Food Chemistry **28** (1988) 269–278

New Methods for the Determination of Aspartame

U. Viplava Prasad, T. E. Divakar, C. S. P. Sastry*

Food and Drug Laboratories, School of Chemistry, Andhra University, Waltair—530 003, India

M. Veerabhadra Rao & O. P. Kapur

Central Food Technological Research Institute, Mysore—570 013, India

(Received 14 August 1987; revised version accepted 15 September 1987)

ABSTRACT

New titrimetric and spectrophotometric methods are proposed for the determination of aspartame. N-bromosuccinimide (NBS), acetous perchloric acid and sodium methoxide are used as titrants, employing some internal indicators in a titrimetric assay. NBS-metol-sulphanilamide is used for the indirect spectrophotometric determination of aspartame. The charge-transfer complexes formed between aspartame and quinones, namely, chloranilic acid and chloranil, are explored for their spectrophotometric determination. Experimental conditions and recoveries in commercial samples and foods are reported.

INTRODUCTION

Aspartame (*N*-L-aspartyl-L-phenylalanine methyl ester), a non-nutritive sweetener with a sweetness of about 150–200 times that of sucrose, has been developed by Mazur *et al.* (1969). Several experiments regarding its safety have been carried out and are summarised in a WHO toxicological monograph (WHO, 1980). Basing on the no-effect level, the FAO/WHO Joint Expert Committee on Food Additives has allocated an ADI of 0–40 mg/kg bw (FAO/WHO, 1980) and it has been approved for use in

* To whom correspondence should be addressed.

Food Chemistry 0308-8146/88/$03·50

several countries. Aspartame is separated from other amino acids and additives by thin-layer chromatography (Skarka *et al.*, 1979; Daniels *et al.*, 1984; Ibe *et al.*, 1985). It is determinable using an amino acid analyzer (Ishiwata & Suzuki, 1975; Skarka *et al.*, 1980; Veseley *et al.*, 1980; Prudel & Davidkova, 1981; Jost *et al.*, 1982) and by spectrophotometry in the visible region using copper sulphate (Goven & Ozol, 1984), 4-dimethylamino benzaldehyde (Ozol, 1984) and benzoquinone (Vechek, 1984) or fluorimetry (Poctova & Kakac, 1982). It is determinable by gas-chromatography (Furda *et al.*, 1975) and high-performance liquid chromatography (Fox *et al.*, 1976; Jost *et al.*, 1982; Scherz *et al.*, 1983; Terada & Sakabe, 1983; Argoudelis, 1984; Daniels *et al.*, 1984; Tsang *et al.*, 1985; Verzella & Mangia, 1985; Tyler, 1984; Webb & Beckman, 1984, Ibe *et al.*, 1985; Tamase *et al.*, 1985; Verzella *et al.*, 1985, Veerabhadra Rao *et al.*, 1987). Simple techniques such as titrimetric methods have not been reported and spectrophotometric methods developed are tedious and time consuming. In this paper, we report the use of internal indicators such as Oracet Blue B (OB B), Sudan Blue GN (SB GN), Sudan Green 4B (SG 4B) (amino anthraquinone dyes) and Resazurin in the non-aqueous titrimetric assay of aspartame using acetous perchloric acid as titrant. Azure A, Azure C, Toluidine Blue O (thiazine dyes), Bromo-Pyrogallol Red (BPR) and Catechol Violet (CV) have successfully been used for its assay using sodium methoxide as titrant. Also, the use of NBS in the oxidimetric assay of aspartame has been explored. NBS-metol-sulphanilamide has been used for the indirect spectrophotometric determination of aspartame. The charge-transfer complexes formed between aspartame and quinones; namely, chloranilic acid and chloranil, have been explored for its spectrophotometric determination. All the proposed methods have been extended for the determination of aspartame in commercial samples and food products.

MATERIALS AND METHODS

All the chemicals and reagents used were of AnalaR Grade. Spectral and absorbance measurements were made with a Perkin Elmer model 575 double beam spectrophotometer and an Elico MK 1 model spectrophotometer.

Preparation of solutions

(*a*) *Standard solutions of aspartame*

A 1 mg/ml solution of aspartame (Searle, Bombay) was prepared by dissolving 100 mg in 100 ml of chloroform:methanol (1:1). A 4 mg/ml solution of aspartame was prepared in glacial acetic acid, methyl isobutyl ketone,

benzene:methanol (3:1) and distilled water separately. The solutions were diluted to working standard solutions with the same solvent whenever required.

