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DETERMINATION OF BIOGENIC AMINES IN MEAT BY COMBINED ION-EXCHANGE AND CAPILLARY GAS CHROMATOGRAPHY

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SUMMARY

A procedure is described for the determination of putrescine, cadaverine and histamine in meat. Crude perchloric acid extracts were pre-separated on a weakly acidic cation exchanger and the amines quantified by capillary gas chromatography. The diamines were analysed as trifluoroacetyl derivatives and histamine was converted into N^{α}-trifluoroacetyl-N^{τ}-ethoxycarbonylhistamine. The accuracy of the determination of diamines was examined by a precipitation pre-separation method and by mass fragmentometric quantification. The proposed procedure allows the sensitive, sufficiently precise and highly specific determination of putrescine and cadaverine in meat.

INTRODUCTION

The freshness of meat is currently assessed on the basis of its bacterial contamination and its sensory properties. Bacteriological methods are usually lengthy for efficient industrial control, and sensory analysis requires a trained panel who are able to detect decomposition with a relatively high degree of accuracy.

Another possibility for the assessment of meat quality is to follow the biochemical changes that take place in meat during spoilage. Fresh meats undergo microbial decomposition in which a bacterial population utilizes meat components as nutrients and degrade them enzymatically. Bacterial metabolites have been considered as chemical indicators of meat freshness[1–4]. We examined whether some biogenic amines which are formed by bacterial decarboxylases from amino acids are suitable for detecting incipient meat spoilage. We aimed our efforts at the diamines putrescine and cadaverine and to a minor extent histamine.

A chemical quality indicator ought to correlate sufficiently with bacteriological and sensory properties. To establish the relationship between bacterial contamination, sensory scores and amine content of spoiling pork as a basis for a chemical test, a reliable analytical procedure is necessary. We chose highly specific quantification methods, *viz.*, capillary gas chromatography (GC) and mass fragmentometry.

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A number of methods have been devised for determining biogenic amines in biological samples. The analytical state of the art is well summarized in the review by Schwedt[5]. We used perchloric acid for the extraction of amines from meat[6–9] and an ion-exchange column chromatography for the pre-separation of a crude extract[10–13]. Prior to GC quantification we converted the diamines into their ditrifluoroacetyl (di-TFA) derivatives[11,14,15]. Histamine was converted into N^{α}-trifluoroacetyl-N^{τ}-ethoxycarbonylhistamine (TFA-ETO-histamine), analogous to its derivatization to N^{α}-heptafluorobutyryl-N^{τ}-ethoxycarbonylhistamine for GC determination in fish and biological samples[12,16].

The method developed for the determination of amines in meat by combined ion-exchange and gas chromatography was examined for its accuracy. The GC quantification was compared with mass fragmentometric analysis and the ion-exchange procedure was checked by a precipitation method[17]. We also measured the precision of the ion-exchange and precipitation methods. Recovery rates for the amines investigated in a meat matrix were determined by a spiking method and the overall procedure was applied to commercial pork with amine concentrations below 0.5 mg per 100 g of tissue.

EXPERIMENTAL

Chemicals and supplies

Cadaverine dihydrochloride (puriss.), ethyl chloroformate (purum), 1,7-diaminoheptane (purum), 1,6-diaminohexane (purum), 1,8-diaminooctane (purum), 1,3-diaminopropane (puriss.), histamine dihydrochloride (puriss), putrescine dihydrochloride (purum) and trifluoroacetic anhydride (puriss.) were obtained from Fluka (Neu-Ulm, F.R.G.). Dichloromethane (p.a.), diethyl ether (p.a.), ethyl acetate (p.a.), ion exchanger (Ionenaustauscher IV, Merck Art. 4835), potassium hydroxide (p.a.), sodium hydroxide (p.a.), sodium tetraphenylborate (p.a.), perchloric acid (p.a.) and hydrochloric acid (p.a.) were purchased from Merck (Darmstadt, F.R.G.).

A WG-11 capillary GC column (50 m × 0.2 mm I.D.) was obtained from Werner Günther Analysentechnik (Düsseldor, F.R.G.) and an AR OV-330 capillary column (50 m × 0.3 mm I.D.) from Macherey, Nagel & Co. (Düren, F.R.G.). A homogenizer (40,000 rpm) was purchased from Edmund Bühler (Tübingen, F.R.G.) and membrane filters (Typ SM 11307-070 G) from Sartorius (Göttingen, F.R.G.).

