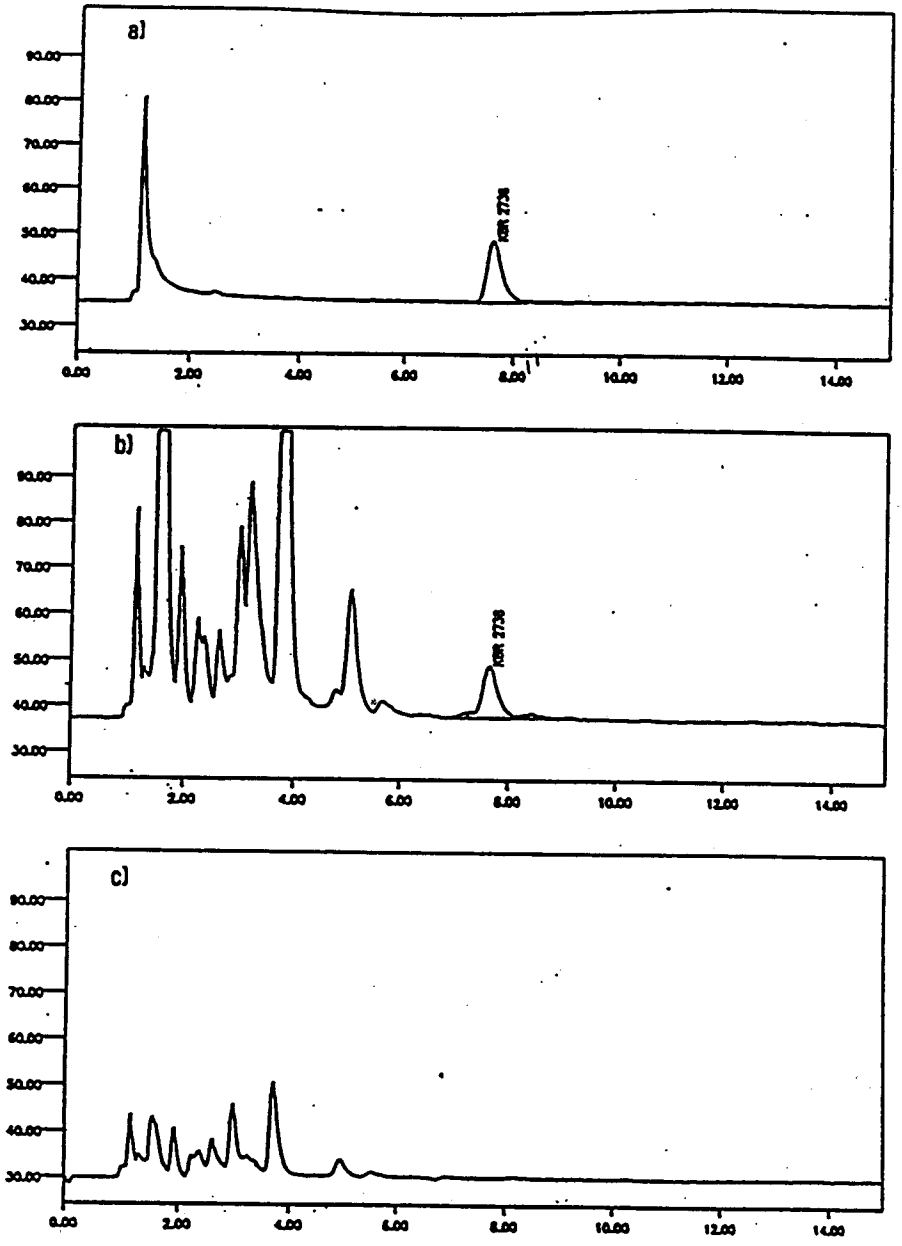

Fig. 14: Determination of the recovery of KBR 2738 in/on grapes (dried marc) by HPLC with an electrochemical detector

A) Standard concentration 0.25 ng/µl (corresponding to 0.05 mg/kg in the evaluation); 10 µl injection volume; solvent = mobile phase; results file KP_KBRA4_WTTR233.RES
B) Addition 0.05 mg/kg A: recovery 93% of standard at a spiking level of 0.05 mg KBR 2738/kg in grapes (dried marc); results file KP_KBRA4_WTTR238.RES
C) Control sample for grapes (dried marc); finding: no KBR 2738 detected; results file KP_KBRA4_WTTR203.RES