(*b*) *Perchloric acid solution*
A 0·1N perchloric acid solution was prepared by mixing 8·5 ml of 70–72% perchloric acid (Riedel, Germany) with glacial acetic acid and adding 25 ml of acetic anhydride in a litre volume. The solution was standardised with potassium acid phthalate dissolved in acetic acid using crystal violet as indicator (Jones, 1959). The solution was further diluted to the required strength when needed.

(*c*) *Sodium methoxide solution*
A 0·1N sodium methoxide solution was prepared by dissolving 2·5 g of sodium metal in 200 ml methanol, followed by dilution to 1 litre with benzene. It was standardised with benzoic acid using thymol blue as indicator (Jones, 1959).

(*d*) *N-bromosuccinimide (NBS) solution*
A 0·02M NBS solution was prepared by dissolving 0·356 g of NBS in a minimum amount of hot water and diluting to 100 ml with distilled water. The solution was standardised iodometrically (Haroun & Khattab, 1978). It was diluted to 0·01M and prepared freshly every day.

(*e*) *Indicator solutions*
0·1% solutions of OB B, SB GN, SG 4B (Chroma-Gesellschaft, Stuttgart) and Resazurin (Gurr Co., Great Britain) were prepared in glacial acetic acid. The solutions (0·1%) of Azure A, Azure C, Toluidine Blue O, BPR (Gurr Co., Great Britain) and CV (BDH, Great Britain) were prepared in methanol. Freshly prepared 1·0% starch indicator was used.

(*f*) *Sodium thiosulphate solution*
A 0·04M sodium thiosulphate (BDH) solution was prepared and standardised using standard potassium dichromate and was diluted to 0·02M when required.

(*g*) *Metol (p-N-methylamino phenol sulphate) solution*
A 0·3% solution of metol was prepared by dissolving 300 mg of metol in 100 ml of distilled water.

(*h*) *Sulphanilamide solution*
A $1{\cdot}163 \times 10^{-3}$M solution was prepared by dissolving 200 mg of sulphanilamide initially in a minimum quantity of dilute hydrochloric acid and subsequently diluting it to 100 ml with distilled water.

(*i*) *Chloranil and chloranilic acid solutions*
0·1% solutions of chloranil and chloranilic acid were prepared separately by dissolving 100 mg each in 100 ml of 1,4-dioxan.

Recommended procedures

Titrimetric determination of aspartame

Method A. Acetous perchloric acid as titrant. To an aliquot of acetous aspartame (20–40 mg) solution taken into a dry conical flask, 15 ml of glacial acetic acid, 0·2 ml of indicator solution (OB B, SB GN, SG 4B or Resazurin) were added and the mixture was titrated with 0·05N acetous perchloric acid solution dropwise until a sharp colour change was observed (blue to pink for OB B and SB GN, bluish-green to wine red for SG 4B and pink to orange red for Resazurin). The amount of aspartame was deduced from its molecular weight and the titre value.

Method B. Sodium methoxide as titrant. An aliquot of sample (25–40 mg) solution was taken in a dry conical flask; 20 ml of methyl isobutylketone (in the case of Azure A, Azure C, Toluidine Blue O) or 20 ml of benzene:methanol (3:1) (in the case of BPR or CV) and 0·2 ml of indicator solution were added and the mixture was titrated against 0·05N sodium methoxide until a sharp colour change was observed. (Blue to pink for Azure A, Azure C and Toluidine Blue O; wine red to blue for BPR and orange red to blue for CV). The amount of aspartame present in the sample was deduced from the molecular weight of aspartame and the titre value.

Method C. Determination of aspartame using NBS by back titration. An aliquot (5–25 mg) of the aqueous solution of aspartame was taken in an Erlenmeyer flask and mixed with 10 ml of 0·02M NBS and the overall acidity was maintained 0·2N with respect to HCl or 2N with respect to acetic acid when diluted to 25 ml. The mixture was kept at room temperature for 2 h, 10 ml of 15% potassium iodide was added and the liberated iodine titrated with 0·04N hypo solution using starch as indicator. A blank titration was performed under similar conditions omitting aspartame. The amount of aspartame (X mg) present is given by:

$$X = M \times \text{N}(V_1 - V_2)$$

where M is the molecular weight of aspartame, N is the normality of the titrant, V_1 is the blank titre and V_2 is the titre for the sample.

Spectrophotometric determination of aspartame

Method D. Indirect spectrophotometric determination using NBS-metol-sulphanilamide. An aliquot of aspartame solution, containing 50–500 μg, was taken in a 25 ml volumetric flask; 15 ml of 1% acetic acid was added to maintain the overall pH of 2·6 when diluted to 25 ml and 0·1 ml of 0·005M NBS were added. The volume was made up to 20 ml and kept for 2 h at room temperature. Then 1·0 ml of metol (0·3%) and, after 1 min, 1·5 ml of sulphanilamide (0·2%), were added and the volume was made up to 25 ml with distilled water. The absorbance was measured at 520 nm against distilled water during the stability period (10–50 min). A blank experiment was also performed. The decrease in absorbance corresponding to aspartame was obtained by subtracting the absorbance of the sample from that of the blank. The amount of aspartame present in the sample was deduced from the standard calibration curve.