Apparatus

A Carlo Erba gas chromatograph (Fractovap 2101) equipped with a flame-ionization detector was used. Analyses were carried out on a WG-11 capillary column and the peak areas on the gas chromatograms were measured with a Spectra-Physics System I integrator.

For gas chromatographic–mass spectrometric (GC–MS) analysis the Carlo Erba gas chromatograph was interfaced to a Varian-MAT CH-7 mass spectrometer with an open coupling. The amines were quantified by a mass fragmentometric technique, using the Spectra-Physics integrator for peak area evaluations.

Extraction of amines

To 20 g of a minced meat sample were added 20 ml of 0.6 *N* perchloric acid and an internal standard solution. The mixture was homogenized at full speed for 5 min;

the flask was submerged in a water-bath for cooling. The suspension was centrifuged and the tissue residue rehomogenized twice with 2 × 20 ml of 0.6 *N* perchloric acid. After centrifugation the supernatants were combined.

Isolation of amines by ion-exchange chromatography

An aliquot of the supernatant (30 ml) was applied to an ion-exchange column. The column (33 × 1.5 cm I.D.) was packed with a weakly acidic cation exchanger (Ionenaustauscher IV) in the H^+ form. Before application to the column the pH of the solution was adjusted to 6.5 with potassium hydroxide. The precipitate was filtered off on a membrane filter and washed with 60 ml of distilled water. The combined filtrate and washing passed through the column at a flow-rate of 12–18 ml/h. Subsequently amines were eluted with 80 ml of 2 *N* hydrochloric acid. The eluate was evaporated to about 6 ml in a rotary evaporator at 30°C *in vacuo*. The column was regenerated with 6 *N* hydrochloric acid, the excess being washed out with distilled water.

Isolation of amines by precipitation with sodium tetraphenylborate

To the centrifuged perchloric acid extract (60 ml) was added 10 *N* sodium hydroxide solution to increase the pH to 3 and precipitated salts were removed with a membrane filter. Amines in the filtrate were precipitated with 15 ml of sodium tetraphenylborate solution (1 g/ml) and the precipitate was separated with another membrane filter. To the filtrate were added (in solution) 0.4 mg of 1,3-diaminopropane and 0.4 mg of 1,8-diaminooctane and the precipitate was combined with the first one by repeated filtration. The whole filter cake was washed with 20 ml of distilled water and rinsed with another portion of distilled water (50 ml) off the membrane filter. To the precipitate were added 50 ml of 4 *N* hydrochloric acid and 100 ml of diethyl ether and the mixture was stirred until the precipitate had dissolved. The ether layer was discarded and the aqueous phase was re-extracted twice with 100 ml of diethyl ether. The aqueous solution was concentrated in a rotary evaporator at 30°C *in vacuo*.

Trifluoroacetylation of amines

A concentrated hydrochloric acid extract (about 6 ml) was transferred into a vial with a PTFE-lined cap and evaporated to dryness *in vacuo*. The solid crust was covered with 2 ml of dichloromethane and 0.7 ml of trifluoroacetic anhydride and left to stand for 15 min in the tightly closed vial. The saturated crust was then crushed, finely suspended by a vibrator and kept at 40°C for 30 min. After cooling, the excess of reagent was removed with a gentle current of nitrogen and the viscous fluid was dissolved in 1–2 ml of ethyl acetate. The solution was injected into the gas chromatograph for diamine analysis.

Preparation of TFA-ETO-histamine

To trifluoroacetylated amines dissolved in ethyl acetate was added an equal volume of ethyl chloroformate and the mixture was shaken by a vibrator and left to stand for 30 min at 40°C. The excess of reagent was left in the solution, otherwise TFA-ETO-histamine was degraded. We observed degradation even after diluting calibration solutions with ethyl acetate. However, ethyl chloroformate can damage capillary columns, as described in the discussion.

