Analytical Methods

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In this lecture I will give a survey on newer and highly sophisticated methods and on trends in analytical chemistry of meat and meat products.
It is the aim of this presentation to give examples for the application of different instrumental methods and to show the efficiency of analytical methods nowadays used in advanced laboratories.
In order to structurize this presentation I will follow the general scheme of analysis (Fig. 1).

CLEAN UP PROCEDURES

The cleaning-up steps in analysis are known as mostly time consuming and tedious. But during the last decade clean-up and concentration steps have been developed which are rapid and easy to handle. In order to control the problem of residues of veterinary drugs in food of animal origin, it is essential that suitable i.e. quick and reliable methods are available.
The main problems arising in developing such quick and reliable methods are the low levels of the compounds to be analyzed as well as the complicated matrix of meat and meat products. Physicochemical methods offer a possibility to overcome these problems.
It can be observed that during past decades the main emphasis of analysts was layed on the development of sensitive and selective detection methods to determine a great many of compounds in the 1-10 ppm range.
But in drug residue analysis the lower ppm and ppb level is necessary to definitely control products. Therefore preconcentration steps have turned out to be as effective as possible to gain the concentration factors which are sufficient to analyse the drug residues.
Extraction of the analyte needs an intense contact of solvent and sample. Mixer e.g. Ultra turrax, or ultra sonic devices are helpful in this respect.
Extraction with water containing solvents is less favourable because of the adsorption of drug molecules on proteins. This causes incomplete extraction. It has been shown that in pig meat tissues sulphadimidin to a great extent was bound to proteins. Dichloromethane or chloroform-acetone mixtures were able to fully extract the analyte.

As a new trend, beside time consuming and tedious extraction by liquid-liquid partition solid phase extraction and immunoaffinity clean up has been introduced into analysis of drugs.

Solid phase extraction becomes very popular due to its simplicity.
Handling time and amount of solvents needed are greatly reduced whilst emulsification problems are totally avoided.
In these procedures the analyte is removed from the sample solution by sorption on a solid phase present in a small column. After an eventual wash step the analyte e.g. the drug is eluted by a small volume of solvent.
Extraction can be performed by a variety of principles: straight-phase and reversed-phase retention, ion exchange and ion pair formation.
The type of sorbent and solvent used has to be selected in relation to the analyte to be extracted.
The retention of an analyte may be based on different and combined types of interaction. As an example retention on bonded silica phase is usually caused by the functional groups bonded to the silica, but may also be due to the interaction between the analyte and the unbonded silanol groups remaining on the silica substrate. These secondary interaction may give rise to a severe problem. It was observed that e.g. tetracyclines have been strongly retained on a reversed phase column so that no complete elution has been achieved.
Recently, fully automated sample pretreatment combined with fast LC, using aqueous samples or sample extracts, is described for analysis of veterinary drugs. This combines on-line continous-flow dialysis - in which the drug molecules pass through a membrane whilst large molecules such as proteins are retained - with on-line preconcentration on a small column.
Reviews with regard to on-line sample handling and trace enrichment in liquid chromatography and to the determination of drugs has been presented by N. Haagsma (1). On-line sample preparation procedures are also described for sulphonamides (2) and nitroburans (3).

Gelpermeation is a well established clean-up technique for organophosphorus and organochlorine pesticides from co-extracted lipids (4). Using Sephadex LH-20 there has been also observed a good separation of drugs from coextractives from liver, kidney and mussel tissues (5). However time is needed and the large eluate volume restricts the application to some extent. But recently GP was applied on-line to the analysis of sulphonamides, thimethoprim and their metabolites in pig tissues (6).

Matrix solid phase dispersion is a recently introduced method based upon dispersion of animal tissue for extracting drug residues from these tissues proceeding HPLC analysis. Homogenized tissue samples are blended with a lipid-solubilizing polymer e.g. C-18 packing material, which gives a semi-dry mixture. In the same manner as certain surfactants do, this material disrupts cell membranes through solubilisation of membrane components, e.g. phospholipides, making by this procedure the cell content accessible to extraction.

For elution the tissue/C-18 mixture is brought in a small e.g. 10 ml syringe-barrel column and eluted stepwise by an extracting solvent in order to isolate the drug residues (Fig. 2).

