(HCB) using the $^{13}C_6$-labelled standard [6]. For the analysis of eggs, sample preparation involves initial treatment with phospholipase, extraction with acetone/hexane and then clean-up of the lipid extract on a water deactivated alumina column. Selected ion monitoring is for m/z 284 and 286 for HCB and 292 and 294 for [$^{13}C_6$]HCB. The GC/MS program involves switching between a number of different ions, grouped for different retention time windows to enable monitoring of altogether 10 organochlorine pesticides plus respective isomers. For the future it is intended to increase the number of isotopically labelled standards utilized which will further increase the complexity of the multiple ion monitoring program.

4. Veterinary Drug Residues in Animal Tissue

Veterinary drug residues in foods, although obvious candidates for isotope dilution approaches to analysis, do present the same logistical problems as pesticides with the same requirement for multi-residue monitoring. For the determination of the sulphonamide drug, sulphamethazine in kidney samples by isotope dilution GC/MS [7], after addition of d_4-sulphamethazine to an acetonitrile slurry of the kidneys and allowing for equilibration, the clean-up involves solvent partition, diazomethane treatment to form the methyl derivative and then HPLC fractionation as a further clean-up stage. In the trapped HPLC fraction GC/MS selected ion monitoring for m/z 227 and 228 for sulphamethazine and 231 and 232 for the deuterated internal standard is carried out for quantification. The limit of detection of the method is around 0.05 mg/kg and the CV is between 3.7 and 5.7% for sulphamethazine spiking levels in the tissue from 0.2 to 1.2 mg/kg. Deuterated sulphamethazine had to be custom synthesized and other stable isotope-labelled drugs are not widely available commercially which hinders development of this approach, as does the fact that many drugs of interest are not amenable to GC. One possible way to overcome this latter difficulty is to maintain the use of isotope dilution but to use MS/MS which also confers advantages of reduced sample preparation and clean-up [8].

References

[1] Startin, J. R., Parker, I., Sharman, M., and Gilbert, J., J. Chromatogr. **387**, 509 (1987).
[2] Castle, L., Mercer, A. J., Startin, J. R., and Gilbert, J., Migration from plasticized films into foods. 3. Migration of phthalate, sebacate, citrate, and phosphate esters from films used for retail food packaging, Food Addit. Contamin., in press (1988).
[3] Startin, J. R., Sharman, M., Rose, M. D., Parker, I., Mercer, A. J., Castle, L., and Gilbert, J., Food Addit. Contamin. **4**, 385 (1987).
[4] Castle, L., Mercer, A. J., Startin, J. R., and Gilbert, J., Food Addit. Contamin. **4**, 399 (1987).
[5] Castle, L., Mercer, A. J., and Gilbert, J., Gas chromatographic-mass spectrometric determination of adipate-based polymeric plasticizers in foods, J. Assoc. Offic. Anal. Chem., in press (1988).
[6] Gilbert, J., Startin, J. R., and Crews, C., Pestic. Sci. **18**, 273 (1987).
[7] Gilbert, J., Startin, J. R., and Crews, C., J. Assoc. Publ. Analysts **23**, 119 (1985).
[8] Finlay, E. M. H., Games, D. E., Startin, J. R., and Gilbert, J., Biomed. Environ. Mass Spectrom. **13**, 633 (1986).

An Isotope Dilution Mass Spectrometric (IDMS) Method for the Determination of Vitamin C in Milk

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The accurate determination of constituents in food is necessary to establish dietary requirements. A non-fat milk powder (NFMP) has been developed as a Standard Reference Material (SRM) for use in validating methods for the analysis of milk and other biological materials. When this SRM was issued, L-ascorbic acid (vitamin C or AA) was measured by HPLC methods developed at NBS and by the AOAC microfluorimetric method.

An IDMS method has been developed as an independent method to measure AA in NFMP, using AA-13 C-1 as an internal standard. After the labeled AA is added to the NFMP and has equilibrated with the unlabeled AA present, samples are treated to remove protein, put over an anion exchange column, and freeze-dried. AA is converted

to the t-butyldimethylsilyl derivative and analyzed by capillary column GC/MS under a strict measurement protocol [1]. The two masses of interest are the $[M\text{-}C_4H_9]^+$ ions at 575 and 576. The ions are monitored using a mass switching system designed to provide accurate measurement of isotope ratios for the narrow GC peaks (12–15 seconds) typical of capillary columns.

Two independent sets of standards were prepared by weight from labeled and unlabeled ascorbic acid. Each set was used as standards to measure, according to the protocol, the weight ratios of the other set as if it were samples. The difference between the measured and weighed weight ratios within each set is calculated as a percent difference, which is a measure of the consistency of each independently prepared set with the other. As an example, the average percent difference for set 1 as standards and set 2 as "samples" was +0.06%. This set is consistent with the other set because the mean of the percent differences is acceptably low.

Analyses of the NFMP Standard Reference Material run in 1984 gave values of 53 (SD=5) μg/g by an HPLC method [2] and 49.6 (SD=4.2) by the AOAC method [2,3]. Analyses run in 1987 by two other HPLC methods [4] gave values of 35 (SD=1) and 41.4 μg/g. Table 1 gives the results of the IDMS analyses; the IDMS overall mean is 40.23 μg/g (SD=0.71).

There are two points to consider. Firstly, the value for AA appears to have fallen over 3 years, suggesting that AA is not stable in this matrix. It must be noted, however, that the measurement methods used in 1984 and 1987 were not the same. Secondly, there is a difference between the HPLC measurements made in the same year, and that one result does not agree with the IDMS result, thus indicating a problem with at least one of the methods.

In summary, L-ascorbic acid can be determined precisely in NFMP by an ID/MS method using a C-13 labeled ascorbic acid as the isotopic diluent. The discrepancy between the HPLC and IDMS methods is being investigated, but since the L-ascorbic acid content of this Standard Reference Material may change rapidly with time, a certified value for AA will not be assigned.

Table 1. Results of ID/MS analyses

Bottle	Sample	μg AA/g NFMP	SD	CV(%)
1	1	40.31		
	2	39.85		
	mean	40.08	0.33	0.82
2	1	41.11		
	2	40.55		
	3	41.16		
	4	41.21		
	5	41.01		
	6	39.68		
	mean	40.79	0.59	1.45
3	1	39.88		
	2	39.37		
	3	39.15		
	4	39.76		
	5	39.91		
	mean	39.61	0.34	0.85
overall mean		40.23	0.71	1.77

References

[1] Cohen, A., Hertz, H. S., and Mandel, J., et. al. Clin. Chem. **26**, 854 (1980).
[2] Certificate of Analysis 1549: Non-Fat Milk Powder.
[3] Tanner, J. T., Smith, J. S., and Angyal, G., et. al. J. Assoc. Off. Anal. Chem. **68**, 110 (1985).
[4] S. Margolis, NBS, private communication.