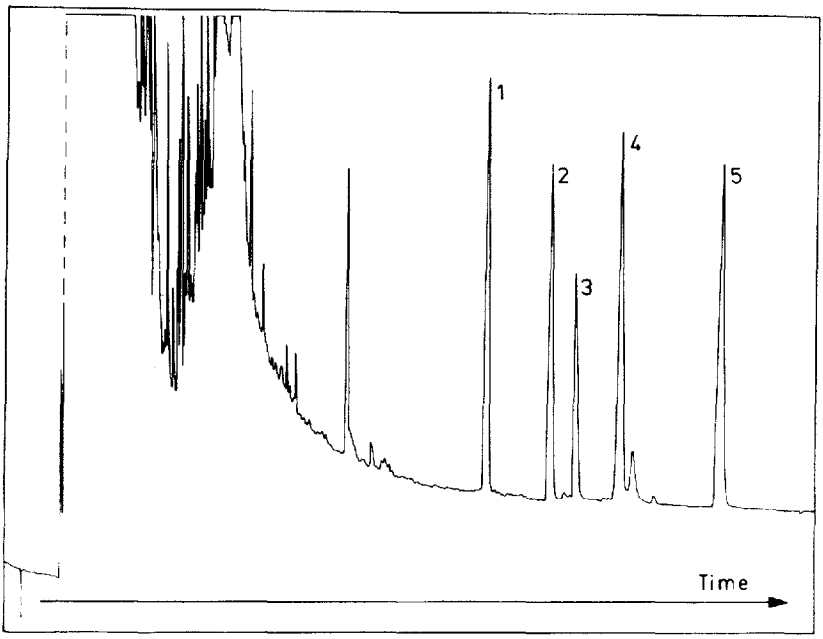

Fig. 1. Chromatogram of a meat extract with flame-ionization detection. WG-11 capillary column (50 m × 0.2 mm I.D.); injection temperature, 210°C; oven temperature, 80°C for 1.5 min, then 220°C; carrier gas, helium; inlet pressure, 3.2 atm. Peaks: 1 = di-TFA-putrescine; 2 = di-TFA-cadaverine; 3 = TFA-ETO-histamine; 4 = di-TFA-1,6-diaminohexane; 5 = di-TFA-1,7-diaminoheptane.

GC and GC–MS analysis

The derivatives of amines were separated mainly on the WG-11 capillary column. The GC conditions are given in Fig. 1. The AR OV-330 capillary column was used only to check the quantitation of diamines on WG-11.

GC calibration graphs were determined for amines in the range 25–250 ng. A number of standard solutions, each containing the internal standard and the amines investigated, were derivatized and separated as described above. The concentrations of both the internal standard and the amines were varied. The peak-area ratios relative to the internal standard were plotted against the concentration ratios of amines. There was a linear relationship over the investigated concentration range. The detection limit was below 8 ng of amine.

For mass fragmentometric quantification, the WG-11 column was also used (Fig. 2). The mass spectrometer was focused on the $(CF_3CONHCH_2)^+$ fragment and its isotope peak (*m/e* 126 and 127). Mass fragmentometric calibration graphs were constructed in the same manner as in GC. The amounts of amines lay in the range 12.5–125 ng. Over this range the relationship between peak area and concentration ratios was linear. The detection limit was about 1 ng of amine.