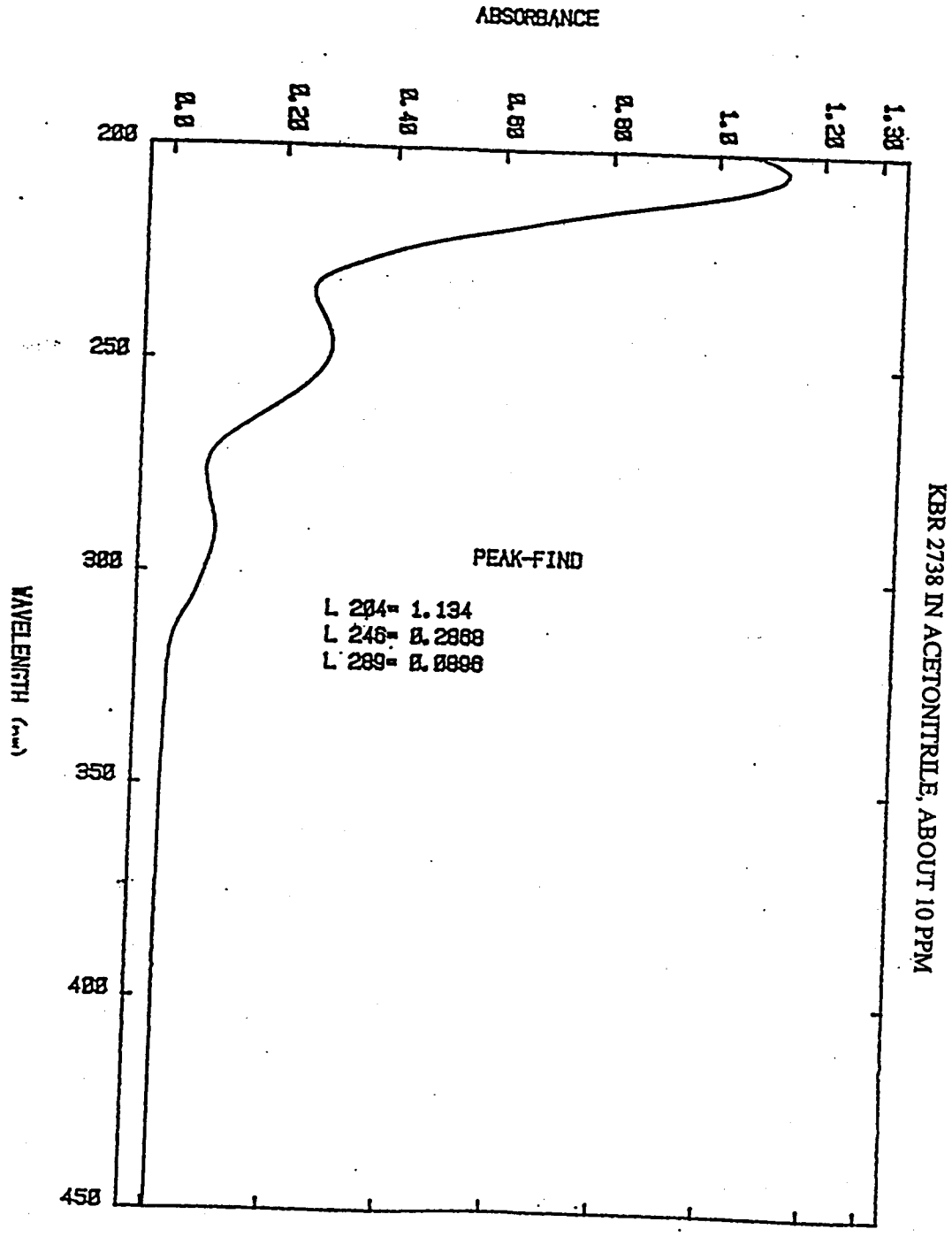

Fig. 15: UV spectrum of KBR

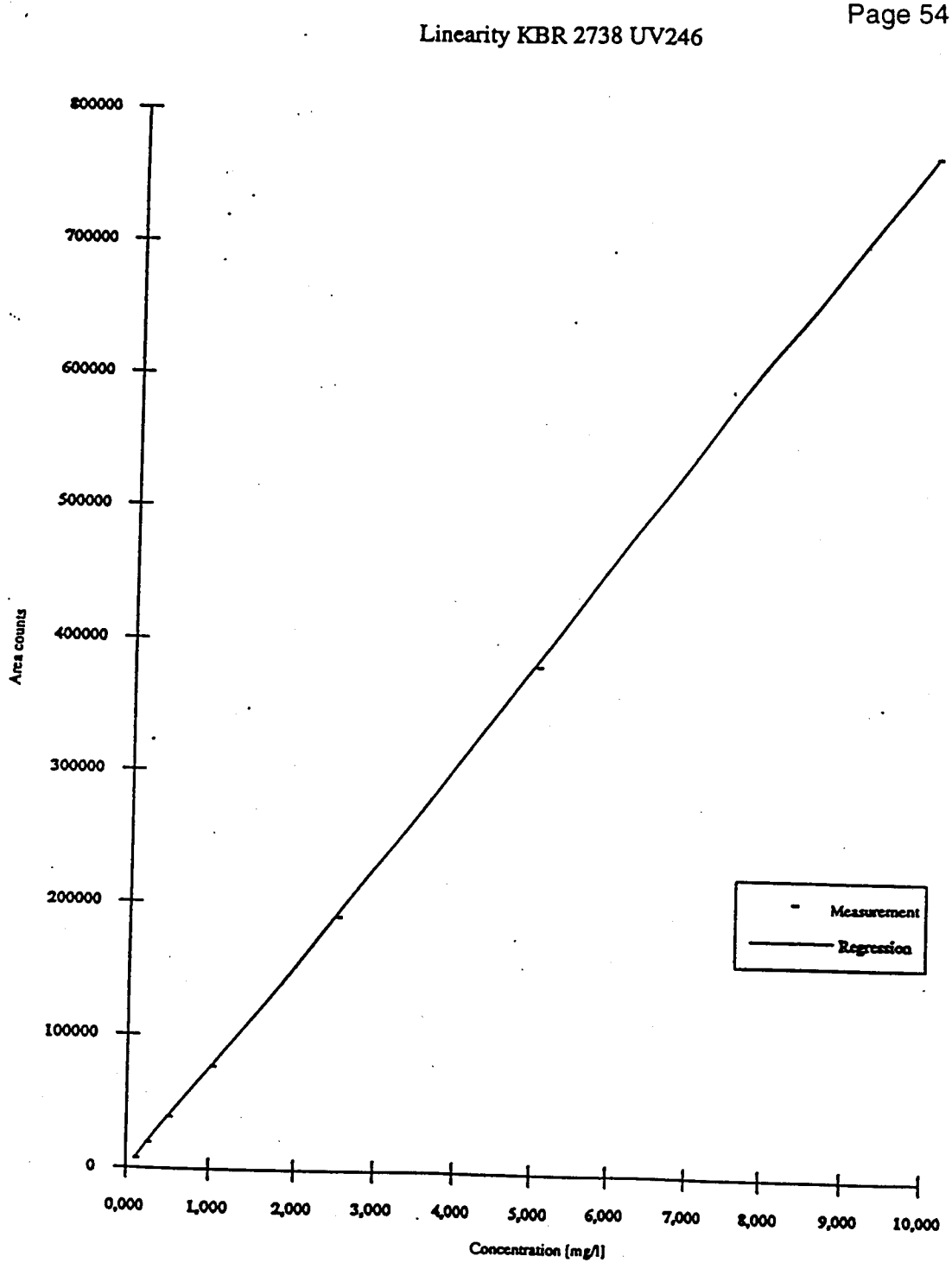

Fig. 16: UV detector linearity in the detection of KBR 2738 at 246 nm

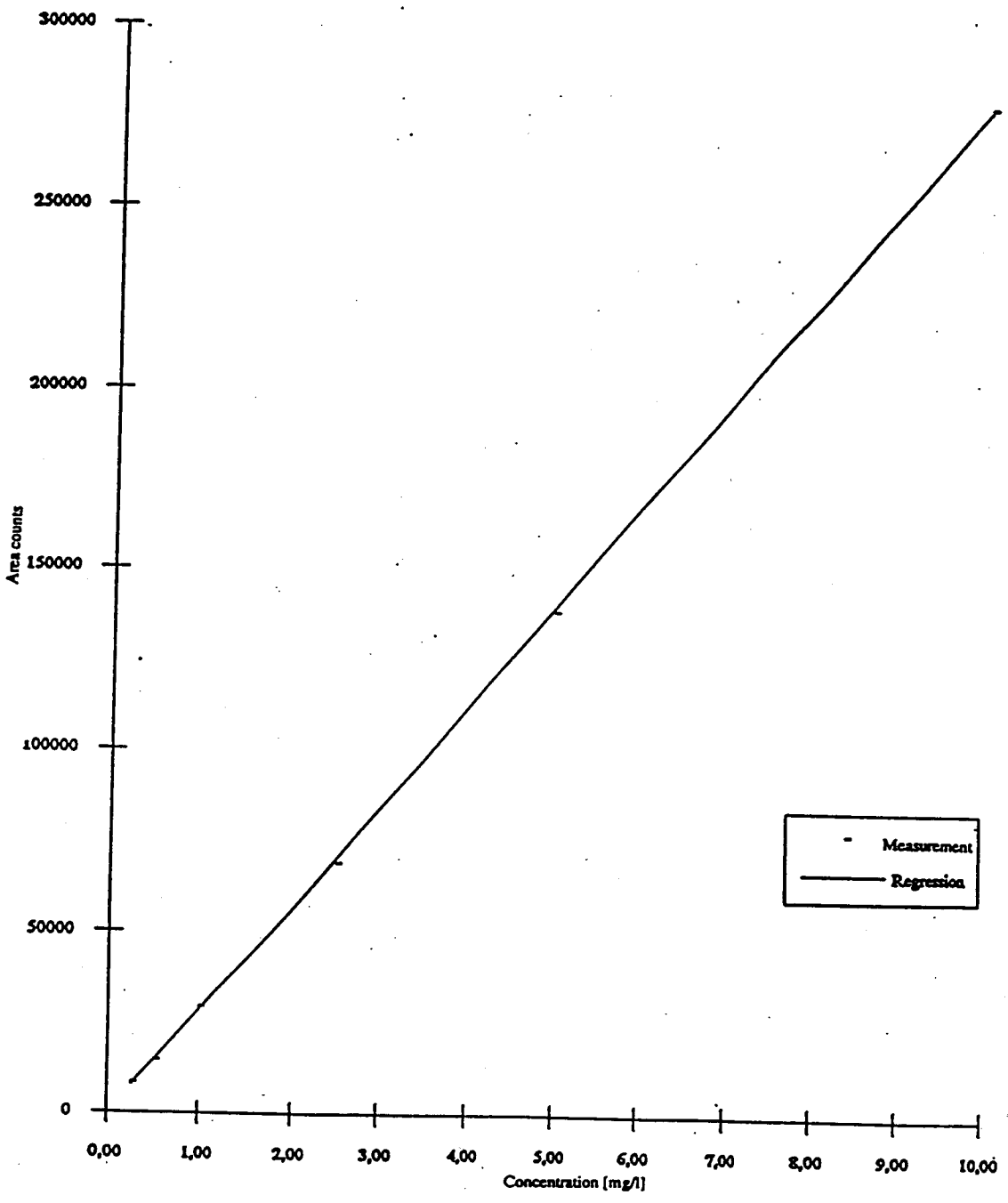

Fig. 17: UV detector linearity in the detection of KBR 2738 at 289 nm

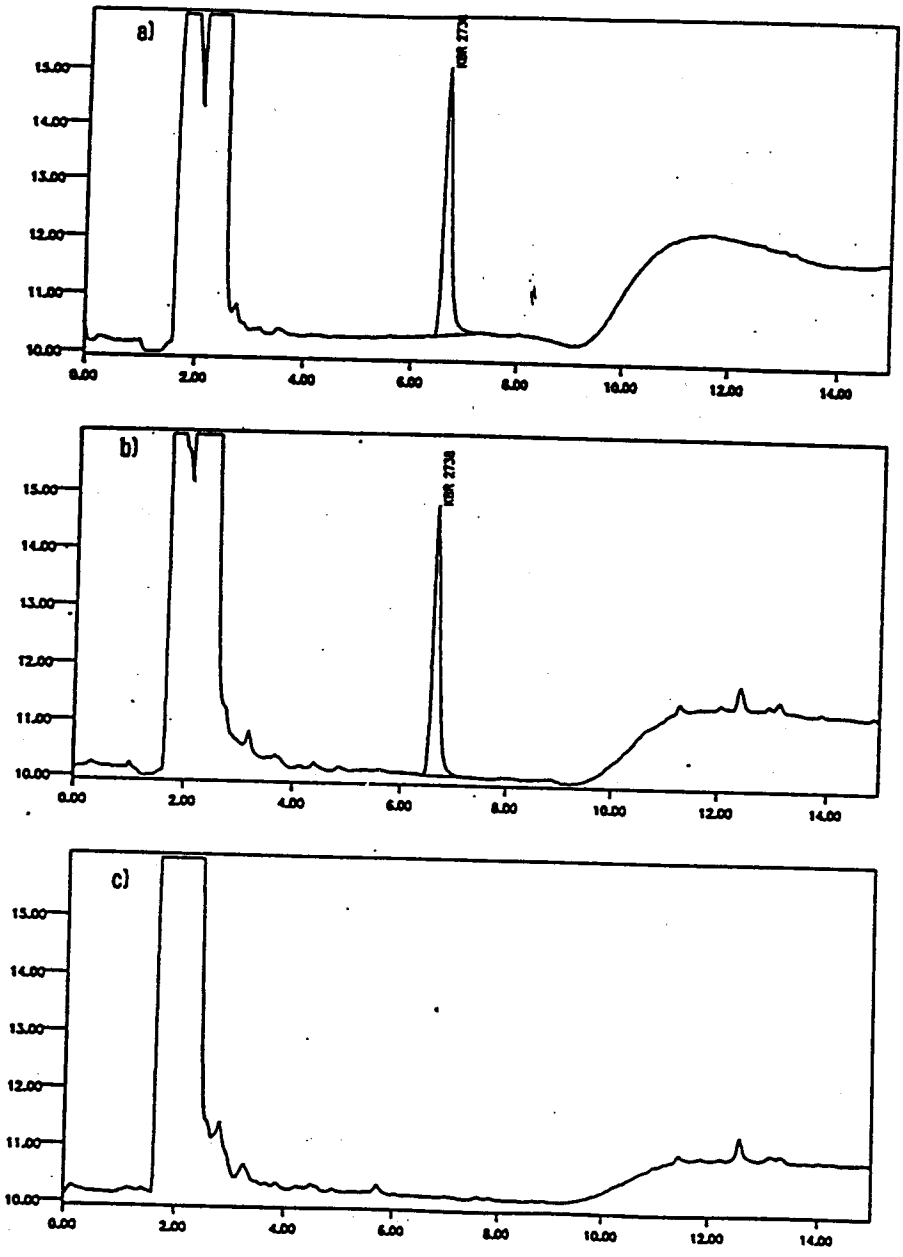

Fig. 18: Strawberries (fruit), UV detection at 246 nm

a) Standard (1.25 ng/µl)
b) Recovery at 5.0 mg/kg
c) Control

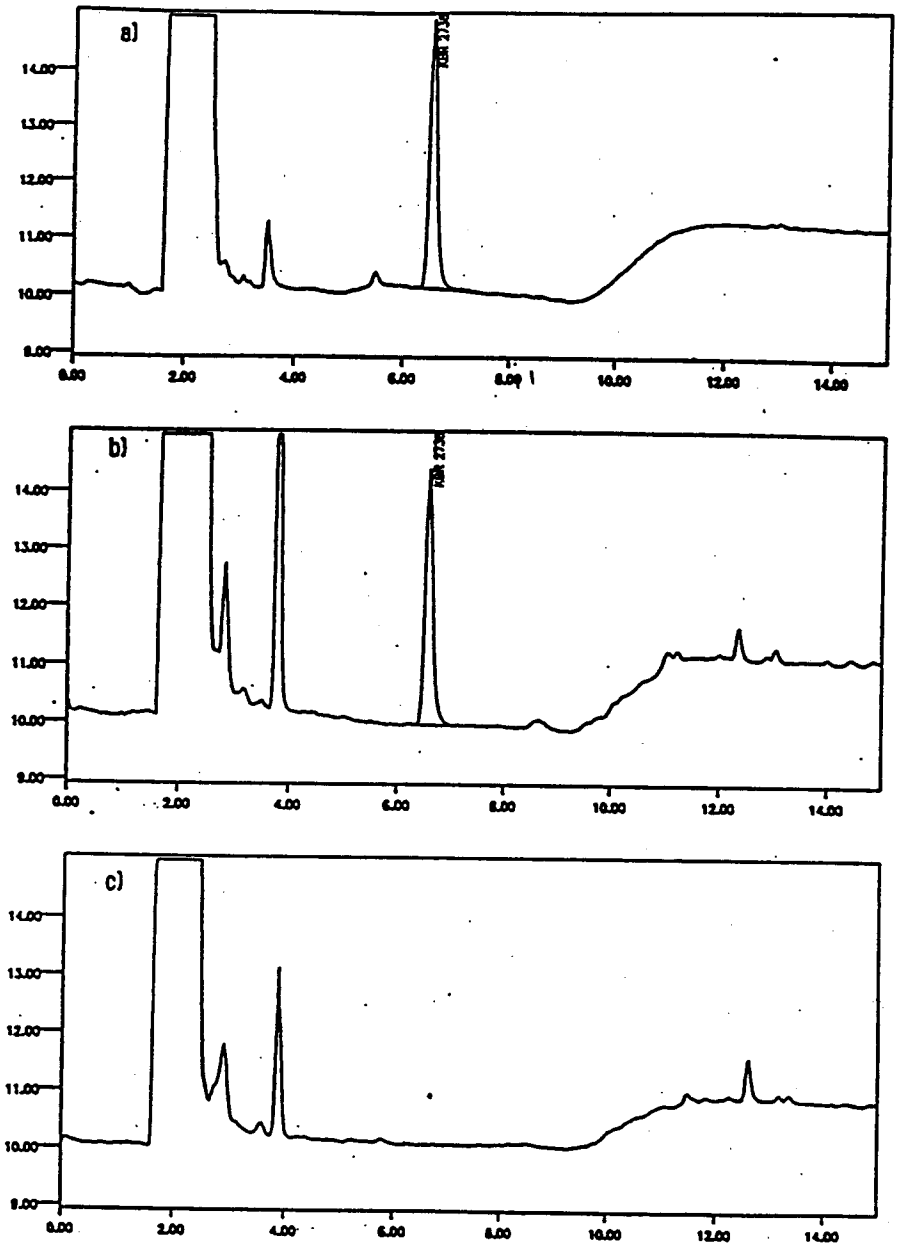

Fig. 19: Cherries (fruit), UV detection at 246 nm

a) Standard (1.25 ng/µl)
b) Recovery at 5.0 mg/kg
c) Control

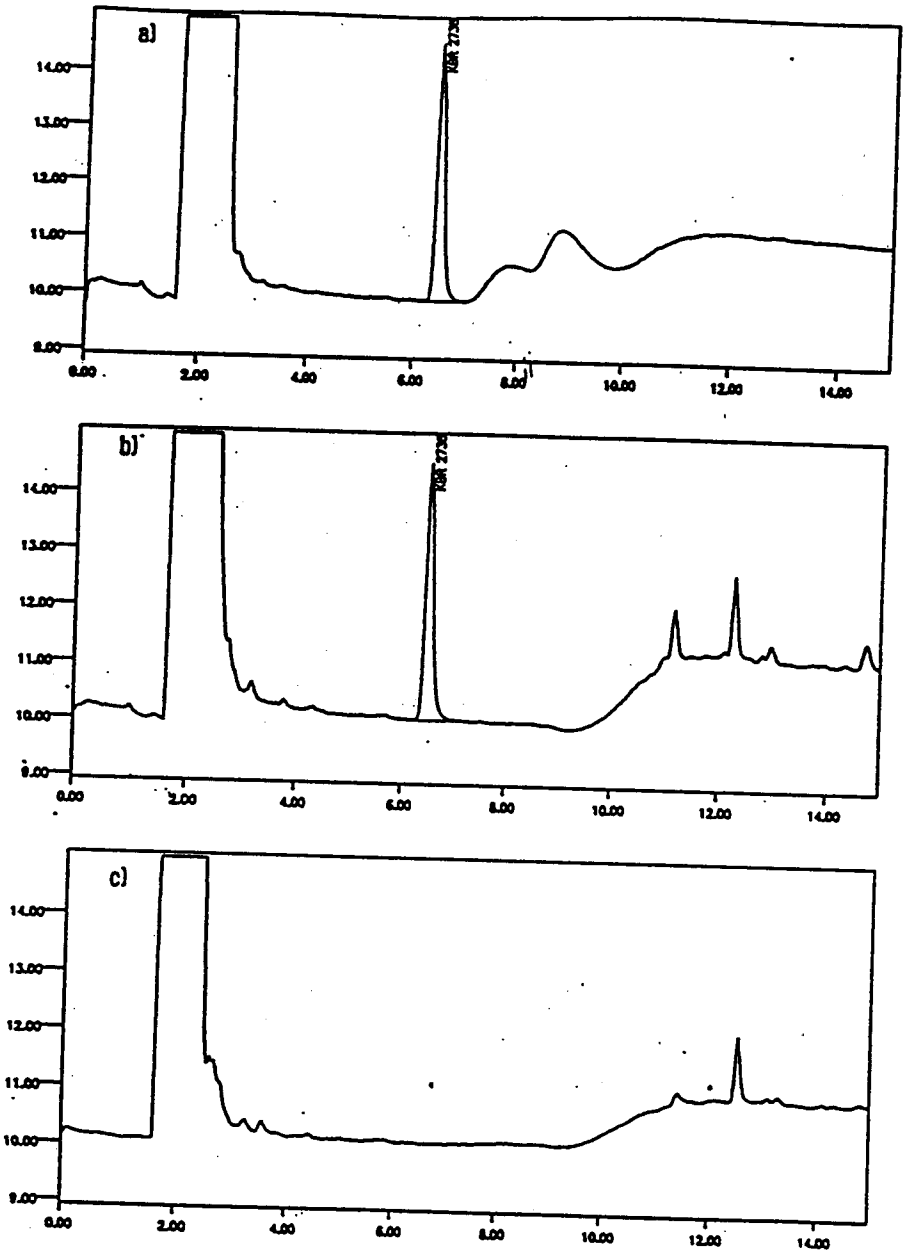

Fig. 20: Peaches (fruit), UV detection at 246 nm

a) Standard (1.25 ng/µl)
b) Recovery at 5.0 mg/kg
c) Control

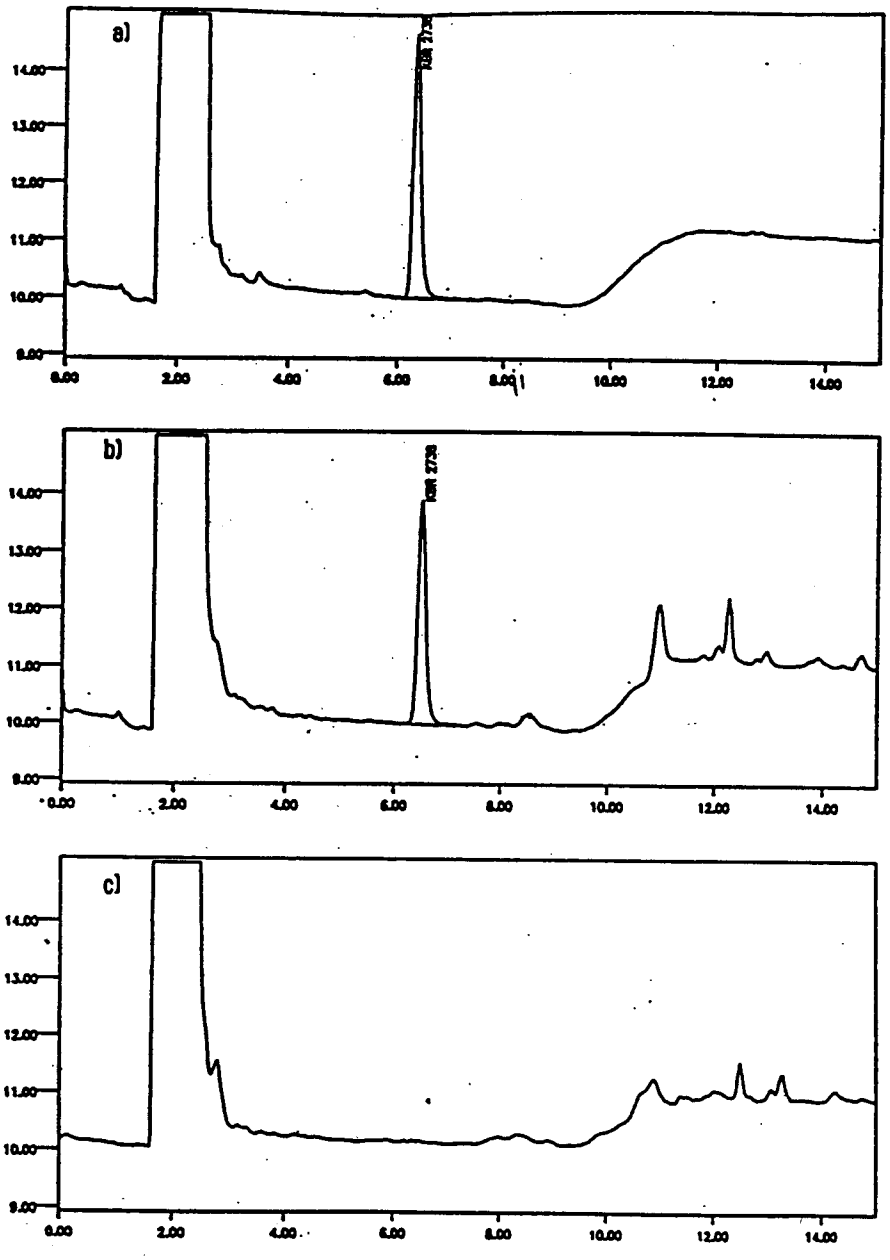

Fig. 21: Plums (fruit), UV detection at 246 nm

a) Standard (1.25 ng/µl)
b) Recovery at 5.0 mg/kg
c) Control

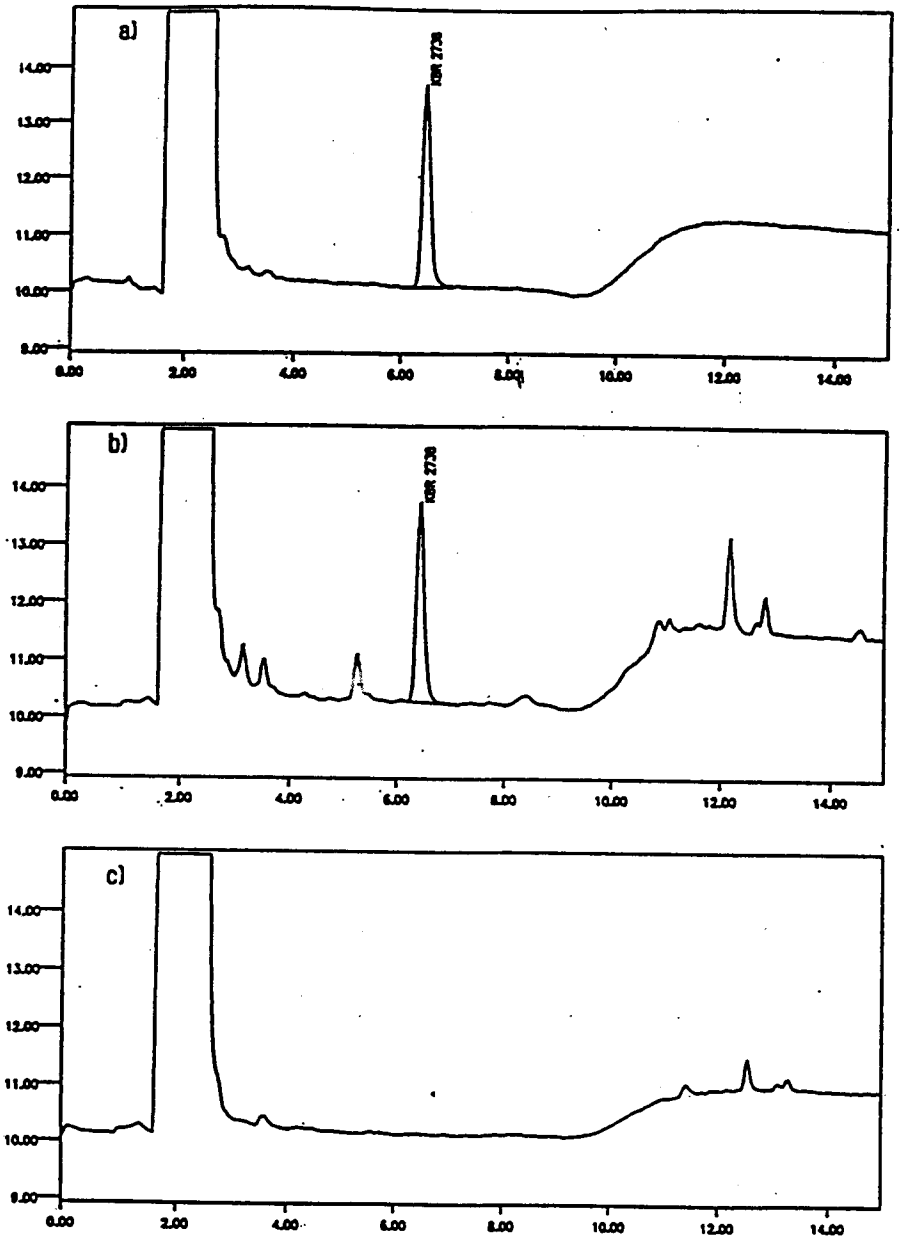

Fig. 22: Grapes (fruit), UV detection at 246 nm

a) Standard (1.0 ng/µl)
b) Recovery at 2.0 mg/kg
c) Control

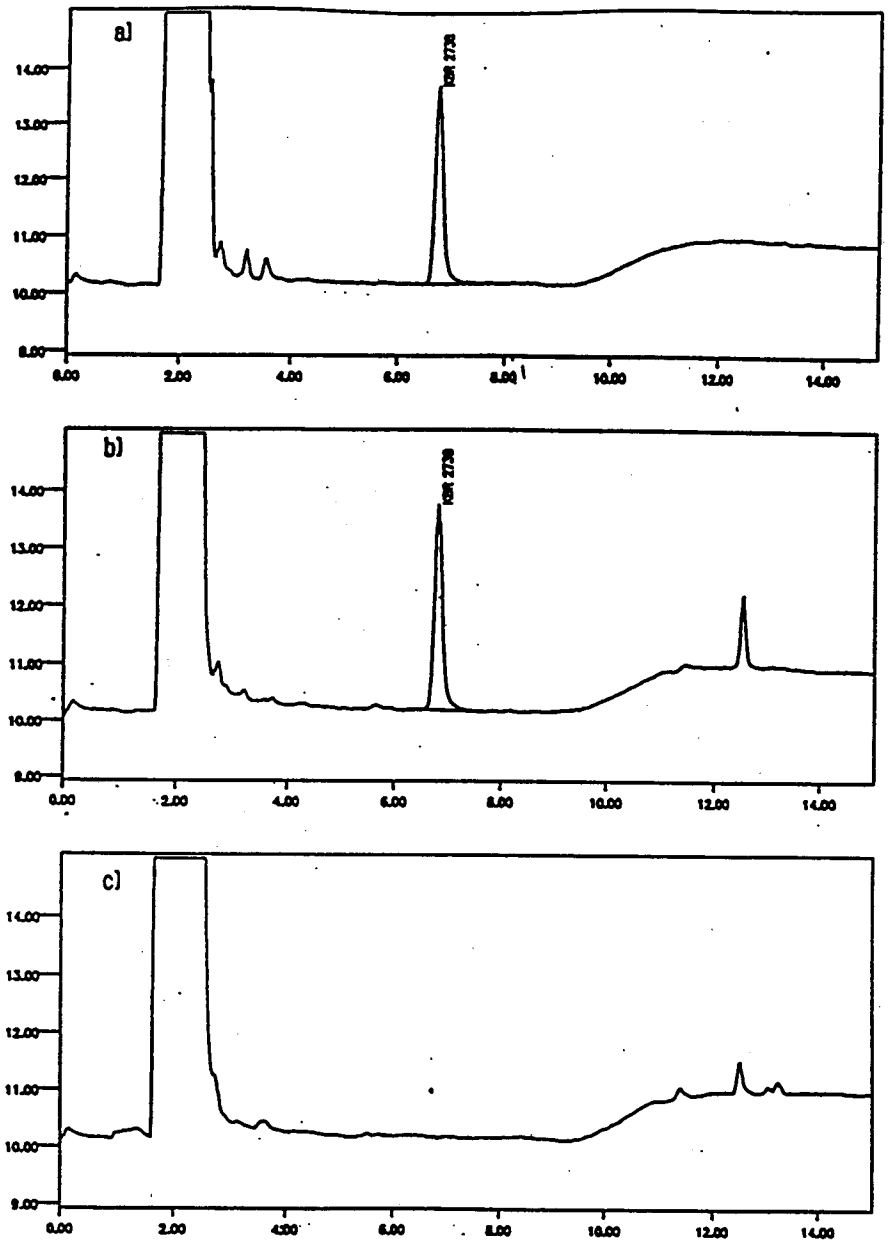