This procedure has been applied to the determination of organophosphates, benzimidazoles and ß-lactam antibiotics (7).

Immunoaffinity clean-up is the newest hit in analysis of veterinary drugs. These techniques are based upon a high specific binding between a haptene, i.e. the drug molecule and an antibody raised against it. Another application of this drug-antibody reaction is its use in clean-up and concentration step prior to physicochemical determination. Its name nowadays is immunoaffinity clean-up (Fig. 3).

In principle the antibodies are covalently bound to an activated support such as cyanogenbromide-activated Sepharose, tresylactivated Sepharose or carbodiimidazol activated trisacryl. In general the immobilized antibody is transfered to a small column. The scheme shows this procedure (Fig. 4).

The sample solution is drawn through the column. Due to immunochemical interactions the drug molecules are retained by the immobilized antibody. The matrix components are passing the column and are washed out in a washing step. Mainly solutions such as phosphate buffered saline are used, which do not influence the immuno-chemical interaction.

After the washing the drug is eluted. Elution can be based on ligand competition, alteration of the ionic strength, solvent charge or buffer and pH-change. Each antibody - mediated clean-up procedure has its own optimal elution conditions.

It has to be taken into considerations that a low flow rate combined with high volumen and low hapter concentration leads to the highest retention - which is contradictory to solid phase extraction technique. Therefore more time is needed for analysis. Due to the great specificity of monoclonal antibodies a large amount of sample can be subjected to antibody-mediated clean-up without retention of matrix components. This opens the possibility to determine very low drug residue levels.

Only a few applications have been described until now. The analysis of trembolon and

17 alpha-trembolon in bovine urine (8), a multi-determination of several anabolics in meat and just recently a method comprising seven antibodies used together (9). It has be pointed out that clean-up is still a step of analysis that consumes some time. But very sensitive and selective methods, especially in the field of immunochemical methods are now available leading back to micro analytical techniques also in clean-up.

CHROMATOGRAPHIC METHODS
Liquid chromatography

HPLC is a preferred method for the separation of veterinary drugs in food of animal origin.

Increasing tendencies go to the on-line and automated application of HPLC.

A very versatile method is the fully automated sample treatment in combination with liquid chromatography, first introduced in practice by Van Gend (10) for the analysis of chloramphenicol in milk.

As an example may serve: An aqueous extract of the sample is introduced from an autosampler with a peristaltic pump into the system and the injection volume is segmented by air to avoid diffusion. In the dialyse block the drugs pass the cellulose acetate membrane. Proteins and other macromolecules remain in the upper phase which is descarded. The analytes in the dialysate are trapped on a first column, more polar coextractives and non-organic compounds are washed out. The analytical separation starts when a valve is switched and the trapped drugs from the first column are backflushed to a second column from which separated drugs enter the detector. Despite the low solubility of drugs in water they are totaly extracted because of the very low concentration of residues. To prevent in vitro metabolism of veterinary drugs the addition of sodium azide to the solution is recommended.

In some cases the selectivity and/or sensitivity of a HPLC-separation and instrumental detection device is not sufficient for identification and quantitation.

In this case the potential of post- and precolumn derivatisation reactions for enhancing selectivity and sensitivity is used (11). In the case of pre column derivatisation all reagents can be used, even aggressive ones.

Examples are the formation of fluorescent compounds from the coccidostats monensin, salimycin and lasalocid, in beef liver with 9-anthryldiazomethane to derivatize the carbonyl group (12).

Post column derivatisation is executed using an additional pump and mixing the reagent in a T-piece with the eluate of the LC column. As an example Neomycin and other aminoglycoside antibiotics are analyzed by post column derivatisation with

o-phthalaldehyde (13).

A very promising new approach to increase specifity in on-line HPLC is the application of photoreaction in a photoreactor. By simply switching light on, due to photochemical reactions a fluorescent or electroactive substance can be created.

An example is the formation of fluorescent hexahydrophenanthren from diethylstilbestrol (DES) (14).

Additional efforts have been undertaken, to develop-methods which are able to give molecular information on the residues analyzed, guaranteeing unambigous identification.

Currently only mass spectrometry offers this information for the sensitive residue analysis.

