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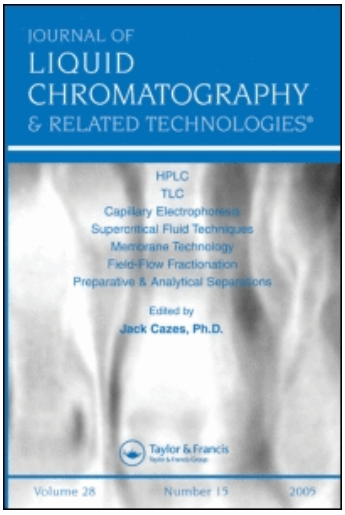

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Determination of Residues of Chemotherapeutic and Antiparasitic Drugs in Food Stuffs of Animal Origin with Liquid Chromatography and UV/VIS Diode-Array Detection

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DETERMINATION OF RESIDUES OF CHEMOTHERAPEUTIC AND ANTIPARASITIC DRUGS IN FOOD STUFFS OF ANIMAL ORIGIN WITH LIQUID CHROMATOGRAPHY AND UV/VIS DIODE-ARRAY DETECTION

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ABSTRACT

An HPLC method is described which enables the qualitative and quantitative analysis of residues of pharmacologically active substances in food stuffs of animal origin. Egg samples were spiked with the therapeutic drugs sulfapyridine and its N-acetyl metabolite, with ethopabat, chloramphenicol, meticlorpindol, metronidazol, ipronidazol, furazolidone and nicarbazin and with pyrazon and benzothiazuron as internal standards in the range of 0.02 to 0.1 mg/kg. The drugs were extracted with acetonitrile and purified by liquid-liquid partition steps. They were separated on a reversed phase column and detected with a multi-signal UV/Vis diode-array detector at 3 different wavelengths: 275, 315 and 360 nm. The peak's identity was confirmed by comparing retention times and UV spectra with those of standards. Peaks were checked for homogenity by comparing spectra at the peak's upslope, apex and downslope. The detection limit was in the range of 0.001 to 0.05 mg/kg.

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INTRODUCTION

Modern intensive animal breeding demands permanent suppression of different diseases, caused e.g. by vira, bacteria, protozoa or fungi. A great number of chemotherapeutic drugs are available for the prevention and control of these diseases. They are different in their chemical structure as well as in their therapeutic effect. Antiparasitic drugs should protect animals against endo- and and ektoparasites. They are routinely applied in a preventive mode mode of action e.g. as feed additives.

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There are several hundreds of admitted pharmacologically active substances. In 1982, in the Federal Republic of Germany (FRG) about 3000 drugs containing more than 200 different substances were admitted [1]. In 1983, about 290 antiparasitic drugs were admitted in the FRG [2]. An index summarizes more than 500 pharmacologically active substances available in FRG [3]. After application, residues of these drugs can be found in food of animal origin like milk, eggs or meat. Because of the toxic nature of the drugs the Bundesgesundheitsamt (Federal Health Institute) in FRG demands the following detection limits for residue analysis, if withdrawal periods should be fixed on account of elimination studies: for sulfonamides and their N-acetyl metabolites 0.1 mg/kg and for nitrofuranes, nitromidazoles and chloramphenicol 0.001 mg/kg [4]. In total, detection limits in the range of 0.001 - 0.1 mg/kg are demanded for more than 70 compounds by the Bundesgesundheitsamt [4]. In the meantime, these detection limits became the character of tolerance levels for residues in meat at meat inspection.

The official supervision should be able to detect residues of the admitted substances as well as of non admitted ones. Because of the high number of compounds to be analyzed, multiresidue methods are very useful for the analysis.

A multiresidue method for the analysis of more than 60 chemotherapeutics and antiparasitics has been developed by Malisch [5]. The method is based on HPLC with UV detection. The advantage of HPLC versus GC is reduced sample preparation [6]. The extracted drugs can be analyzed directly without time consuming and cumbersome derivatization steps. The advantage of HPLC versus thin layer chromatography is improved separation. In addition, liquid chromatographs can be connected to UV/Vis diode-array detectors for selective multi-signal detection. With fast scanning diode-array detectors spectra can be acquired during a peak's elution. Spectra from unknown compounds can be overlayed with those of a standard to confirm a peak's identity. Applications and limitations of this technique are discussed in this paper.

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MATERIALS AND METHODS

Principle

Residues of certain chemotherapeutic drugs (sulfonamides and their N4-acetyl metabolites, nitrofuranes, chloramphenicol and diaphenylsulfon) and antiparasitics (nitroimidazoles, benzimidazoles, meticlorpindol, nicarbazin and ethopabat) in eggs, milk or meat are

extracted with acetonitrile. After a first purification by liquid-liquid partitioning with hexane the co-extracted water is separated with sodium chloride/methylene chloride. The advantage of acetonitrile in comparison to other organic solvents is based on its miscibility: acetonitrile is miscible with water, but not with solutions of salt in water. Therefore pharmacologically active substances can be extracted from water-containing food with acetonitrile in homogeneous phase. Afterwards, the co-extracted water can be separated easily by addition of sodium chloride [7].

The organic phase is concentrated and purified in miniaturized steps first with water/methanol/acetonitrile - hexane and than with water - ethylacetate. After this, the sample is concentrated to a few microliters.

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Reagents

For extraction and purification steps

(a) buffer, pH about 2.1 (1 m citric acid, 1 m KH_2PO_4)

(b) acetonitrile (HPLC grade)

(c) n-hexane (dist.)

(d) dichlormethane (dist.)

(e) water (dist.)

(f) ethylacetate (dist.)

(g) acetonitrile/methanol/water (1:1:1 v:v:v)

(h) sodium chloride

(i) pH indicator paper (e.g. 0.5 - 5 and 5.5 - 9)

(j) internal standard: solution of 0.5 mg/ml benzthiazuron (Ehrensdorfer, D-8900 Augsburg, FRG) and 0.5 mg/ml pyrazon (=chloridazon) (Promochem, D4230 Wesel, FRG) in methanol.