Fig. 23: Grapes (must), UV detection at 246 nm

a) Standard (1.0 ng/µl)
b) Recovery at 2.0 mg/kg
c) Control

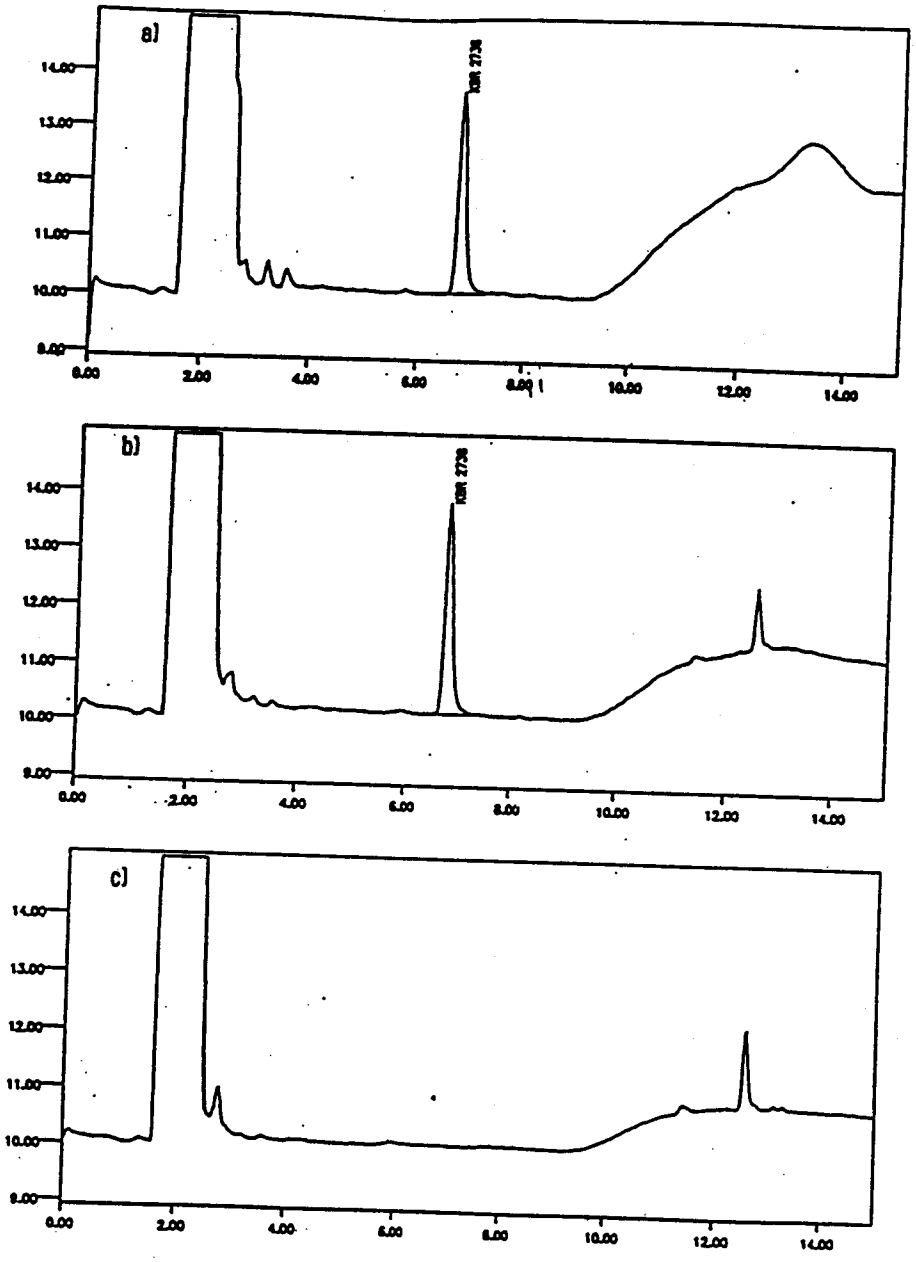

Fig. 24: Grapes (juice), UV detection at 246 nm

a) Standard (1.0 ng/µl)
b) Recovery at 2.0 mg/kg
c) Control

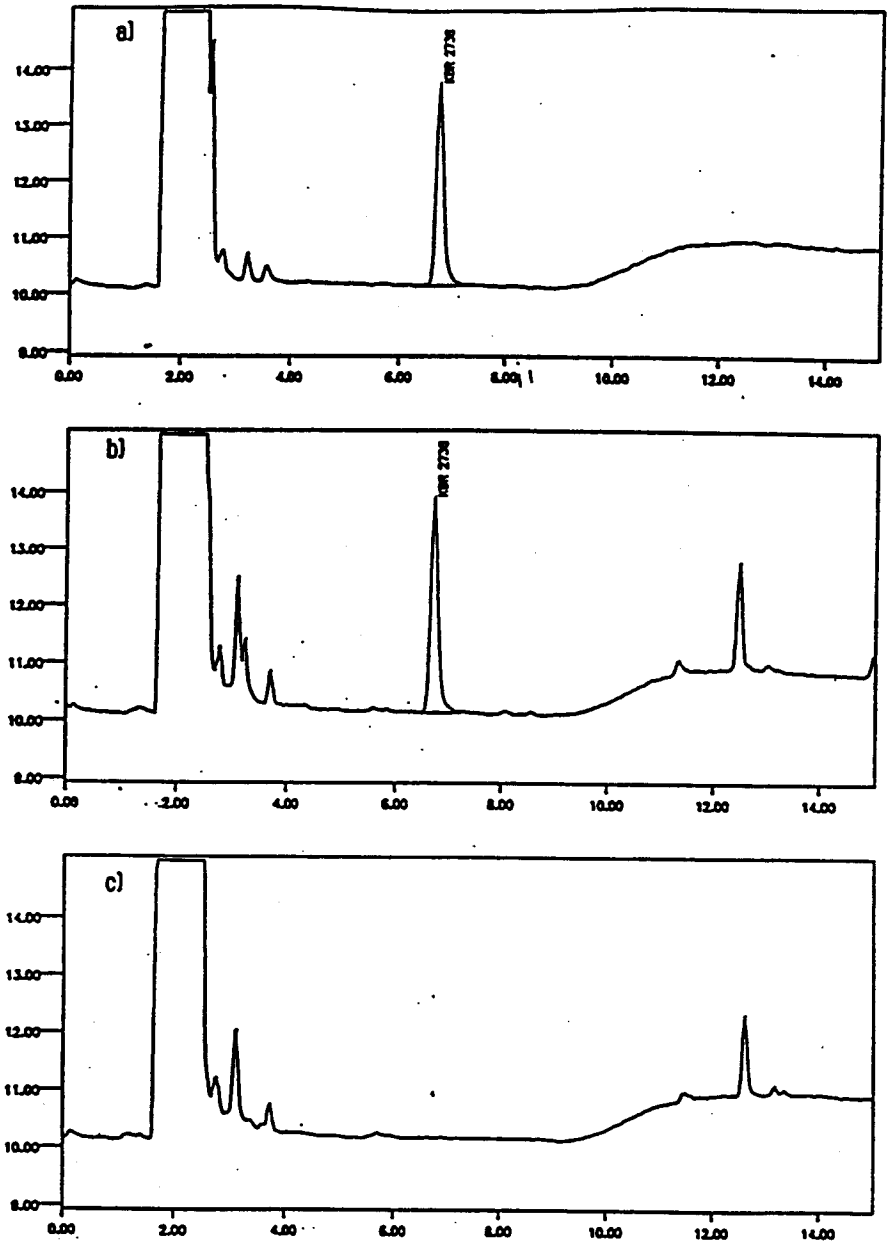

Fig. 25: Grapes (wine), UV detection at 246 nm

a) Standard (1.0 ng/µl)
b) Recovery at 2.0 mg/kg
c) Control

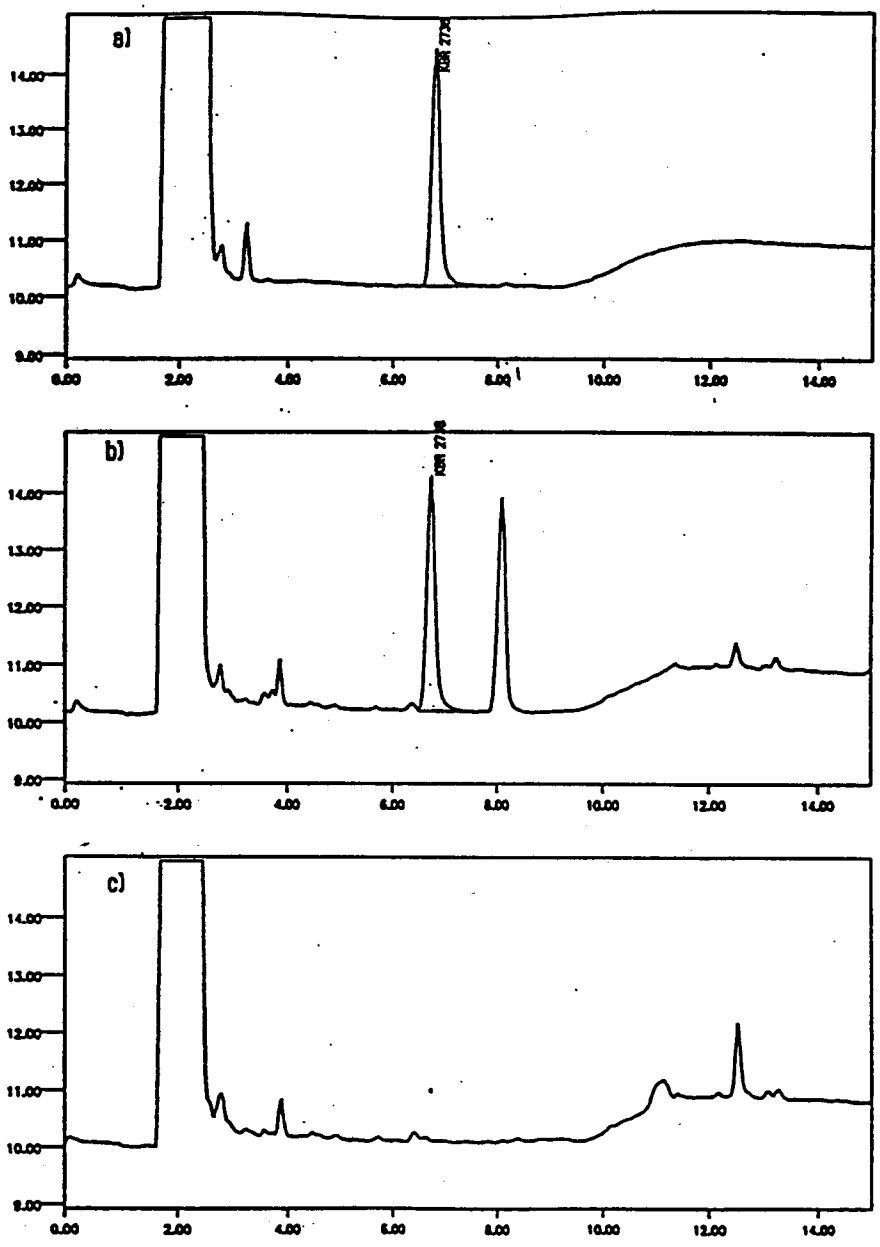

Fig. 26: Grapes (raisins), UV detection at 246 nm

a) Standard (1.25 ng/µl)
b) Recovery at 5.0 mg/kg
c) Control

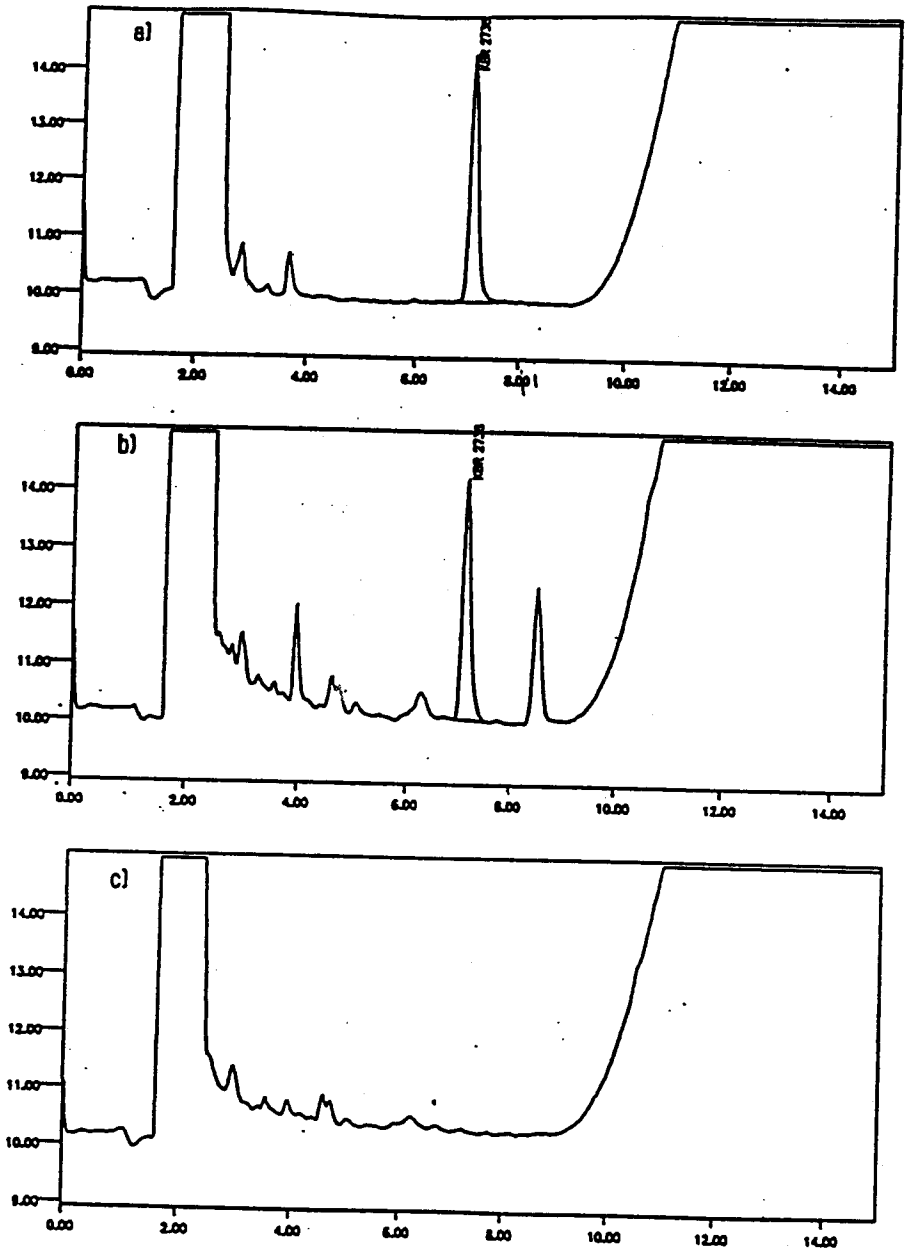

Fig. 27: Grapes (raisin waste), UV detection at 246 nm

a) Standard (1.25 ng/µl)
b) Recovery at 5.0 mg/kg
c) Control

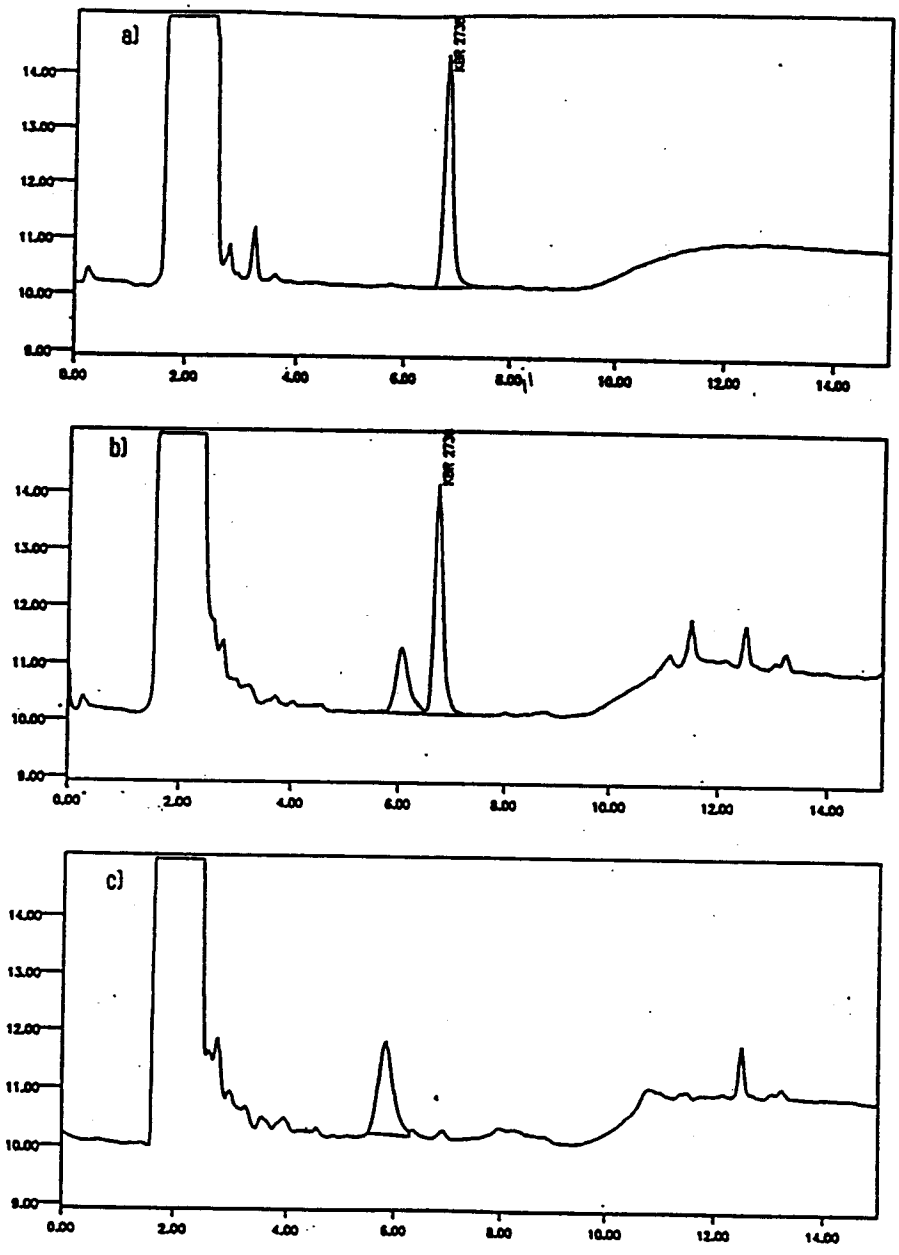

Fig. 28: Grapes (moist marc), UV detection at 246 nm

a) Standard (1.25 ng/µl)
b) Recovery at 5.0 mg/kg
c) Control

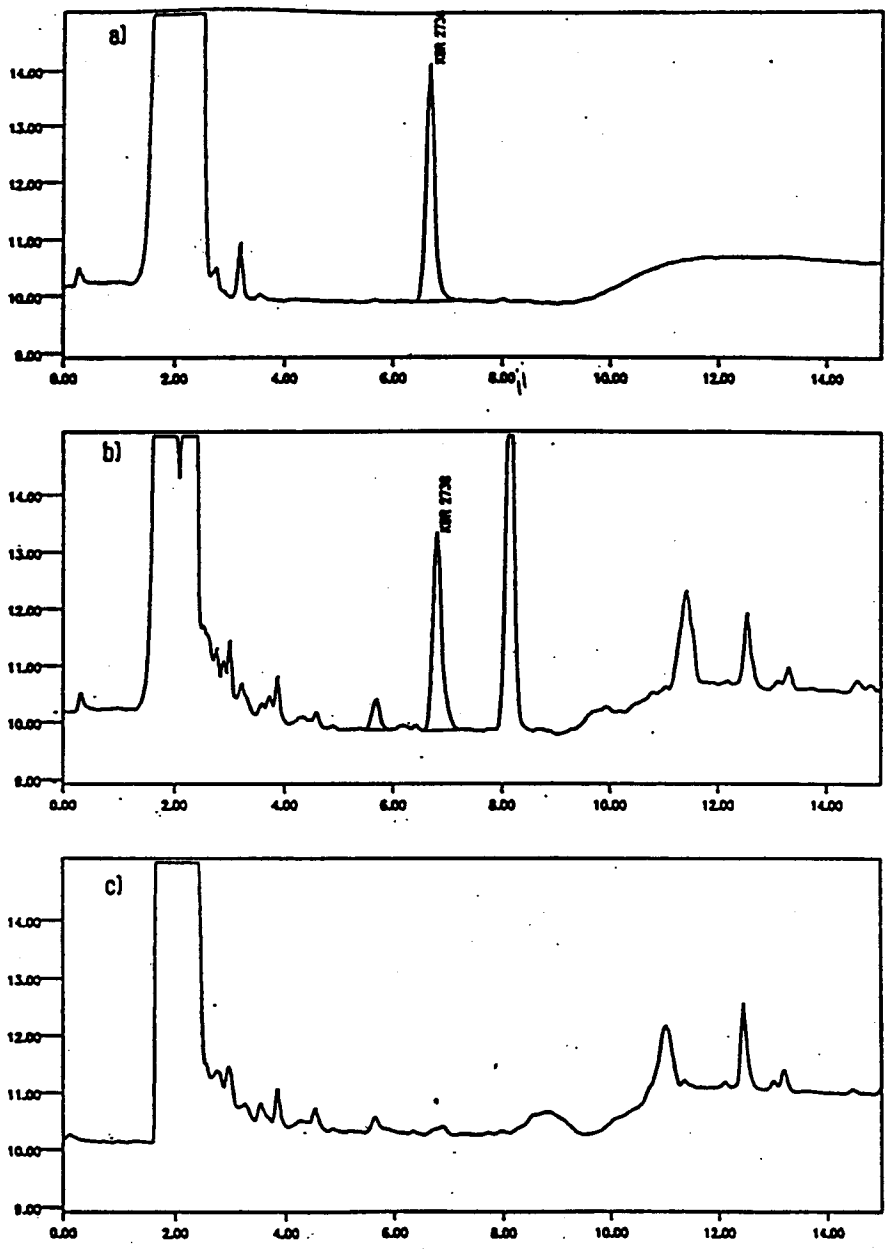

Fig. 29: Grapes (dried marc), UV detection at 246 nm

a) Standard (1.25 ng/µl)
b) Recovery at 5.0 mg/kg
c) Control

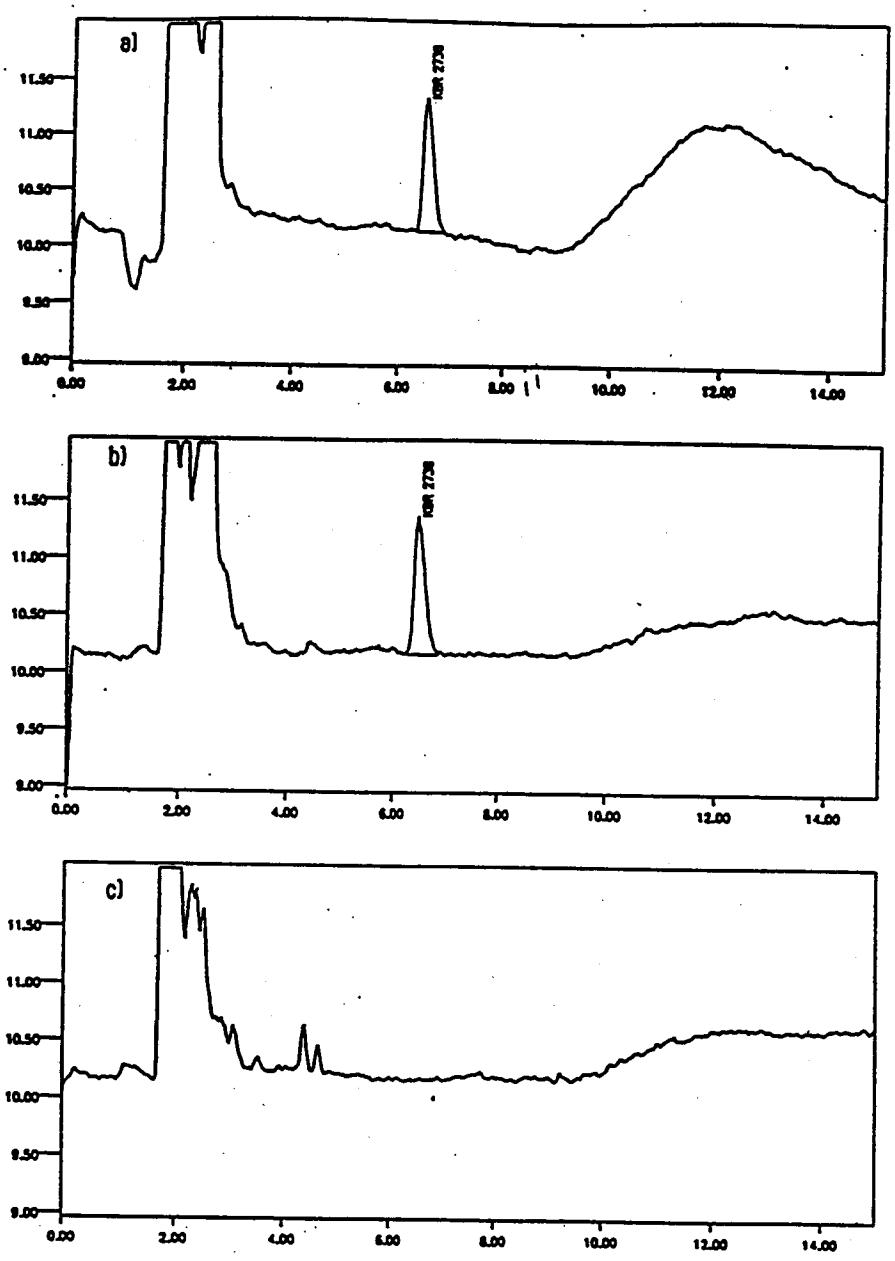

Fig. 30: Strawberries (fruit), UV detection at 289 nm