Many years the efforts of coupling HPLC/MS were not successful. But now there seems a break through of thermospray systems also with respect to drug analysis.

As an example for confirmation of the results using thermospray MS the analysis of Nicarbrazin in chicken tissue may serve. The principle of thermospray which can be used in conventional RP-HPLC flow rates, can be easily explained. Aqueous mobile phase containing an electrolyte e.g. ammoniumacetate passes at a flow rate of 1-2 ml/min through an electrically heated stainless steel capillary situated in a special heated ion source. The end of the capillary lies opposite a vacuum line. This results in a super sonic jet stream of vapor, containing little electrically charged droplets. As the droplets move to the hot source area they continue to vaporize. The electric field at the liquid surface increases until ions present in the liquid phase are ejected from the droplets.

Ions are sampled through an exit aperture in the mass analyzer. Ions can be formed either by direct ion evaporation of a sample ion or similar to chemical ionisation (CI) processes in a two-step process, e.g. ammonia ions ejected from a droplet reacts with a sample molecule in the gas phase and generates a sample ion that is mass analyzed.

HPLC is applied to several other problems in meat science and technology.

One challenging problem are mutagens in heated meat. From the list of mutagens identified in cooked meat one can see that analysis of these substances is necessary in the lower ppb range.

HPLC is used as purification step of the extracts as well as for the separation itself.

A standard procedure is extraction with methanol, purification using acid/base partition and HPLC on a sulfopropenyl cation exchange column. The separation and quantification of the mutagens was carried out by HPLC on RP-18-phase using either electrochemical or UV-detection (16).

HPLC in combination with fluorescence detection offers a very sensitive method of analysis.

The tranquilizer Carazolol, used for diminishing stress in cattle during transportation

can be analyzed by the following methode:
Carazolol is extracted by methanol from meat homogenates. The extract is purified by passing an Extrelut-column. Subsequently the basic fraction is extracted.
For confirming the results excitation spectra as well as emission spectra can be recorded. The limit of detection amounts to 0,5 µg/kg (17).

Gaschromatography

New trends in analysis of quality of meat and meat products by gaschromatography can be observed in two areas.
First the application of newer specific detectors namely the atomic emission detector is now commercially available (Fig. 5).
Just at that place some words on GC-detectors, their types and selectivity as well as their dynamic range is advisable. From the table presented (Fig. 6) one can see minimum amounts detected and selectivity achieved by various detector types.
The second area of fast development is the combination of sensory and instrumental analytical methods e.g. in aroma extract dilution analysis (AEDA). This technique was introduced first by Rothe in the early 70s, and successfully applied to different problems by the working group of Grosch (21).
Starting with quality assessment and quality assurance flavour analysis can give insights into the effect of differences in feeding on characteristic flavour. The distinct flavour of sheep meat especially that of older animals is a reason for its low consumption rate.
F. Caporasco et al. (20) extracted the subcutan adipose tissues of sheep and separated the extract into acid, basic and neutral fractions. The apparatus and scheme is shown here (Fig. 7).
It was found out from a total number of 51 different volatiles identified, 14 are suggested as important contributors to the overall flavour quality.
Olfactory analysis of a six membered sensory panel accompagnied the study. Also sniffing analysis was carried out.

The typical flavour of meat develops during heating processes and is due to the thermal and oxidative degradation of sugars, amino acids, unsaturated acyl lipids and thiamine.
In the case of cooked beef, more than 500 volatiles have been identified but only a relatively small number of these volatiles are responsible for the smell of this typ of meat.
So the question of character impact compounds raises. In this respect the technique of GC/Sniffing analysis is important. Using this technique the working group of Grosch (21) developed a measure called flavour dilution factor ("FD-factor"). The scheme of

separation and a chromatogram are shown in the following figures (Fig. 8, 9).
As a result shown in the aromagram compounds having high aroma values were identified and exhibit a meat like aroma. The two compounds showing highest aroma values and possessing a meat-like odor have been identified as 2-methyl-furanthiol and its disulfide. The odor threshold level is shown in the Fig. 10.
The Aroma Extract Dilution Analysis (AEDA) shows the significant contribution of these compounds to the meat like odor of cooked beef. In addition compounds contributing to the roasted note of cooked beef have been identified, namely 2-acetyl-1-pyrrolin, which has been identified previously in crust of white bread.