(k) sulfapyridine and chloramphenicol (Serva, D-6900 Heidelberg, FRG), N-acetyl sulfapyridine (friendly gift by Dr. Arnold, Bundesgesundheitsamt, D-1000 Berlin, FRG. According to MS-spectrum with Chemical Ionisation: diacetylated), ethopabat and nicarbazin (MSD AGVET, D-8022 Gruenwald, FRG), meticlorpindol (Dow Chemical, D-6000 Frankfurt, FRG), ipronidazol (Hoffmann LaRoche, D-7889 Grenzach-Wyhlen, FRG), metronidazol (Bayer AG, D-5600 Wuppertal, FRG), furazolidone (Sigma, D-8024 Deisenhofen, FRG)

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Eluent for HPLC

(a) buffer, pH 4.8 (0.02 m sodium acetate)

(b) acetonitrile/water (60:40 v:v)

Apparatus

Important note: active surfaces of glassware must be avoided. Therefore it is necessary to clean the used glassware not in an automatic dish washer with hot alkaline solutions but by hand with a few drops of a cleansing fluid in water, afterwards with water, methanol and ethylacetate.

For extraction and purification steps

(a) brown glassware: wide-bore Erlenmeyer, 300 ml; round-bottom flask, 500 ml; peak bottom flask, 10 ml; separatory funnel, 500 ml; screw-cap tubes, 10 ml, with teflon coated seal

(b) stirring rod (e.g. Ultra-Turrax with TP18/2N-rod and Thyristor regulator

(c) Tissue homogenizer (e.g. Braun Multiquick, Waring Blendor)

(d) centrifuge including high-grade steel centrifuge beakers (250 ml); if missing: Witt's vessel with Buchner funnel (diameter 9 cm) and filter paper (cleaned by extraction with methanol/ethylacetate 1:1 for 3d)

(e) small centrifuge (for 10 ml screw cap tubes)

(f) disposable pipettes.

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For HPLC

(a) glass vials for an automatic sampler

(b) HPLC system: all chromatograms were recorded with a Hewlett-Packard 1084B liquid chromatograph, equipped with an autosampler, an HP 1040M diode array detection system and an HP 79994A ChemStation.

(c) HPLC column: 250x4.6 mm, RP18-modified silicagel (Spherisorb ODS), 5 um

Procedure

Preparation of samples

(a) egg samples: homogenize 2 to 3 eggs with the Ultra-Turrax stirring rod. Adjust the pH to 5-6 by adding dropwise buffer solution while stirring.

(b) milk: extract the milk after adjusting the pH-value to 5-6.

(c) meat: cut the meat to pieces and mince it. Normally a pH adjustement is not required for the meat.

Extraction

(a) Egg and meat samples:

Mix 50 g of the food with 20 ul of the internal standard and with 150 ml acetonitrile in an Erlenmeyer flask and homogenize for 1 min with the Ultra-Turrax. Homogenization in a Waring Blendor is especially recommended for meat. Suck the homogenisate under slightly reduced pressure into another Erlenmeyer flask. Decant the filtrate into a separatory funnel, rinse the Erlenmeyer flask with 100 ml hexane and decant it also into the separatory funnel. (Note: centrifugation of the homogenized extract and decanting of the supernatant liquid instead of sucking is also possible)

(b) Milk samples

Homogenize 50 g of the prepared food in a centrifuge glass with 20 ul of the internal standard and with 150 ml acetonitrile for 1 min with the Ultra-Turrax. Centrifugate for 10 min with 3000 rpm, decant the supernatant liquid into a separatory funnel and add 100 ml hexane.

Degreasing, separation of water and concentrating

Shake the separatory funnel for 30 s and let the lower acetonitrile phase off into an Erlenmeyer flask. Dispose the hexane phase and return the acetonitrile phase into the separatory funnel. Add sodium chloride (3 to 4 g) and shake for solving. Rinse the Erlenmeyer flask with 100 ml dichloromethane and add it to the separatory funnel. Shake the solution and than wait until phase separation. Dispose the lower phase (water)

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with the unresolved salt and decant the upper organic phase into a rounded flask. Concentrate the extract in a rotation evaporator at a water-bath temperature of 35 deg C until dryness.

Purification by partition

Dissolve sequentially the residue in the round-bottom flask with 2 ml each of first water, than methanol and last acetonitrile (dissolve more dirty extracts with supersonant treatment for 10 s) and transfer the solution by means of a glass funnel into a screw cap tube. Add 3 ml of hexane (with rinsing the round bottle flask) , shake the test tube and centrifugate for 3 min at 3600 rpm. Siphone the upper hexane phase (by means of a water jet blast or a pipette) and dispose it. With egg samples repeat this degreasing procedure once again. For this rinse the round-bottom flask with 3 ml hexane and add the hexane to the water/methanol/acetonitrile mixture in the tube. Shake the mixture, centrifugate and siphon the hexane phase off.

Return the lower phase to the round-bottom flask and evaporize the methanol/acetonitrile portion by means of a rotation evaporizer. There should remain about 0.5 to 1 ml water which is transferred again to the tube. Rinse the round-bottom flask with a little bit water and add the water to the tube up to a mark of 2.5 ml. After this, rinse the round-bottom flask with 5 ml ethylacetate and transfer it to the tube. Shake and centrifugate for 3 min at 3600 rpm. Siphon the upper ethylacetate phase by means of a pipette into a peak-bottom flask. Repeat this procedure once again with 5 ml ethylacetate. Concentrate the combined ethylacetate extracts to dryness, resolve it in 350 ul acetonitrile/methanol/water and transfer it into a vial.

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To separate solid particles centrifugate the vial for 1 min at 1300 rpm.

HPLC Analysis

Mobile phase A: buffer pH 4.8 (0.02m sodium acetate),
B: acetonitrile/water
Gradient elution starting with 8 % B, constant for 5 minutes, than after 7 min - 20% B, 14 min - 23% B, 16 min - 33% B, 19 min - 40% B, 21 min - 50% B, 26 min - 60 % B, 30 min - 80 % B, 33 min - 90% B, 43 min - 90% B, 55 min - 8 % B.
Flow rate: 1.5 ml/min, Injected volume: 20 ul

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RESULTS AND DISCUSSION

Spiked sample for demonstration

9 drugs were selected as representative samples for the demonstration of the multi-signal detection and peak confirmation 2 internal standards were used. Table 1 lists these substances, their concentration in the external standards and the spiked concentrations in the food samples. The amounts of the substances are roughly in the range of the detection limits demanded by the Bundesgesundheitsamt For this demonstration, egg samples were spiked with the drugs. For a comparison, blank samples were extracted and analyzed in the same way as the spiked samples.