a) Standard (1.25 ng/µl)
b) Recovery at 5.0 mg/kg
c) Control

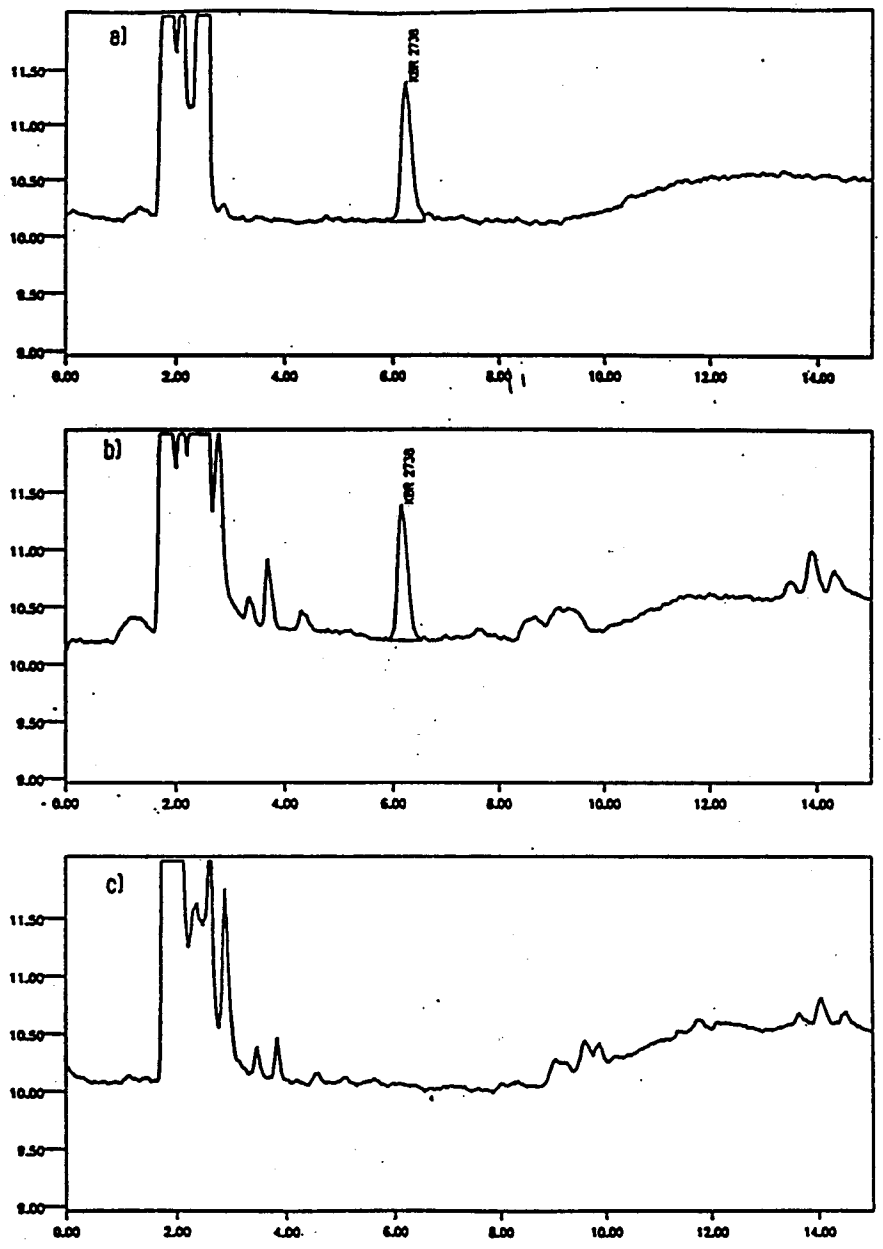

Fig. 31: Cherries (fruit), UV detection at 289 nm

a) Standard (1.25 ng/µl)
b) Recovery at 5.0 mg/kg
c) Control

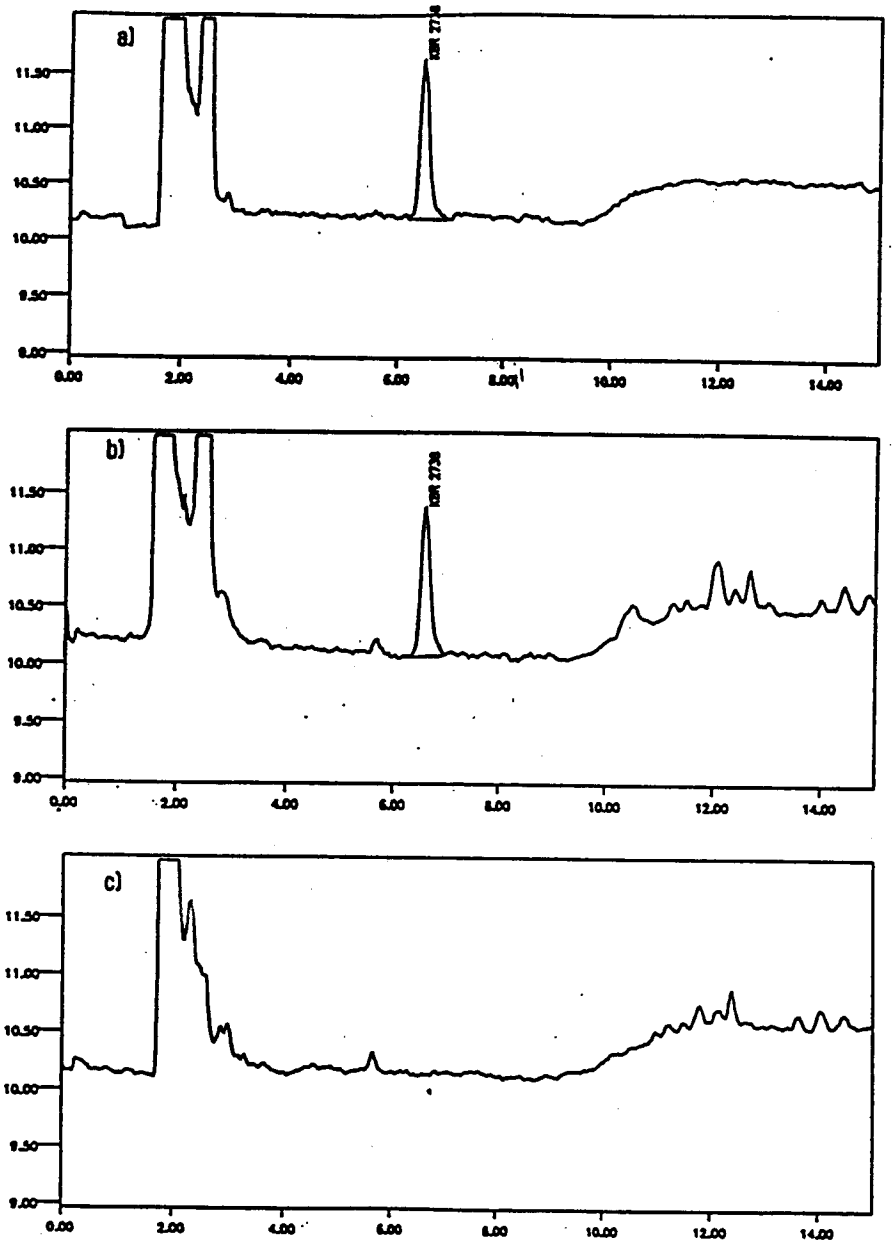

Fig. 32: Peaches (fruit), UV detection at 289 nm

a) Standard (1.25 ng/µl)
b) Recovery at 5.0 mg/kg
c) Control

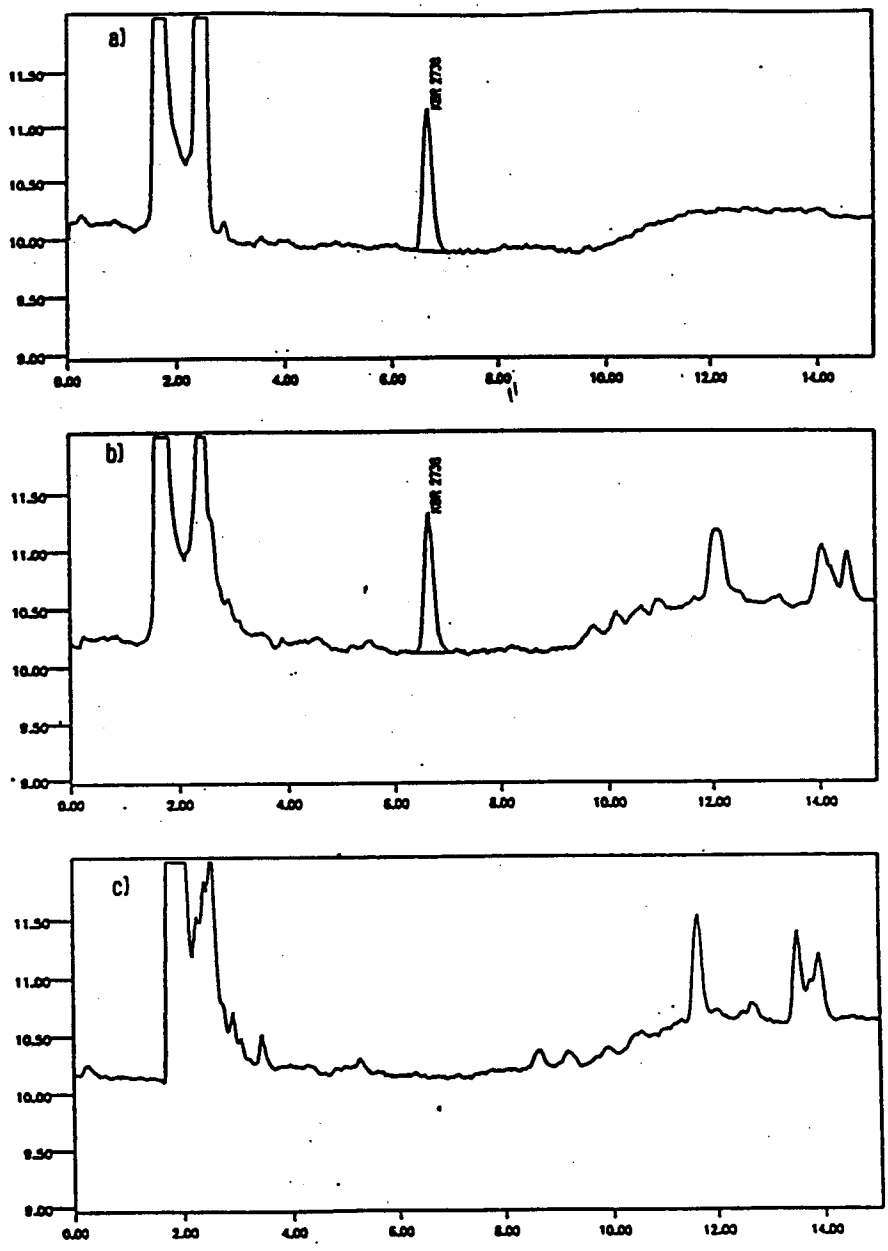

Fig. 33: Plums (fruit), UV detection at 289 nm

a) Standard (1.25 ng/µl)
b) Recovery at 5.0 mg/kg
c) Control

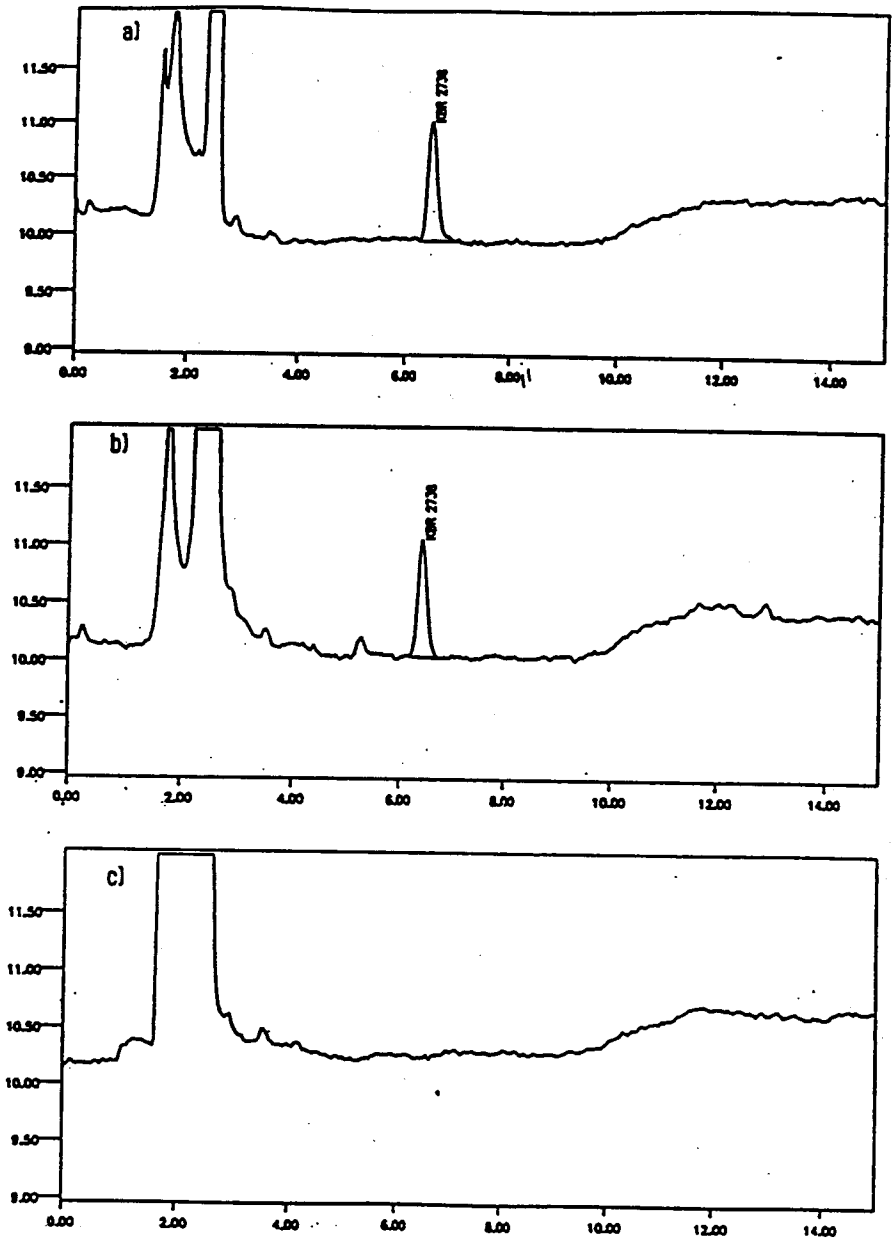

Fig. 34: Grapes (fruit), UV detection at 289 nm

a) Standard (1.0 ng/µl)
b) Recovery at 2.0 mg/kg
c) Control

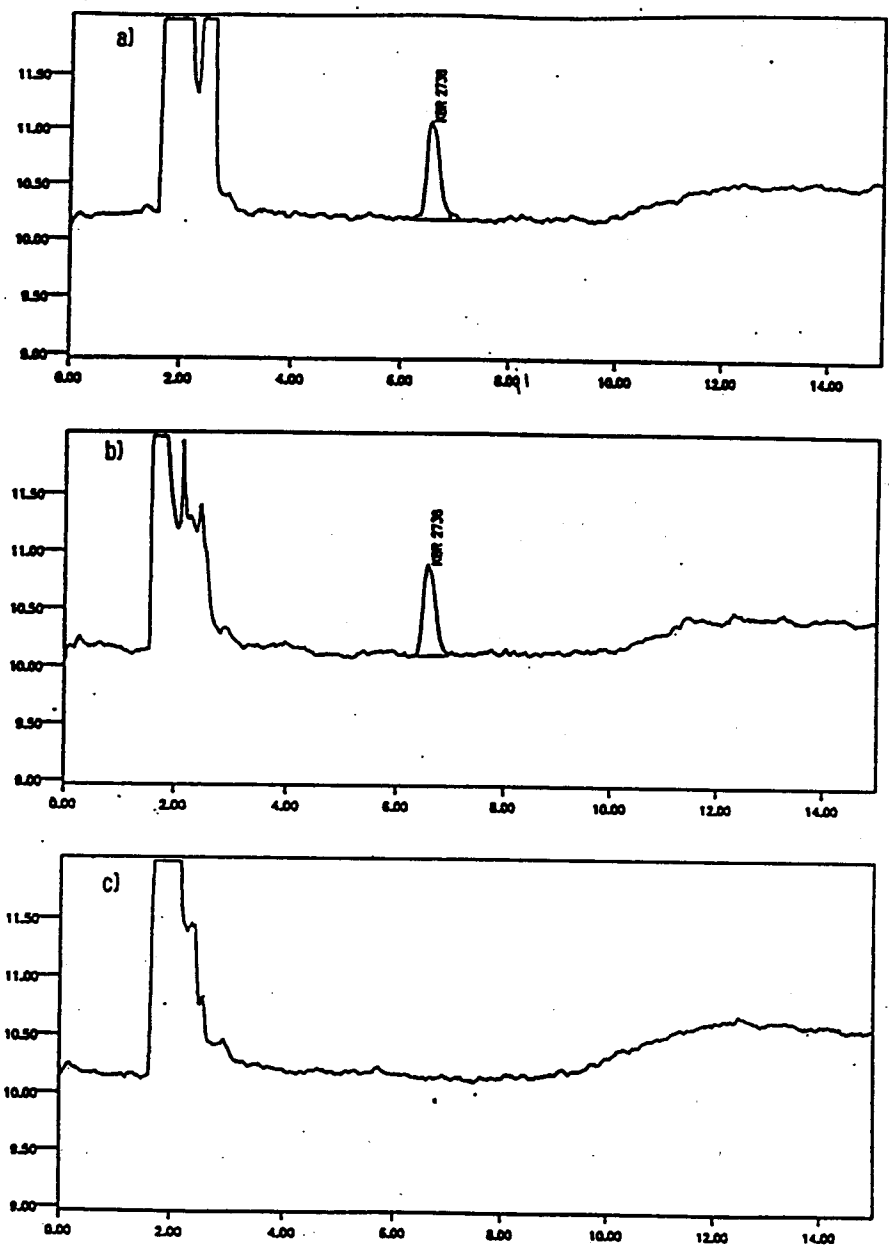

Fig. 35: Grapes (must), UV detection at 289 nm

a) Standard (1.0 ng/µl)
b) Recovery at 2.0 mg/kg
c) Control

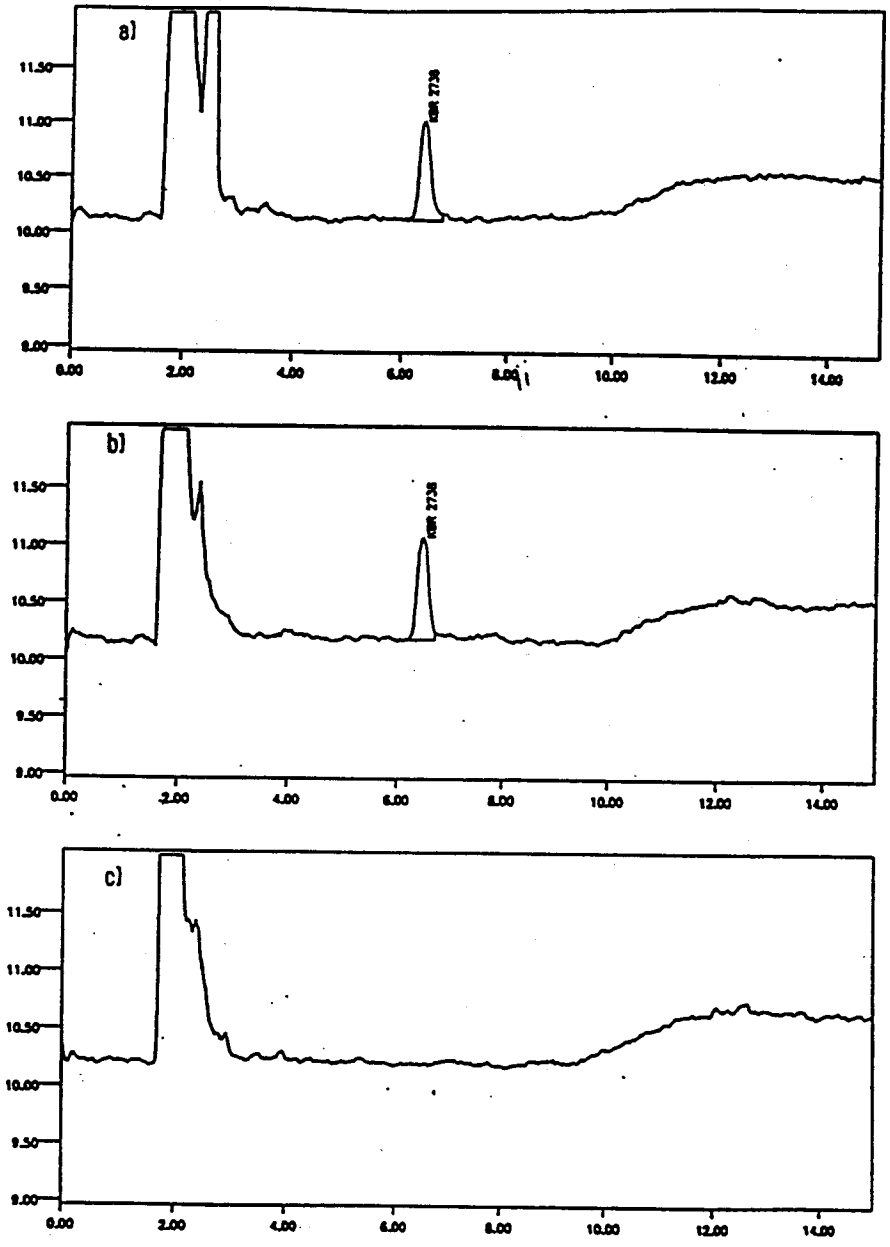

Fig. 36: Grapes (juice), UV detection at 289 nm

a) Standard (1.0 ng/µl)
b) Recovery at 2.0 mg/kg
c) Control

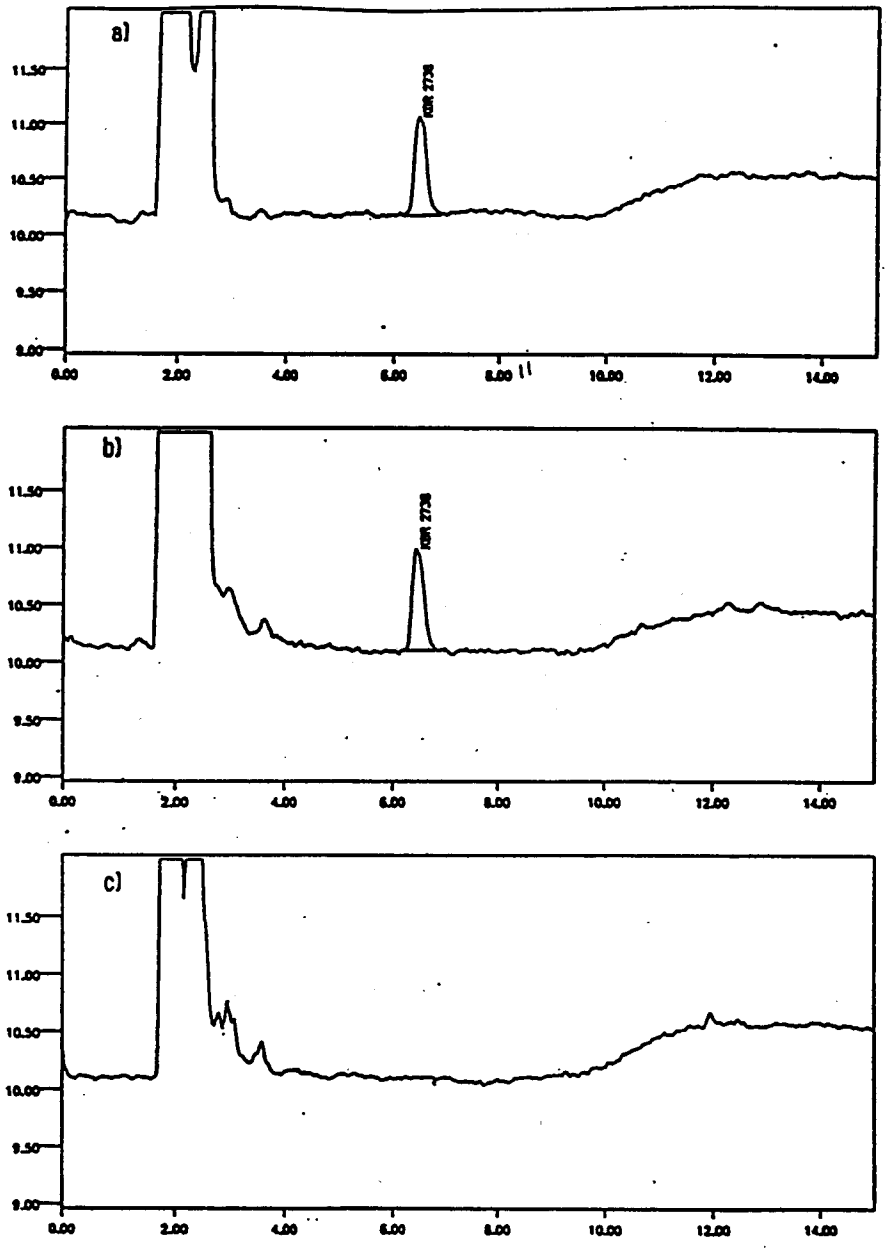

Fig. 37: Grapes (wine), UV detection at 289 nm

a) Standard (1.0 ng/µl)
b) Recovery at 2.0 mg/kg