Method E. Spectrophotometric determination using chloranilic acid. An aliquot of aspartame solution (120–1250 μg) in chloroform:methanol (1:1) was taken in a 10 ml flask; 1 ml of chloranilic acid and 1 ml of dimethylformamide were added, the volume was made up to 10 ml with dioxan and the absorption was measured at 520 nm against a reagent blank. The amount of aspartame was deduced from the calibration curve.

Method F. Spectrophotometric determination using p-chloranil. An aliquot of the aspartame solution (250–4500 μg) in chloroform:methanol (1:1) was taken into a 10 ml volumetric flask; 1 ml of 0·1% *p*-chloranil and 1 ml of dimethylformamide were added, the volume was made up to 8 ml with dioxan and the flask was heated on a water-bath maintained at 65°C for 30 min. The solution was cooled and made up to volume with dioxan and the absorbance was measured at 520 nm against the reagent blank. The amount of aspartame was deduced from its calibration curve.

Analysis of commercial samples of aspartame

Commercial samples of aspartame were treated similarly as in a standard preparation and the recommended procedures were followed for their determination.

Recovery experiments from dry beverages mixes

In order to find the suitability of the proposed spectrophotometric methods for the determination of aspartame in food products, malted beverage mix and ragi-malt samples were spiked with aspartame at the 500 and 1000 ppm levels and were analysed after extraction following the recommended

procedure. To accurately weighed beverage powder, 20 ml of methanol was added, shaken for 15 min, filtered and the remaining powder washed with more solvent. The combined filtrates were evaporated and the residue was dissolved in distilled water (while using method D) or chloroform : methanol (1:1) (while using methods E & F) and made up to a known volume with the corresponding solvent. Taking an aliquot, the recommended procedure was followed for its determination.

RESULTS AND DISCUSSION

The maximum error and the per cent purity of commercial samples obtained in the titrimetric determinations (acetous perchloric acid, SB GN; sodium methoxide, Azure A; NBS) of aspartame are in agreement with their label declarations (Table 1).

The optical characteristics of the spectrophotometric methods for the determination of aspartame by the indirect method employing NBS-metol-sulphanilamide and the direct method using chloranilic acid and chloranil are given in Table 2. The precision (given in terms of per cent range of error at confidence limits of the 0·05 level and the per cent relative standard deviation) and accuracy (given in terms of % error) in the analysis of six replicate samples by the proposed methods are given in Table 2. The purity assay of commercial aspartame and aspartame tablets and the per cent recovery of aspartame added to a malted beverage mix and ragi-malt samples are given in Table 3. It can be seen from Table 3 that all the methods are sensitive (especially the NBS-metol-sulphanilamide method), reprodu-

TABLE 1
Titrimetric Methods for the Determination of Aspartame

	Method	*Maximum error (%)*[a]	*% Purity*	
			Commercial aspartame declared (99%)	*Aspartame tablets declared (20%)*
A	Acetous perchloric acid and SB GN	0·5	99·1	20·1
B	Sodium methoxide and Azure A	0·8	99·2	20·1
C	NBS	0·7	98·6	20·1

[a] Average of three determinations.

TABLE 2
Optical Characteristics and Precision for the Determination of Aspartame in the Spectrophotometric Methods

Sample No.	*Parameter*	*Chloranilic acid system*	*Chloranil system*	*NBS-metol-sulphanilamide system*
1.	Absorption maximum	520 nm	520 nm	520 nm
2.	Beer's law limits (μg/ml)	12–205	25–450	2–20
3.	Regression equation[a]	0·003 2 + 0·002 8C	0·002 1 + 0·001 3C	−0·001 + 0·023C
4.	Correlation coefficient	0·999 6	0·999 8	0·999 8
5.	% relative standard deviation at confidence limit (0·05 level)	1·75	1·9	1·9
6.	% range of error[b]	±1·8	±1·7	±2·02

[a] Found in this work; it must be determined independently by the users of the method.
[b] Value based on six determinations.

cible and suitable for the determination of aspartame in commercial samples, as well as food products, even when present in microgram amounts.