Table 1. List of spiked substances and internal standards and their respective concentrations

Spiked substances	Concentration
Sulfapyridine	14 ng/ul resp. 0.1 mg/kg
N-Acetylsulfapyridine	14 ng/ul resp. 0.1 mg/kg
Ethopabat	14 ng/ul resp. 0.1 mg/kg
Chloramphenicol	7 ng/ul resp. 0.05 mg/kg
Meticlorpidol	7 ng/ul resp. 0.05 mg/kg
Metronidazol	7 ng/ul resp. 0.05 mg/kg
Ipronidazol	7 ng/ul resp. 0.05 mg/kg
Furazolidone	3 ng/ul resp. 0.02 mg/kg
Nicarbazin	3 ng/ul resp. 0.02 mg/kg
Spiked internal standards	
Pyrazon	14 ng/ul resp. 0.1 mg/kg
Benzothiazuron (chlorizadon)	14 ng/ul resp. 0.1 mg/kg

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Multi-signal detection

The investigated compounds show different UV absorbance maxima in a range of 270 to 360 nm. Therefore, in order to analyze all the individual compounds at their highest sensitivity, three different wavelengths are selected for the chromatographic signals: 275, 315 and 360 nm. The chromatograms of the standard mixture obtained at the various wavelengths are overlayed and displayed in figure 1.

All compounds, besides ipronidazol (No 6) and chloramphenicol (No 7) are separated. It is clearly shown, that the relative response at various wavelengths is different for the individual compounds. At these 3 wavelengths all compounds are detectable. Generally, besides the signals as displayed in figure 1 we also acquire

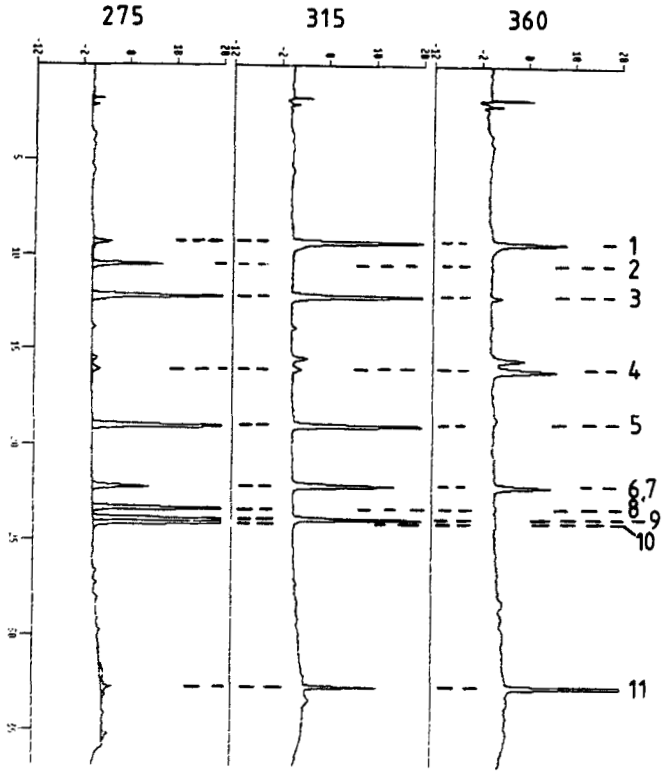

Figure 1. Chromatogram of the external standard at different wavelengths.
1.Metronidazol 2.Meticlorpindol 3.Sulfapyridine 4.Furazolidone
5.Pyrazon 6.Ipronidazol 7.Chloramphenicol
8.N-Acetylsulfapyridine 9.Ethopabat 10.Benzothiazuron
11.Nicarbazin

signals at 250, 290 and 330 nm. The detection system is programmed such, that after each chromatographic run first the 3 signals of the main interest at 275, 315 and 360 nm are printed. After this the other three signals and finally the analysis report is printed. In this way, a complete information on the entire analysis over the wavelength range from 250 to 360 nm is obtained.

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Detection limits

Because of the various detection limits as required for the different drugs the sensitivity must be differentiated for the various signals.

As shown in table 1, sulfapyridine and acetylsulfapyridine are added in the range of the required detection limits. Chloramphenicol was 50 fold and and furazolidone (representative for nitrofuranes) was added at 20 fold higher concentration than reqired by the Bundesgesundheitsamt. With these concentrations, the problems involved with the complex analysis are demonstrated easier.

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Sulfonamides are generally detected at 275 nm and nitrofuranes at 360 nm. As it is shown in figure 1, the attenuation for the signal at 275 nm is sufficient for the detection of sulfonamides and their acetylmetabolites: 0.1 mg/kg result in a more than full scale response. For the detection of the nitrofuranes, the attenuation must be much lower.

In chromatography, the mininimum detectable level is determined by signal height and noise. When using this criterion to determine the minimum detectable level for our experiments, the minimum detectable amount for pure substances would be in the range of 0.001 mg/kg.

In our analyses, the detection was not limited by the noise of the chromatographic baseline but by the recovery and especially by the noise caused by chromatographic peaks from the sample matrix.

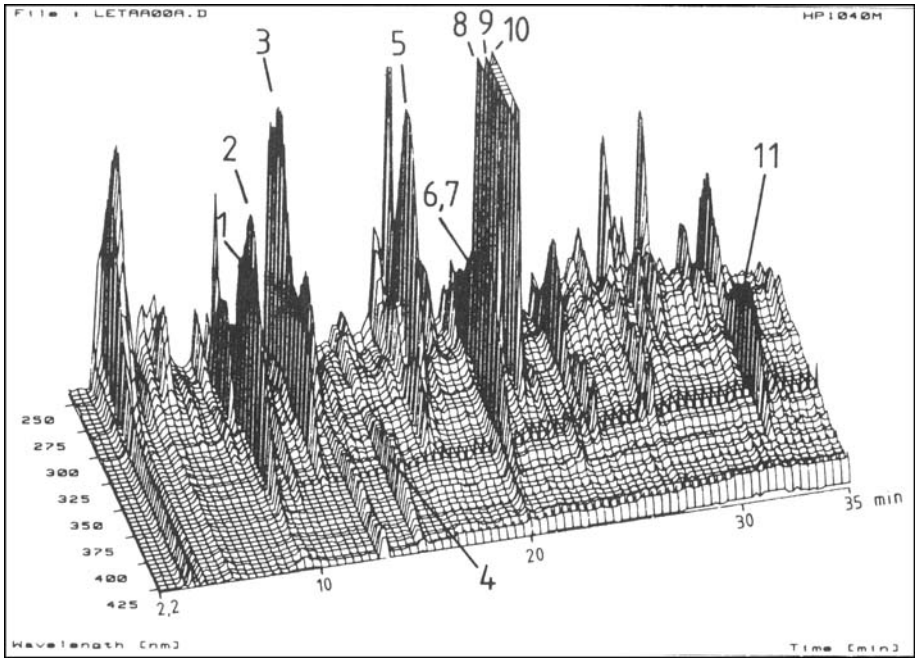

Figure 2. 3-D plot of a spiked egg sample (For peak identification see figure 1)