c) Control

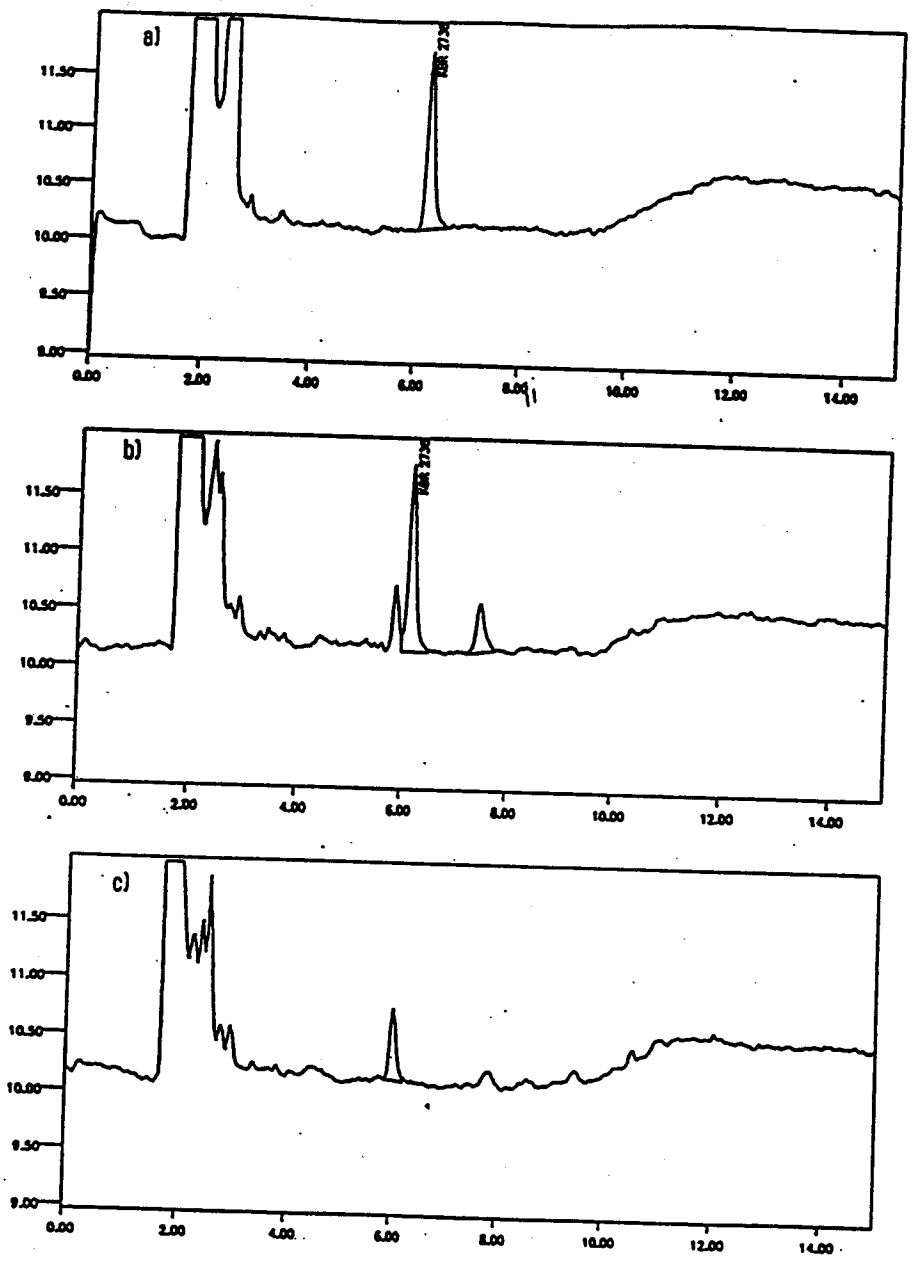

Fig. 38: Grapes (raisins), UV detection at 289 nm

a) Standard (1.25 ng/µl)
b) Recovery at 5.0 mg/kg
c) Control

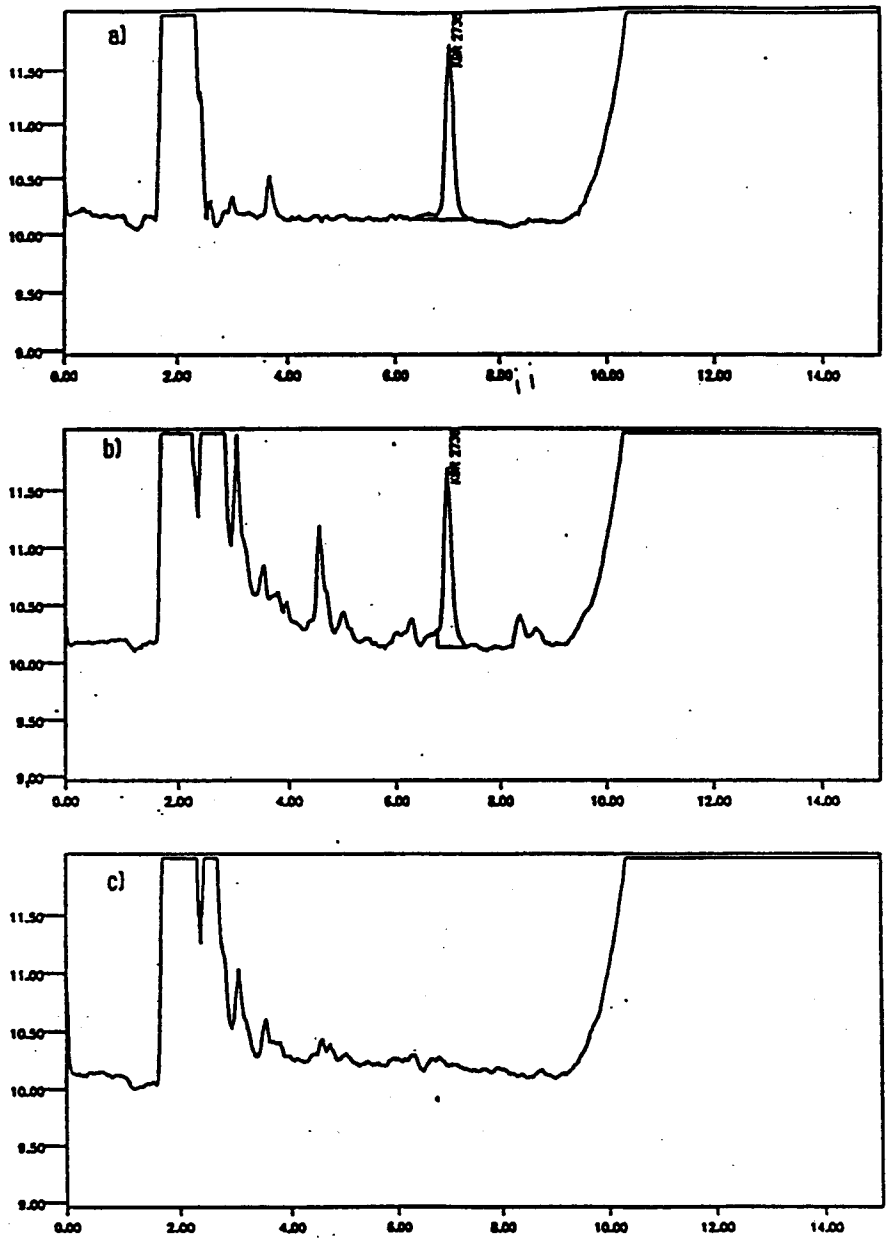

Fig. 39: Grapes (raisin waste), UV detection at 289 nm

a) Standard (1.25 ng/µl)
b) Recovery at 5.0 mg/kg
c) Control

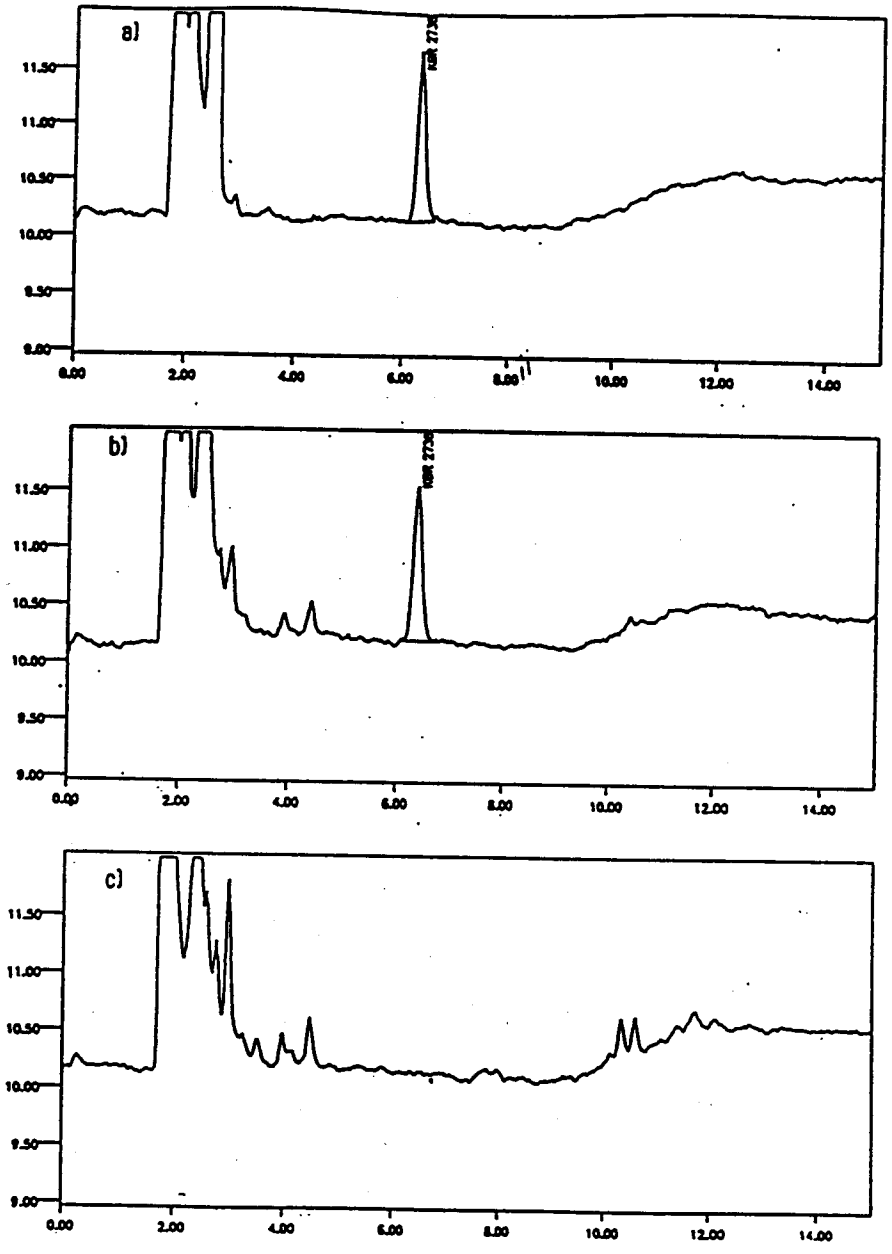

Fig. 40: Grapes (moist marc), UV detection at 289 nm

a) Standard (1.25 ng/µl)
b) Recovery at 5.0 mg/kg
c) Control

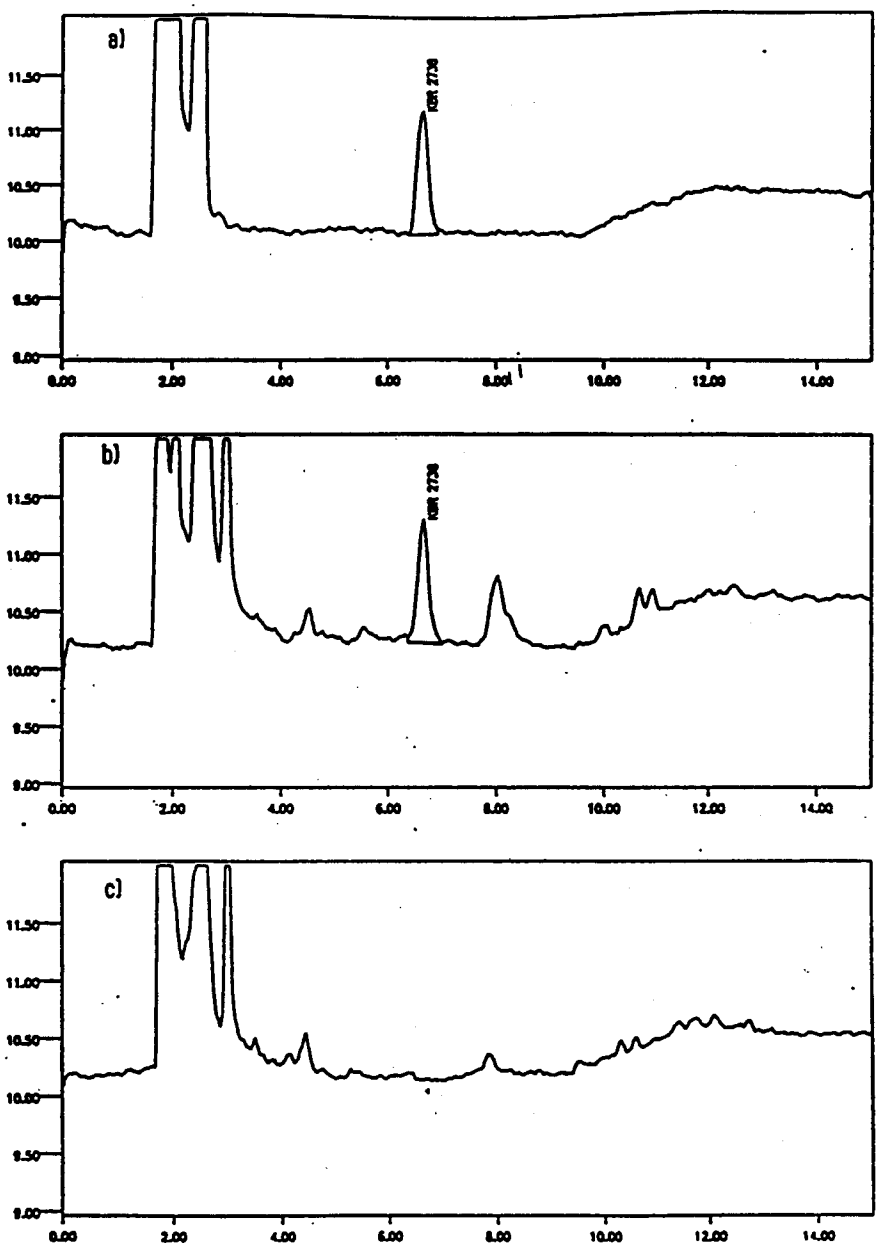

Fig. 41: Grapes (dried marc), UV detection at 289 nm

a) Standard (1.25 ng/µl)
b) Recovery at 5.0 mg/kg
c) Control

Signatures:

Study Director: _____ 19.06.95
Dr. J. Bachmann Date

Manager of Testing Facility: _____ 22.06.95
Dr. J. Köhler Date

Archiving:

All raw data on the study and an authorized copy of the method report will be kept in the central GLP archive of PF-F, Bayer AG, D-51368 Leverkusen for as long as the GLP regulations require. The test and reference substances will be kept in the archive of PF-E/FT-EA, Bayer AG, D-51368 Leverkusen for as long as the quality of the substance as shown by the results of follow-up analyses make this seem reasonable.