Another problem nowadays food chemists are faced with, is the so called "warmed oven flavour (WOF)" which is a result of the autoxidative spoilage of fatty acids started by iron liberated from myoglobins. This off-odor is recognized if boiled frozen meat is reheated.
Also this problem may be analyzed by GC/sniffing analysis.
In connection to flavour analysis the atomic emission spectrocopic-(AES)-detection is of considerable interest.
An objective of flavour analysis is to identify components which are not yet in spectral libaries.
Knowing which elements are present in an unknown can be an invaluable aid in identifying the molecule.
Commercial available Atomic Emission Detectors are capable of specifically detecting any organic element (and many metals) and can be used to determine, which elements are present in unknowns.

Thinlayer Chromatography

HPTLC is a very cheap and quick method for separation of analytes. Nowadays equipment is available to spot extracts precisely and to separate under exact defined conditions.
Detection using UV, visible or fluorescence light direct or after derivatisation can be executed.
Polycyclic aromatic hydrocarbons (PAH) play an important role in smoked meat technology. Smoking conditions have to be checked very carefully to prevent to high residues of PAH.
We use a TLC-separation on acetylcellulose plates and an in-situ fluorescence determination of the different PAHs.
With this equipment concentrations down to e.g. 0,01 ppb Benzo(a)pyren can be quantified (18).

HPTLC also serves very well as multiresidue method. The screening of pesticides using different solvents and combinations of spraying agents is possible.

In a recent publication (19) it has been demonstrated, that more than 150 different pesticides can be analyzed.

The detection via cholinesterase spraying agents on Kieselgel plates offers the possibilities of very sensitive and reliable analysis of groups of pesticides.

Also an RP-18 reversed phase plate using silver nitrite/UV light or chlorine/ or toluidin staining are useful approaches to quick screening and/or quantitation of pesticide residues.

IMMUNOASSAY

As an example the use of monoclonal antibodies for the immunoassay of mutagenic compounds produced by cooking meat is chosen (22).

Grilling, frying and broiling of meat is an important source of mutagens of the Aminoimidazoarene (AIA) group, which count to the most potent carcinogens known until now (Fig. 11).

AIAs are present in 0,1-15 ppb levels in cooked beef. Although e.g. HPLC is a possible tool for the determination of these compounds due to high sensitivity, selectivity and low sample preparation requirements immunoassay have attracted alteration.

To produce antibodies to small organic molecules the chemical of interest must first be conjugated to carrier proteins (Fig. 12). In the AIA-case keyhole limpet hemocyanin (KLH) was used. The site and typ of conjugation are important because they influence the specificity of the antibodies used.

In this example mentioned, the authors produced monoclonal antibodies by immunisation of mice with 2-KLH-IQ, to respond with antibodies capable to recognize IQ preferentially since the distinctive portion of the hapten is available to the immune system.

Also 8-KLH-Me/Qx immunisation was the used to respond with antibodies that recognize many members of the AIA-class, since the aminoimidazo ring is presented unmodified to the immunesystem.

This experiments ended up with a set of antibodies that recognice four AIAs. This immunoassay has sufficient sensitivity to quantify AIAs in that concentration found in well-done-ground beef.

ISOELECTRIC FOCUSSING (IEF)

The differentiation of the race of animals is done using electrophoretic, as well as

chromatographic and immunoserological methods of analysis.

Electrophoretic and immunoserological methods relay on the identification of specific proteins or break-down-products of that proteins.

A very potent analytical method in this respect is Isoelectric Focussing (IEF).

Water extracts from meat or pressed and diluted meat juices are directly spolted on a polyacrylamid gel.

For identification the typical and characteristic myoglobin bounds with or without additional staining reactions or enzymatic reactions to visualize the specific proteins are applied.

Due to their genetically different protein pattern different breeds of animals can be differentiated by their native proteins very easily. Much more difficult is the quantitation in mixtures and the qualitative analysis in processed, especially in heated meat products.

IEF has been used in our institute (23) e.g. for the determination of pig meat in beef meat products which have been heated up to 75°C inner temperature. To enhance the sensitivity ultrathin polyacrylamide gel layers and a highly sensitive silver staining procedure has been used. The determination is carried out using 1-2 specific protein bounds as indicator for the presence or absence of pig meat.