The interference of chromatographic peaks is best demonstrated in figure 2, which shows a 3-dimensional plot of an egg sample spiked with a standard mixture as shown in figure 1. The absorbance is plotted as a function of run time and wavelength. As it can be seen, the interference is much higher at lower wavelengths and at higher retention times. In the range of 350 to 400 nm, only a few matrix compounds do absorb. Therefore, furazolidone (No. 4) and nicarbazin (No. 11) are quite well distinguished from the matrix peaks. From figure 2 it is obvious, that the detection limit of all the drugs is far below the spiked amounts.

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Figure 3 to 5 show a comparison of chromatograms of spiked and blank egg extracts and of standard mixtures acquired at 275 (figure 3), 315 (figure 4) and at 360 nm (figure 5). The attenuation at 315 nm was a factor of 2 and at 360 nm a factor of 7 lower than at 275 nm. With these attenuations, the detection limits are sufficient for most of the drugs. Problems arise only with chloramphenicol. In the FRG the tolerance level is 0.001 mg/kg in milk and egg samples. For this analysis, a gas chromatographic method with electron capture detection is required [8].

Figures 3 to 5 demonstrate in addition, that the drugs of interest are found with good recoveries and detected without disturbance and with good sensitivity. The selectivity of the selected wavelengths becomes obvious.

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Natural coextracted substances

Milk, eggs and meat are complex matrices. The natural components nicotinamide and riboflavin (as well as its decomposition products lumiflavin and lumichrome) are extracted and detected, nicotinamide at 275 nm, riboflavin, lumiflavin and lumichrome at 360 nm). One major peak in extracts from milk and meat is of unknown origin. This peak is eluted near sulfapyridin and detected at 275 nm. Processing of food e.g. cooking or smoking results in disturbing peaks, as well as addition of spice e.g. piperine and vanillin or decomposition products built during too long storage od food e.g. biogenic amines. Specific feed components (e.g. coffeine of theobromine , reported to be present in cocoa press cake, used as feed component) may carry over over into food and may be detected.

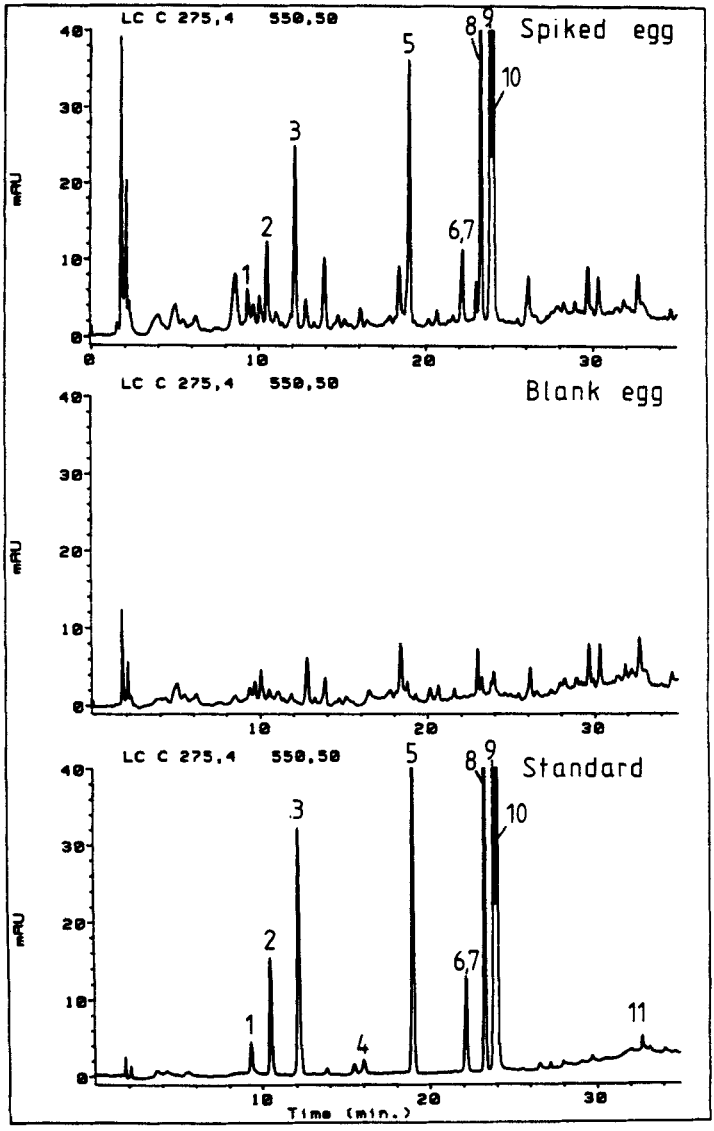

Figure 3. Chromatograms of the standard, a blank and a spiked egg sample at 275 nm.