Reference:

Deutsche Forschungsgemeinschaft (1987)
Rückstandsanalytik von Pflanzenschutzmitteln, Mitteilung VI der Senatskommission für Pflanzenschutz-, Pflanzenbehandlungs- und Vorratschutzmittel, Methodensammlung der Arbeitsgruppe Analytik, 1.-9. Lieferung, VCH Verlagsgesellschaft, Weinheim.

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for original signature.

GLP-Bestätigung (Statement of Compliance)

Prüfnummer: (Study Number)	**P 612 4 1008**
Prüfsubstanzen: (Test Substance)	KBR 2738
Prüfleiter: (Study Director)	Dr. J. Bachmann PF-E/MR, Geb. 6610 D-51368 Leverkusen Tel. 02173/38-3886
Prüfeinrichtung: (Testing Facility)	Bayer AG PF-E/MR, Geb. 6610 D-51368 Leverkusen
Titel der Prüfung: (Titel of the Study)	Methode zur Bestimmung der Rückstände von KBR 2738 in pflanzlichen Probenmaterialien durch HPLC

Die vorliegende Methodenvalidierung ist keine sogenannte *nichtklinische experimentelle Prüfung* im Sinne des § 19a Abs. 1 Chemikaliengesetz (ChemG). Die oben genannte Prüfung wurde trotzdem nach den Grundsätzen der Guten Laborpraxis (GLP) gemäß Anhang 1 ChemG in Verbindung mit § 19a Absatz 1 ChemG vom 25.07.1994 durchgeführt.