As higher the products are heated the less sensitive is the determination limit.

In the case of sterilized meat products there are nowadays kits commercial available, using ELISA technique. Using this method the determination of pig, beef and chicken in meat heated products is possible.

INDUCED COMPLED PLASMA EMISSION (ICP) AND ATOMIC ABSORPTION SPECTROSCOPY (AAS)

These two techniques for the analysis of inorganic ionic constituent are nowadays well established.

ICP techniques are of advantage in determining a number of trace elements in one sample simultaneously. A proper digestion step has to precede. Nowadays mostly temperature controlled acid digestion in decombustion bombs is used for most of the elements. Salinity content has to be controlled very carefully avoiding matrix effects on the signal.

AAS with graphit furnace and background correction (Zeeman- Effect or D_2-Lamp) is now state of the art.

Some elements e.g. arsenic and selenium are determined by their volatile hydrids, mercury is determined using the vapor formed from metallic mercury formed by reduction.

Main topics in meat analysis are in this field essential trace elements as well as toxic trace elements.

Meat is known as a food rich in essential trace elements e.g. iron, zinc and selenium. With respect to that, meat therefore acts as an important constituent for an adequate nutrition of man.

Potential toxic elements e.g. lead, cadmium and mercury can be found only in minute amounts in meat, whilst the amount in liver and kidney is times 2 to 10 respectively higher. It has to be pointed out that concentrations normaly present in meat or viscera are proportional higher than in other foodstuffs but are considerable below any harmful dose.

In a duplicate diet study we checked the total uptake of Hg, As, Cd and Pb in Austria (24). Fig. 13 presents the results, showing that there is no harmful situation given at present.

Some meat of game has been found higher burdened with these heavy metals due to environmental pollution.

QUICK-TESTS

Where YES/NO-results are satisfactory, card or cup tests based on enzyme-immunochemical reactions may be sufficient. These quick tests which indicate presence or absence of a drug by a colour change can be very sensitive and are commercially available for chloramphenicol, sulfamethazine or benzylpenicillin (penicillin G). The extended use of these kits led to a significant increase in the numbers of samples tested by the monitoring program of the US Food Safety and Inspection Service (FSIS). While in 1980 65.000 samples were analyzed this number rose in 1988 to 300.000 samples and in 1989 to 450.000 samples (25).

SCREENING-TESTS

A screening procedure with higher specifity is the Charm-test II which originally was developed for residues in milk (4) but now has been modified also for meat testing. With this technique 7 antibiotic families can be detected and identified within 12 to 15 minutes: beta-lactams, macrolides, tetracyclines, aminoglycosides, novobiocin, sulfonamides and chloramphenicol. Most antibiotic drugs act by specifically binding to a site in a microbe causing an interruption of metabolic activity, for example beta-lactam drugs bind to an enzyme immobilized on the cell wall; tetracyclines and aminoglycosides bind to sites on ribosomes. This is used as principle of the Charm-test. Two different microorganisms are required to provide the necessary sites for the 7 families. Because the functional chemical group of the drug is involved with the binding site and not a

side chain as in most cases of immunoassays, a single receptor detects all members of a family. Known amounts of C-14 or H-3-labelled antibiotics and microorganism are added to the sample. A residual antibiotic in the food sample competes for sites on the microorganism with the labelled antibiotic. As lower the antibiotic concentration in the milk sample, the more labelled drug will bind to a site. For quantitation the amount of tracer binding is correlated with the concentration of drug in the sample. To identify a specific drug in a family, a confirmatory assay must be performed.

These screening methods can give very precise results when it is known that there is only one particular drug from one family present.