RESULTS AND DISCUSSION

Derivatization

Trifluoroacetylation of diamines resulted in complete conversion of both amino

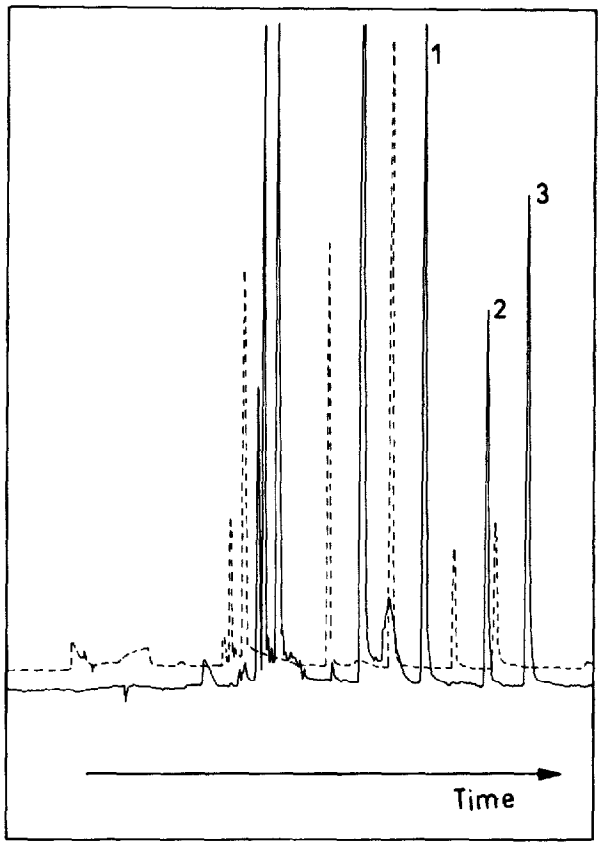

Fig. 2. Mass fragmentogram of a meat extract. GC conditions: WG-11 capillary column (40 m × 0.2 mm I.D.); injection temperature, 210°C; oven temperature, 80°C for 1.5 min, then 230°C; carrier gas, helium; inlet pressure, 3.0 atm. MS conditions: electron energy, 70 eV; trap current, 300μA. Solid line, *m/e* 126; broken line, *m/e* 127. Peaks: 1 = di-TFA-1,3-diaminopropane; 2 = di-TFA-putrescine; 3 = di-TFA-cadaverine.

groups. No partially acylated amines were observed in the gas chromatograms and the incorporation of two trifluoroacetyl groups was confirmed by the mass spectra of the derivatives. These spectra correspond to the those obtained by previous workers[14,18,19].

Histamine was converted in a two-step derivatization into N^{α}-trifluoroacetyl-N^{τ}-ethoxycarbonylhistamine, as its mass spectrum confirms (Fig. 3). This derivative shows a fragmentation pattern similar to that of N^{α}-heptafluorobutyryl-N^{τ}-ethoxycarbonylhistamine[20]. The distinct molecular ion (*m/e* 279) is followed by the fragments $M^+ - 0C_2H_5$, $M^+ - CF_3$ and $M^+ - COOC_2H_5$ (*m/e* 234, 210 and 206).

As with the heptafluorobutyrylethoxycarbonyl derivative, the position of the latter group on the imidazole ring (N^{τ} or N^{π}) is not clear. However, the N^{τ}-position is more likely because of steric hindrance of the other position[20]. Mita *et al.*[20] ascertained almost 100% conversion of histamine to its heptafluoroethoxycarbonyl derivative.

As mentioned in the description of the derivatization procedure, TFA-ETO-histamine had to be kept in an excess of ethyl chloroformate, otherwise it degraded. We could have performed a series of histamine analyses on one WG-11 capillary column

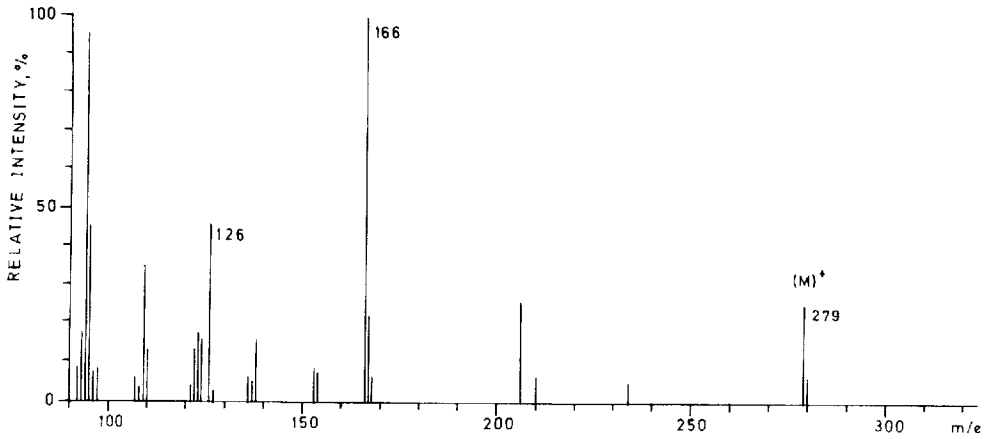

Fig. 3. Mass spectrum of TFA-ETO-histamine. Electron energy, 70 eV; trap current, 300 μA.

without lowering its resolution power, but other WG-11 capillaries were rapidly severely damaged with ethyl chloroformate. A similar experience could have led Mita *et al.*[16] to remove an excess of this reagent in the mass fragmentometric determination of histamine in biological samples. Ethyl chloroformate was also removed after modifying amino, phenolic hydroxyl and other functional groups for GC determination[21–23].