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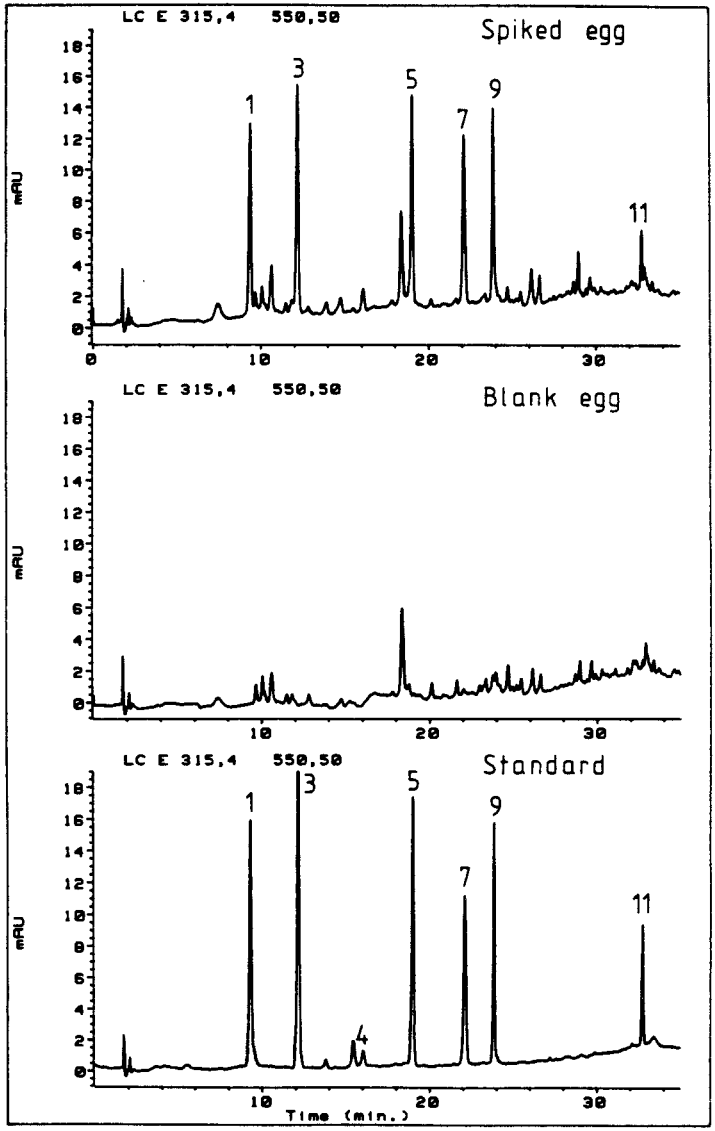

Figure 4. Chromatograms of the standard, a blank and a spiked egg sample at 315 nm

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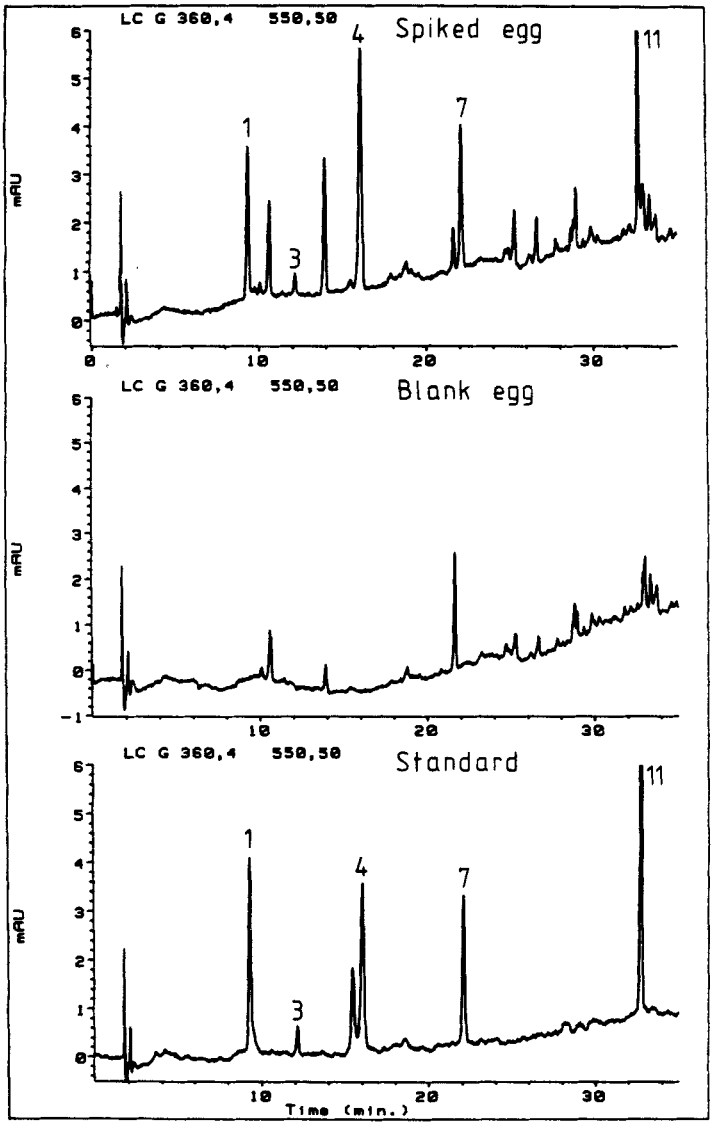

Figure 5. Chromatograms of the standard, a blank and a spiked egg sample at 360 nm

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Spectra Acquisition with a pilot signal

In chromatographic routine analysis, generally peaks are identified by comparing the retention times with those of a standard. In the most real life samples, especially when working at trace levels it is very risky to rely on retention times only, because other compounds from the sample might have the same or similar retention times as the residues of interest. Therefore, an additional method for peak confirmation is required.

Some information on the chemical nature of a compound can be obtained from the UV/Vis spectrum which shows absorbance as a function of wavelength. With diode-array detectors compounds can be scanned during a peaks elution within a few milliseconds.

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The UV/Vis diode array detector as used for our experiments has two possibilities to store spectra during a run. Spectra can be acquired and stored in fixed time rates up to a rate of 8 spectra per second or in peak controlled mode, where for a peak four spectra are stored: in the peak upslope, at the apex, at the downslope and at the baseline following the peak. In routine analysis, the first method requires too much memory on the storage device, therefore the second method was used.

The problem with the peak controlled mode was to find a signal wavelength which could be used as a pilot signal to acquire the spectra during a run with the 3 different interesting wavelengths. As it can be seen from figures 1 and 3 to 5, at none of the 3 selected wavelengths all compounds are detected.

Two other wavelengths have been considered as pilot signals: one was in the low UV range e.g at 220 nm where all investigated compounds absorb, or a signal at 315 nm with a large bandwidth of 120 nm. Because of a lower risk of interfering matrix peaks the the second method was chosen. The wide bandwidth ensured, that all compounds absorbing in a range between 255 to 375 nm contributed to the signal. Figure 6 shows two chromatograms acquired at the same wavelength of 315 nm with two different bandwidths of 4 and 120 nm. With the broader bandwidth, all peaks are detected in contrast to the signal with a bandwidth of 4 nm only, where 4 peaks are missing.