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Fig. 1

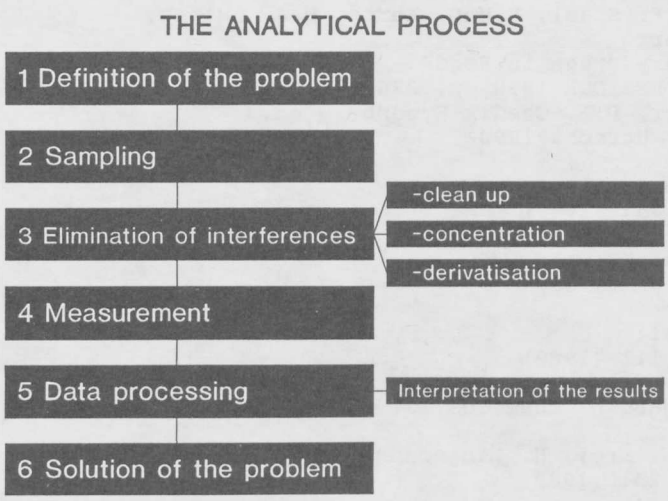


Fig. 2

Representation of a column filled with tissue/C-18 mixture

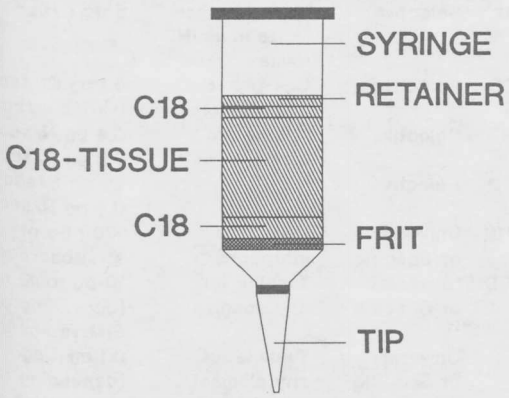


Fig. 3

Schematic representation of immunoaffinity clean-up column

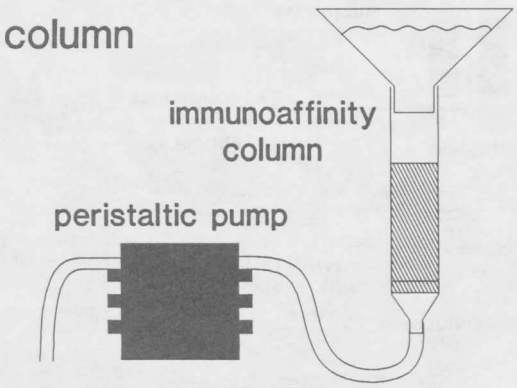


Fig. 4

Schematic representation of immunoaffinity clean-up

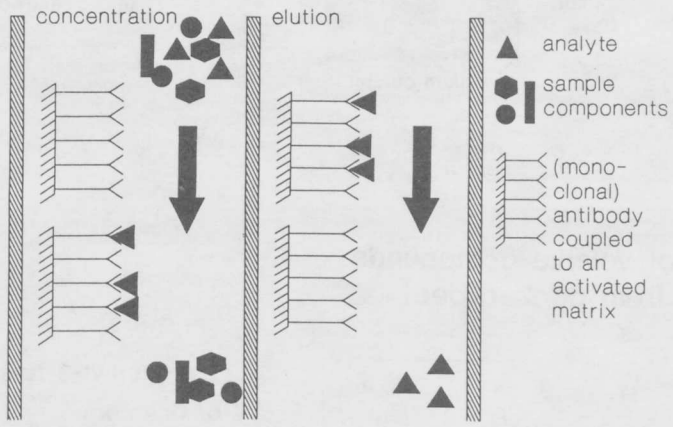


Fig. 5

GC-AES block diagram

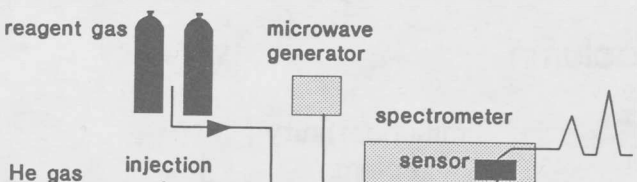


Fig. 6

General Characteristic of GC Detectors

Name	Type	Selective For:	Minimum	Linear Dyn.
FID	Selective	Materials that ionize in air/H2 flame	5 pg C/sec	10^7
ECD	Selective	Gas-phase electrophoreses	0.1 pg Cl/sec (varies w/str.)	10^4
NPD	Selective	N,P, hetero atoms	0.4 pg N/sec 0.2 pg P/sec	10^4 10^3
FPD	Selective	P,S	20 pg S/sec 0.9 pg N/sec	10^3 10^3
FTIR	Universal or Specific	Molecular vibrations	1000 pg of str. absorber	10^5
MSD	Universal or Specific	Tunable for any species	10 pg to 10 ng (depending on SIM vs scan)	10^3
AES	Universal or Specific	Tunable for any element	0.1 pg-1 ng (depending on element)	