Precision of the isolation methods

The overall precision of the ion-exchange procedure was determined by analysing ten samples taken from a centrifuged perchloric acid extract. To each meat extract an equal amount of 1,7-diaminoheptane was added and the samples were separately chromatographed. Table I gives the relative standard deviations for putrescine, cadaverine and the sum of both. The latter quantity was of interest as a potential spoilage indicator; it could be determined with a relative standard deviation (R.S.D.) of 8%.

The overall precision of the precipitation method was determined in the same manner also using 1,7-diaminoheptane as an internal standard. The R.S.D. for the sum of putrescine and cadaverine was 12% (Table II).

Check of the GC quantification

The GC analysis of diamines on a WG-11 capillary columns was checked for possible errors due to overlapping substances. Putrescine and cadaverine were quantified in three different meat extracts by GC and mass fragmentography with 1,3-diaminopropane as an internal standard (Table III). The differences between the GC and

TABLE I

PRECISION OF THE DETERMINATION OF DIAMINES BY THE ION-EXCHANGE METHOD

Parameter	*Putrescine*	*Cadaverine*	*Total*
Mean concentration (mg per 100 g)	0.18	1.28	1.46
Number of determinations	10	10	10
R.S.D. (%)	21	9	8

TABLE II

PRECISION OF THE DETERMINATION OF DIAMINES BY THE PRECIPITATION METHOD

Parameter	*Putrescine*	*Cadaverine*	*Total*
Mean concentration (mg per 100 g)	1.09	1.97	3.06
Number of determinations	9	9	9
R.S.D. (%)	21	7	12

mass fragmentographic results corresponded to the precisions of these methods (the former determination had an R.S.D. of 6% and the latter 7%).

Three meat extracts were quantified by GC on WG-11 and AR OV-330 columns, using 1,7-diaminoheptane as an internal standard (Table IV). In this comparison also the differences did not exceed the range of variation of the GC analysis.

TABLE III

COMPARISON OF GC AND MASS FRAGMENTOGRAPHIC (MF) RESULTS

Sample	*Method*	*Concentration of amines (mg per 100 g)*			*Comparison (%)**		
		Putrescine	*Cadaverine*	*Total*	*Putrescine*	*Cadaverine*	*Total*
A	GC	0.95	1.78	2.73			
	MF	0.97	1.53	2.49	102.1	85.9	91.2
B	GC	0.49	1.47	1.96			
	MF	0.54	1.56	2.10	110.2	106.1	107.1
C	GC	0.57	0.22	0.79			
	MF	0.59	0.18	0.77	103.5	81.8	97.4

* MF results based on corresponding GC results = 100%.

Both checks indicate that the separation of diamines on a WG-11 capillary column was not disturbed by interfering substances.

Comparison of the isolation methods

The ion-exchange and precipitation procedures were used to check the accuracy of the pre-separation step. Five meat samples with different amine contents were ana-

TABLE IV

COMPARISON OF GC RESULTS ON TWO CAPILLARY COLUMNS

Sample	*Column*	*Concentration of amines (mg per 100 g)*			*Comparison (%)**		
		Putrescine	*Cadaverine*	*Total*	*Putrescine*	*Cadaverine*	*Total*
A	WG-11	1.34	1.75	3.10			
	AR OV-330	1.31	1.58	2.90	97.7	90.2	93.5
B	WG-11	1.13	3.12	4.25			
	AR OV-330	1.09	3.18	4.21	96.4	101.9	99.0
C	WG-11	0.33	2.32	2.65			
	AR OV-330	0.31	2.60	2.91	93.9	112.0	109.8

* AR OV-330 results based on WG-11 results = 100%.