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UV-Spectra for peak confirmation

When using UV spectra for peak confirmation, the spectra of the unkown peaks are normalized and overlayed with the standard

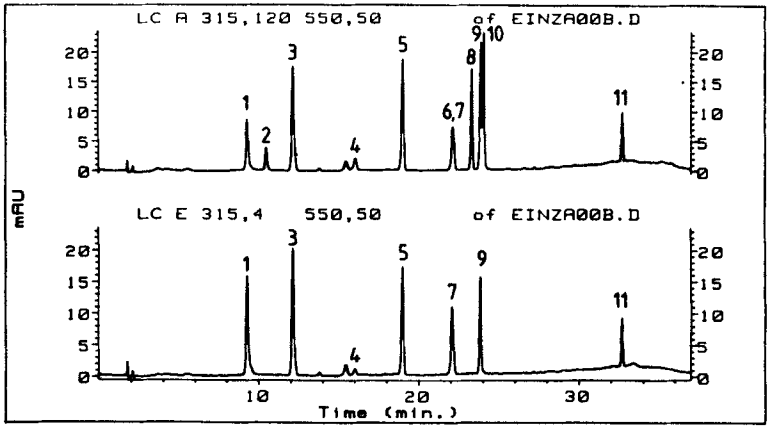

Figure 6. Chromatograms of the standard at 315 nm with different signal bandwidths (upper trace: 120 nm, lower trace: 4 nm)

spectra. It is very important, that the chromatographic conditions, especially the mobile phase composition, are identical for the sample and standard analysis. When working at high concentrations (above 1 mg/kg), the sample spectra are expected to be identical with the standard spectra. In the range below 0.1 mg/kg small deviations between the sample and standard spectra may occur.

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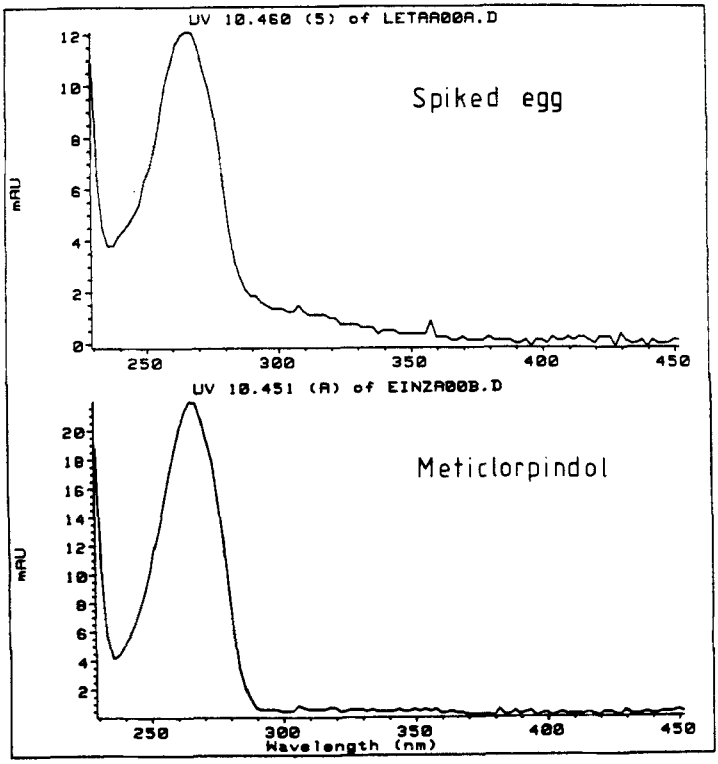

Figure 7. UV spectra of Meticlorpindol from sample and standard

Figure 7 to 9 show comparisons between sample and standard spectra of meticlorpindol, sulfapyridin and furazolidone. The amounts are 0.05 mg/kg for meticlorpindol, 0.1 mg/kg for sulfapyridin and 0.02 mg/kg for furazolidone. Because of the lower amounts, the spectra of furazolidone are noisy. As indication for low and therefore critical concentrations a value of 10 mAU in the intensity of UV-spectra should be considered. Sulfapyridine (30 mAU) and meticlorpindol (12 mAU) exceed this value, furazolidon (5 mAU) falls below.

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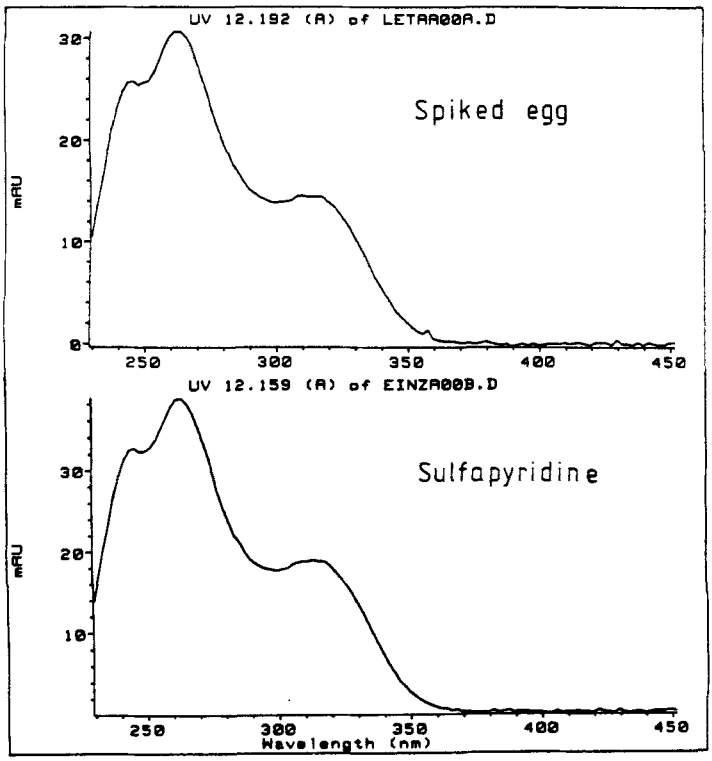

Figure 8. UV Spectrum of Sulfapyridine from sample and standard

Another problem is that at lower levels the spectral influence of the mobile phase becomes significant especially in the low UV range. Therefore, before comparing a sample spectrum with a standard, a reference spectrum should be subtracted, which should represent closely the mobile phase. Mainly in gradient analysis it is very important to acquire the reference spectrum at a retention time which is very close to the sample spectrum.