Unterschrift Prüfleiter:

i.V. F. Nüßlein
(Dr. J. Bachmann)

21.6.95
(Datum)

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for English translation.

Referat GLP	
Quality Assurance Statement	
Report No.: MR-144/94	**Study No.:** P 61241008
Title of report: Methode zur bestimmung der Rückstände von KBR 2738 in/auf Erdbeere, Kirsche, Pfirsich, Pflaume und Traube sowie in Verarbeitungsprodukten von Traube durch HPLC	

Die Durchführung der Prüfung wurde inspiziert und der Bericht wurde von der Qualitätssicherungseinheit der Bayer AG, PF-S, überprüft. Die Daten der Inspektionen sind im folgenden angegeben:

Date of Protocol Inspection:	Date of Report to Management:
July 28, 1994	July 29, 1994
Date of Study Inspection:	Date of Report to Management:
August 8, 1994 September 6, 1994	August 9, 1994 September 6, 1994
Date of Final Report Audit:	Date of Report to Management:
June 21, 1995	June 21, 1995

The results reported in this study accurately reflect the raw data.

L.P.Herrmann L. P. Herrmann
Quality Assurance Unit, PF-S

Date: 21.06.95

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for English translation.

Unterschriften:

Prüfleiter (Study Director)	: i.V. F. Nüßlein Dr. J. Bachmann	19.6.95 Datum
Leiter der Prüfeinrichtung (Manager Testing Facility)	: J. Köhler Dr. J. Köhler	22.6.95 Datum

Archivierung:

Alle Rohdaten zur Prüfung und eine autorisierte Kopie des Berichts zur Methode werden im zentralen GLP-Archiv von PF-F, Bayer AG, D-51368 Leverkusen solange aufbewahrt, wie es die GLP-Regularien verlangen. Prüf- und Referenzsubstanzen werden im Archiv von PF-E/FT-EA, Bayer AG, D-51368 Leverkusen solange aufbewahrt, wie die Qualität der Substanzen eine Nachanalyse als sinnvoll erscheinen läßt.

Literatur:

Deutsche Forschungsgemeinschaft (1987)
Rückstandsanalytik von Pflanzenschutzmitteln, Mitteilung VI der Senatskommission für Pflanzenschutz-, Pflanzenbehandlungs- und Vorratschutzmittel, Methodensammlung der Arbeitsgruppe Analytik, 1.-9. Lieferung, VCH Verlagsgesellschaft, Weinheim.

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for English translation.