TABLE V

COMPARISON OF RESULTS OBTAINED BY ION-EXCHANGE AND PRECIPITATION METHODS

Sample	*Method*	*Concentration of amines (mg per 100 g)*			*Comparison (%)**		
		Putrescine	*Cadaverine*	*Total*	*Putrescine*	*Cadaverine*	*Total*
A	Precipitation	3.02	6.59	9.61			
	Ion exchange	2.82	6.28	9.10	93.3	95.2	94.6
B	Precipitation	0.09	0.44	0.53			
	Ion exchange	0.09	0.47	0.56	100.0	106.8	105.6
C	Precipitation	1.47	1.90	3.38			
	Ion exchange	1.35	1.95	3.30	91.8	102.6	97.6
D	Precipitation	1,29	3,60	4,89			
	Ion exchange	1.26	3.56	4.82	97.6	98.8	98.5
E	Precipitation	1.23	2.17	3.40			
	Ion exchange	1.25	2.16	3.41	101.6	99.5	100.2

* Ion-exchange results based on precipitation results = 100%.

lysed by both methods. The perchloric acid extract from each sample was divided into two equal parts and cleaned up by ion-exchange and precipitation, with 1,7-diaminoheptane as an internal standard.

In the ion-exchange procedure smaller ions can be discriminated, whereas in the precipitation method a loss of large ions cannot be excluded. In either instance systematic deviations between the results of these methods would arise. However, the determined diamine concentrations (Table V) scattered in both directions and no systematic trend could be recognized. The scatter corresponded to the precisions of these methods. The ion-exchange and precipitation procedures provided identical results with comparable accuracy.

Recovery rates

To determine the recoveries of putrescine, cadaverine and histamine, a dry meat homogenate was split into two equal samples and perchloric acid and 1,7-diaminoheptane were added to each one of the samples being fortified with the amines investigated. The recovery rates, determined by means of the internal standardmethod, are summarized in Tables VI and VII and averaged 100 ± 12%.

Amine content of pork

The ion-exchange pre-separation and GC analysis were used to determine amine

TABLE VI

RECOVERY RATES IN THE ION-EXCHANGE METHOD

Sample	*Amine concentration in meat (mg per 100g)*			*Added amount (mg per 100 g)*			*Recovered amount (%)*		
	Putrescine	*Cadaver-ine*	*Histamine*	*Putrescine*	*Cadaver-ine*	*Histamine*	*Putrescine*	*Cadaver-ine*	*Histamine*
A	0.56	0.43	0.22	2.00	2.00	2.00	110	105	112
B	0.87	1.23	0.45	1.00	1.00	1.00	93	96	98
C	0.38	0.67	0.27	0.50	0.50	0.50	95	89	94

TABLE VII

RECOVERY RATES IN THE PRECIPITATION METHOD

Sample	*Amine concentration in meat (mg per 100 g)*			*Added amount (mg per 100 g)*			*Recovered amount (%)*		
	Putrescine	*Cadaverine*	*Histamine*	*Putrescine*	*Cadaverine*	*Histamine*	*Putrescine*	*Cadaverine*	*Histamine*
A	0.14	1.65	–	1.33	1.33	–	94	100	–
B	0.36	0.72	0.21	1.00	1.00	1.00	99	93	102
C	1.08	2.35	0.89	2.00	2.00	2.00	95	104	93

concentrations in commercial fresh pork obtained from different meat markets. In these samples putrescine averaged 0.06 mg per 100 g (n = 13; R.S.D. = 87%) and cadaverine 0.18 mg per 100 g (n = 13; R.S.D. = 49%). These values agree well with those obtained by other analytical methods. Nakamura *et al.*[24] determined the diamines in fresh pork by high-performance liquid chromatography (HPLC). The mean values of putrescine and cadaverine were 0.09 and 0.41 mg per 100 g respectively. Yamamoto *et al.*[22] cleaned up their extracts on a strongly acidic cation exchanger and quantified the diamines as ethoxycarbonyl derivatives by GC. In unspoiled pork they found putrescine and cadaverine in concentrations 0.25 and 0.26 mg per 100g, respectively.

Our histamine values for fresh pork ranged between 0.02 and 0.27 mg per 100 g, with an average of 0.12 mg per 100 g. Taylor *et al.*[25] found nearly the same levels (0.03–0.36 mg per 100 g; mean 0.12 mg per 100 g) using a selective extraction and fluorimetric detection with *o*-phthalaldehyde.

In conclusion, the use of combined ion-exchange and capillary gas chromatography permits the determination of diamines in meat with good sensitivity and precision and with a high degree of specificity. The method gives results that agrees well with those obtained by HPLC and, although more complicated is free from many of the possible interferences seen with less specific methods.

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