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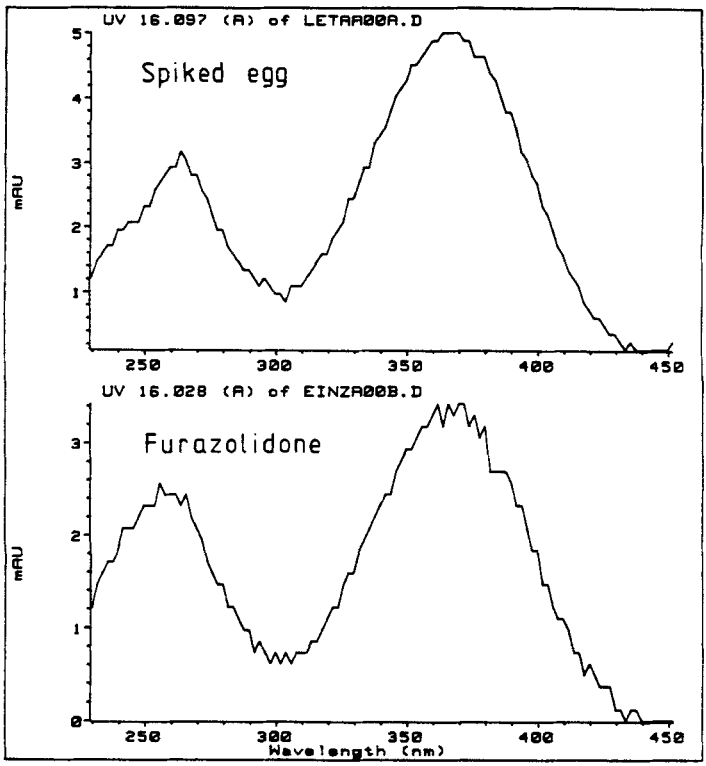

Figure 9. UV Spectra of Furazolidone from sample and standard

The influence of a proper retention time for a reference spectrum is demonstrated in figure 10 which compares the nicarbazin spectrum in spiked eggs at two different retention times of the reference spectrum. It demonstrates, that the UV spectrum looks different, when using a wrong reference spectrum.

At very low concentations, this problem becomes even more significant. Increasing the injected sample amount to 100 ul or higher

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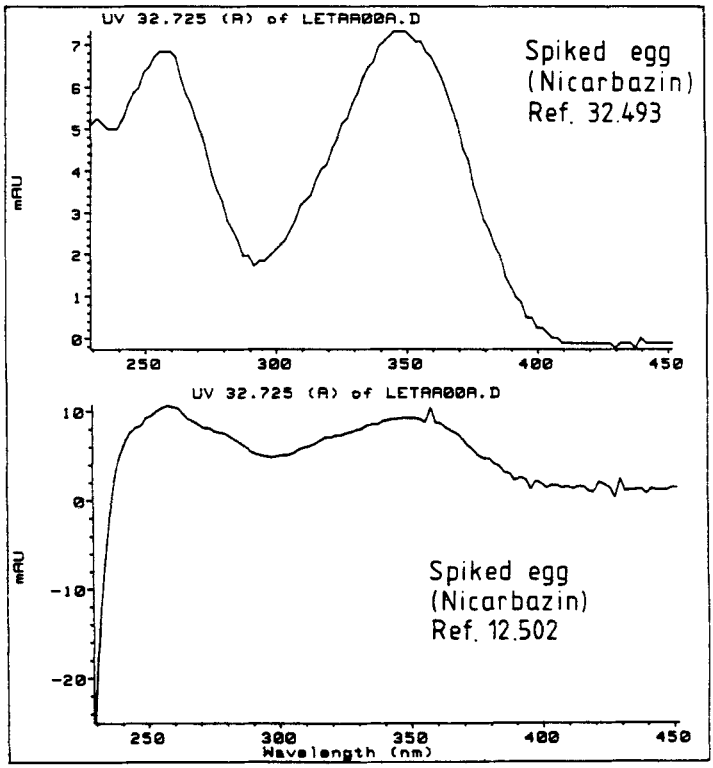

Figure 10. UV Spectra of Nicarbazin from sample with two different reference spectra

results in a higher response and herewith in a lower detection limit [9]. However, it has been demonstrated that chromatographic resolution possibly is lost at the beginning of the chrotomatogram, resulting in overlapping peaks [10]. If higher sample amounts are injected, the solvent should be exactly the same as the mobile phase composition at the time of sample injection. Therefore, instead of acetonitrile/ methanol/water (1:1:1 v:v:v) the extract should be solved in buffer, if a gradient starts with 100 % buffer. But then it is not ensured, that less polar substances are dissolved quantitatively. Problems with UV spectra occur also, if a peak consists of more than one compound. Figure 11 shows a comparison of the metronidazol standard spectrum with a spectrum acquired at the same time in a blank egg sample and at different sections of the metronidazol peak in the spiked sample. The difference in the UV spectra of metronidazol in the spiked egg between upslope, apex and downslope of the peak demonstrates that there is an impurity in the tail of the peak. This unknown peak was not detected at 315 nm (see fig 4, Peak No 1: metronidazol).

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Figure 12 shows the UV spectra acquired at different peak sections of the non-separated compounds ipronidazol/chloramphenicol. The upslope spectrum is similar to ipronidazol, the downslope to the chloramphenicol standard spectrum. The examples in figure 12 and 13 demonstrate, that before a peak spectrum is compared with a standard, the peak should be checked for purity first.