Fig. 7

Apparatus for the isolation of total volatiles from liquid ovine fat

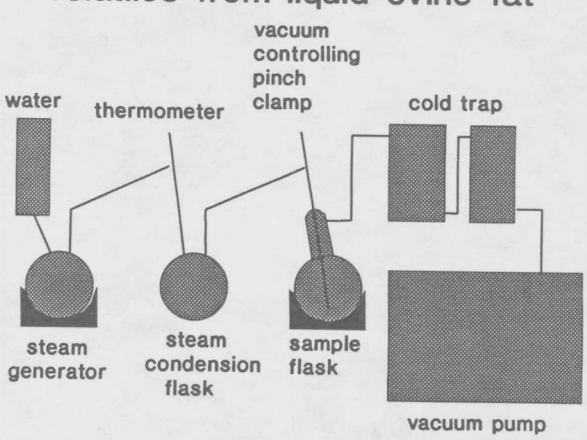


Fig. 8

Scheme of separation of total volatile fraction into acidic, dasic, and neutral fractions

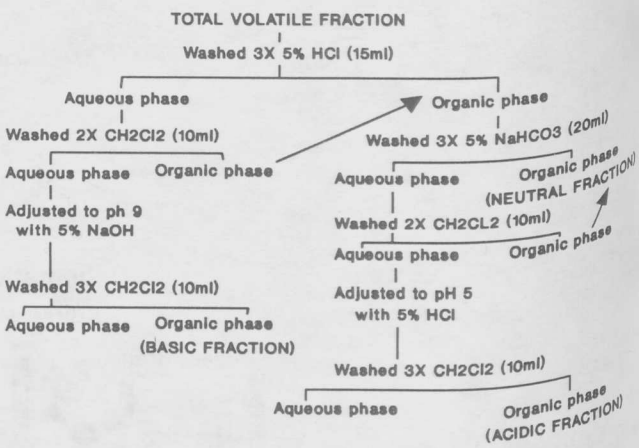


Fig 9/1

Aromagram of volatile compounds obtained from cooked beef

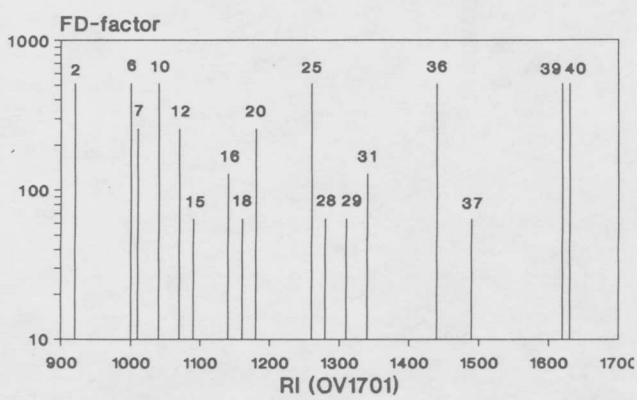


Fig. 9/2

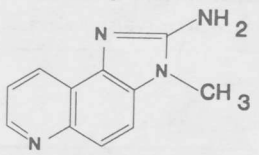
2 2-Methyl-3-furanthiol
6 Unknown
7 2-Acetyl-1-pyrroline
10 Methional
12 1-Octen-3-one
15 2-Octanone
16 2-Acetylthiazole
18 2(E)-Octenal
20 Phenylacetaldehyd
25 2(E)-Nonenal
28 2-Decanone
29 Unknown
31 2(E),4(E)-Nonadienal
36 2(E),4(E)-Decadienal
37 2-Dodecanone
39 beta-Ionone
40 Bis(2-methyl-3-furyl) disulfide

Fig. 10

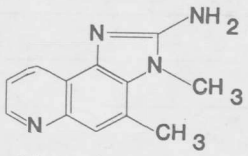
Geruchsschwellenwerte schwefelhaltiger Furane

Verbindung	Geruchsschwelle (ng/l Luft)
2-Methyl-3-furanthiol	0.0025 -0.01
2-Methyl-3-(methylthio)furan	7.6 -30.4
2-Methyl-3-(methyldithion)furan	0.02 -0.08
Bis(2-methyl-3-furyl)disulfid	0.0006 -0.0024
2-Furfurylsulfid	0.004 -0.02
2-Furfurylmethylsulfid	0.4 -1.6
2-Furfurylmethyldisulfid	0.04 -0.17
Difurfuryldisulfid	0.00015 -0.0006

Fig 11/1

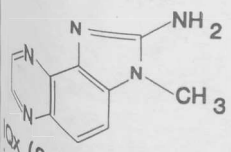
Compound	Source	Amount (ng/g)
	Beef	0.02-0.6
	Pork	----
	Sardine	4.9-20
	Salmon	0.3-1.8

IQ (2-amino-3-methyl imidazol[4,5-f]quinoline)

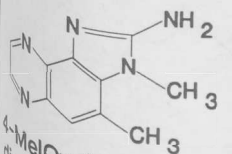
	Beef	<0.1
	Pork	----
	Sardine	17
	Salmon	0.6-2.8

4-MeIQ (2-amino-3,4-dimethylimidazol[4,5-f]quinoline)

Fig. 11/2

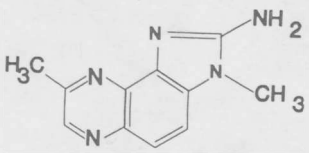
Compound	Source	Amount (ng/g)
	Sausage + Creatine	----

IQx (2-amino-3-methyl imidazol[4,5-f]quinoxaline)

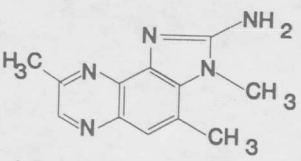
	Pork	----

4-MeIQx (2-amino-3,4-dimethylimidazol[4,5-f]quinoxaline)

Fig. 11/3

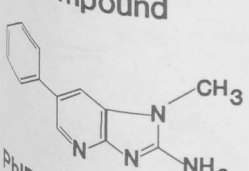
Compound	Source	Amount (ng/l)
	Beef	0.5-2.4
	Pork	----
	Mackerel	0.8

8-MeIQx (2-amino-3,8-dimethyl imidazol[4,5-f]quinoxaline)

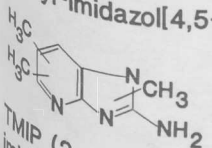
	Beef	0.5-1.2
	Pork	----
	Mackerel	0.08

4,8-DiMeIQx (2-amino-3,4,8-trimethylimidazol[4,5-f]quinoxaline)

Fig. 11/4

Compound	Source	Amount (ng/l)
	Beef	15
	Pork	---

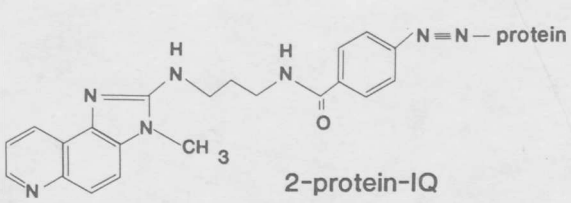
PhIP (2-amino-1-methyl-6-phenyl-imidazol[4,5-b]pyridine)

	Beef	---

TMIP (2-amino-dimethyl-imidazopyridine)

Fig 12

Structures of the AIA mutagens found in cooked beef



2-protein-IQ

IQ was linked through the 2-position to amino groups in proteins using a similar linkage chemistry to form 2-protein-IQ. MABS produced using the 2-protein-IQ immunogen were named IQ-1 and IQ-2.

Table 13 Comparison of two Austrian investigations on heavy metal in the diet

	(mg/person and month)			
	Investigation 1978		Investigation 1988	
Element	Range	Mean	Range	Mean
Lead	1,15 –3,23	1,86	1,04 –3,97	1,98
Cadmium	0,33 –1,16	0,72	0,34 –1,69	0,74
Arsenic	0,25 –1,68	0,83	0,15 –0,55	0,26
Mercury	0,110–0,180	0,140	0,0330–0,4223	0,138