From our experiments it can be concluded, that when analyzing drugs in the range <0.1 mg/kg, peaks from the sample matrix

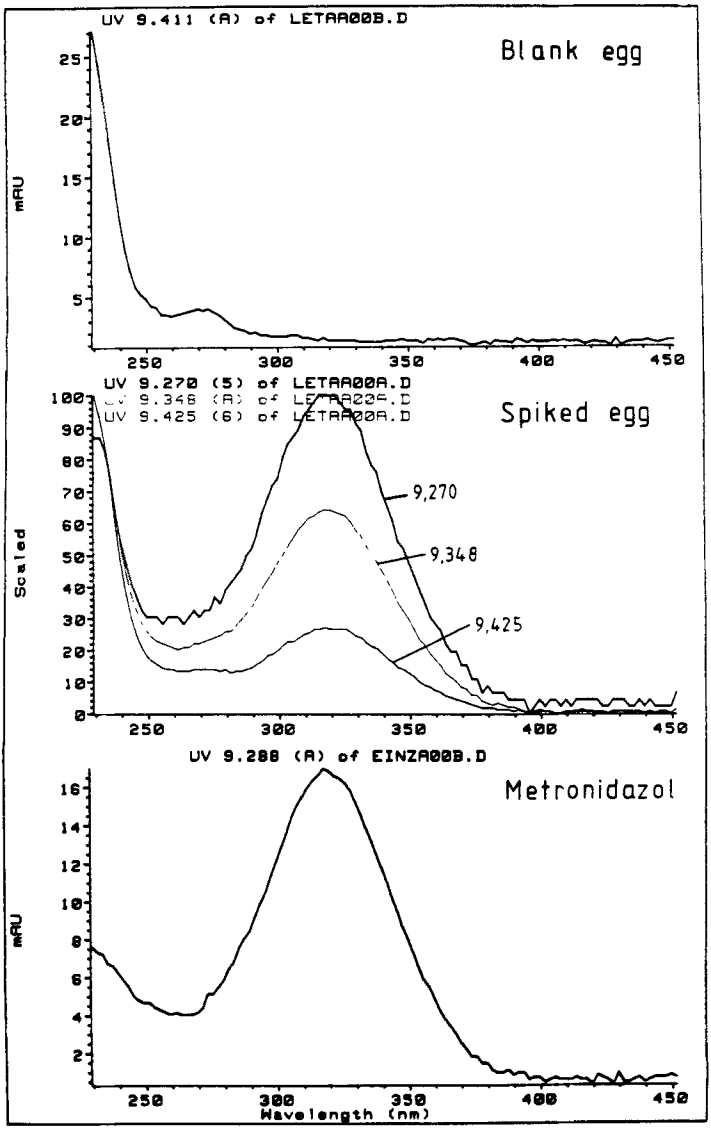

Figure 11. Overlay of spectra of Metronidazol and an impurity

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might chromatographically interfere. If the spectra of the matrix compounds are different from the spectra of the drugs, peak inhomogenity can be detected by a spectra comparison. A recommendation is to check each peak for purity by normalizing and overlaying spectra in the upslope, apex and downslope of a peak before comparing the spectra with a standard. Interfering peaks dont't cause false positive but false negative results, because the spectrum of a suspected substance in an extract of a sample becomes different from the corresponding spectrum of a drug.

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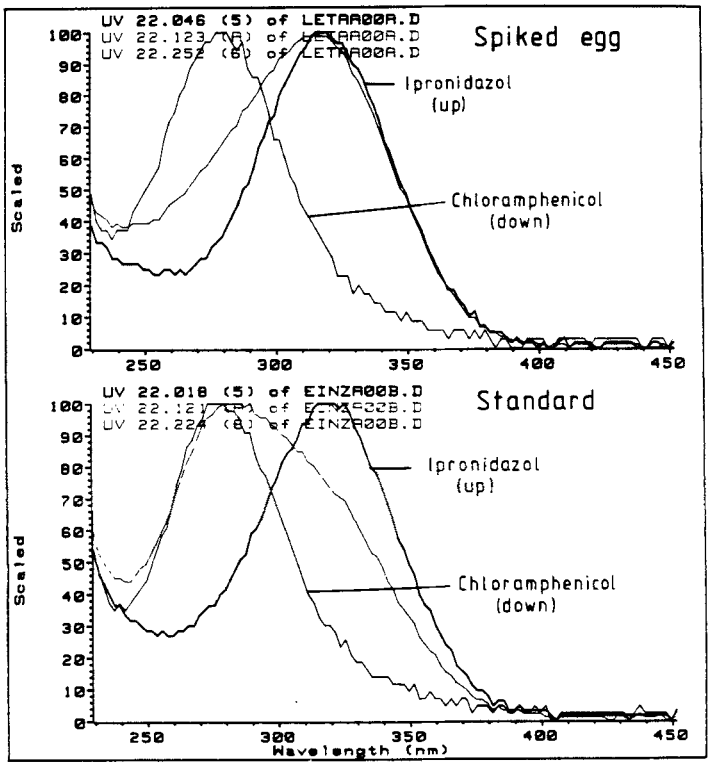

Figure 12. Overlay of spectra from Ipronidazol and Chloramphenicol

If the absorbance is higher than 10 mAU and the spectra of the external standard and sample are exactly the same, so far we never made a wrong positive identification because in this case influence from spectrum noise, mobile phase and minor matrix components are eliminated. If the absorbance is lower special care must be taken with the interpretation of UV/Vis spectra and generally it is strongly recommended to confirm a peaks identity with an other independent specific and sensitive analysis method. In any case UV/Vis spectra are valuable hints for positive peak identification.

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REFERENCES

1. Somogy, A. (1982) Bundesgesundheitsbl. 25, 365-370

2. Deutsche Forschungsgesellschaft (1983), Rueckstaende in Lebensmitteln tierischer Herkunft (Residues in food of animal origin), Mitteilung X der Kommission zur Pruefung von Rueckstaenden in Lebensmitteln, S. 14-15, Verlag Chemie, Weinheim (FRG)

3. Petrausch, R. (1983) Delta Index, 2nd edition, Delta medizinische Verlagsgesellschaft, D-1000 Berlin 51 (West)

4. Bundesgesundheitsamt: "Erste Bekanntmachung ueber Nachweisgrenzen und Fristen zum Einreichen von Unterlagen ueber Rueckstandsnachweisverfahren und Kontrollmethoden fuer Tierarzneimittel" of Dec 20, 1984, Bundesanzeiger vom 28.12.1984, No 242, 14137

5. Malisch, R. (1986) Z.Lebensm.Unters.Forsch. 182, 385-399

6. Petz, M. (1983) Z.Lebensm.Unters.Forsch. 176, 289-293

7. Holtmannspoetter, H., H.P.Thier (1982) Dtsch Lebensmittel Rdsch 78, 347-350

8. Malisch, R. (1987) Z.Lebensm.Unters.Forsch. 184, 467-477

9. Malisch, R. (1986) Z.Lebensm.Unters.Forsch. 183, 253-266

10. Malisch, R. (1987) Arch. Lebensmittelhyg. 38, 41-47