All the proposed indicators used in the non-aqueous titrimetric assay are perfectly reversible. The colour change at the end point may be due to the formation of a protonated secondary amine in the case of amino anthraquinone dyes and formation of salt through tetravalent sulphur (present in orthoquinoid form) leading to the formation of sulphonium base in the case of thiazine dyes. The colour change in the case of BPR and CV may be due to the breakage of the five membered ring and conversion of one of the benzene rings to the quinoid form. The consumption of one mole of NBS per mole of aspartame may be explored by the substitution of the proton on the peptide nitrogen by bromine as methylamine fails to consume NBS under similar conditions. The formation of a purple red charge-

TABLE 3
Spectrophotometric Methods for the Determination of Aspartame

Method	*Maximum error* (%)	% *Purity*		% *Recovery*	
		Commercial aspartame declared (99%)	*Aspartame tablets declared (20%)*	*Malted beverage mix*	*Ragi malt*
D	0·9	99·1	19·9	99·0	98·4
E	0·8	99·2	20·1	99·2	99·3
F	1·2	99·2	20·05	98·8	98·4

transfer complex is based on the reaction between sulphanilamide and oxidized metol (*p*-*N*-methyl aminophenol). The excess NBS reacts with metol to form *p*-*N*-methylbenzoquinone monoimine which forms a charge-transfer complex with sulphanilamide at pH 2·6 (Sastry *et al.*, 1981). The composition of the coloured species formed immediately upon mixing aspartame and chloranilic acid solution was found to be 1:1 through a mole ratio method which suggests the formation of charge-transfer complex between aspartame and quinone molecules.

The proposed methods are simple, sensitive, accurate and could be used for the routine determination of aspartame in commercial samples and food products. Titrimetric (A–C) and visible spectrophotometric (D–F) methods are suitable for the determination of semimicro and micro amounts of aspartame, respectively.

ACKNOWLEDGEMENT

The authors, MVR and OPK, thank the Director, Central Food Technological Research Institute, Mysore, for providing facilities and encouragement during the course of this work. TED thanks CSIR for the financial assistance provided to him.

REFERENCES

Argoudelis, C. J. (1984). Isocratic liquid chromatography method for simultaneous determination of aspartame and other additives in soft drinks, *J. Chromatogr.*, **303**, 256–62.

Daniels, D. H., Joe, F. L., Jr, Warner, C. R. & Fazio, T. (1984). Liquid chromatographic determination of aspartame in dry beverage bases and sweetener tablets with confirmation by thin-layer chromatography, *J. Assoc. Off. Anal. Chem.*, **67**, 513–15.

Fox, L., Anthony, G. D. & Lau, E. P. K. (1976). High-performance liquid chromatographic determination of L-aspartyl-L-phenylalanine methyl ester in various food products and formulations, *J. Assoc. Off. Anal. Chem.*, **59**, 1048–50.

Furda, I., Malizia, P. D., Kolor, M. G. & Vernieri, P. J. (1975). Decomposition products of L-aspartyl-L-phenylalanine methyl ester and their identification by gas–liquid chromatography, *J. Agri. Food Chem.*, **23**, 340–3.

Goven, K. C. & Ozol, T. (1984). Determination of aspartame by spectrophotometric method, *Acta Pharm. Pharmacy*, **26**, 28–30.

Haroun, I. & Khattab, F. (1978). Semimicro determination of tetracyclines using *N*-bromosuccinimide. *Indian J. Pharmacy*, **40**, 12–14.

Ibe, A., Saito, K., Nakazato, M., Kikuchi, Y., Fuzima, K., Naoi, Y. & Nishima, T. (1985). Detection and determination of aspartame in foods by thin-layer chromatography and high-performance liquid chromatography. *Shokuhin Eiseigaku Zasshi*, **26**, 1–6 (*Anal. Abstr.*, **47**, 11F15, 1985).

Ishiwata, A. & Suzuki, Y. (1975). Determination of L-aspartyl-L-phenylalanine methyl ester in foods, *Shokuhin Eiseigaku Zasshi*, **16**, 420–1 (*Anal. Abstr.*, **32**, 2F11, 1977).

Joint FAO/WHO Expert Committee on Food Additives (1980). *Evaluation of certain food additives*, 21st report, Technical Report Series No. 653, WHO, Geneva, 20.

Jones, A. G. (1959). *Analytical chemistry, some new analytical techniques*, Butterworth Scientific Publications, London, 161–2.

Jost, R., Monti, J. C. & Schaufelberger, U. (1982). Analysis of dipeptide sweetener aspartame, *Int. J. Vitam. Nutr. Res.*, **52**, 229 (*Anal. Abstr.*, **44**, 6F10, 1983).

Mazur, R. H., Schalatter, J. M. & Goldkamp, A. H. (1969). Structure–taste relationships of some dipeptides, *J. Amer. Chem. Soc.*, **91**, 2684–91.

Ozol, T. (1984). Spectrophotometric determination of aspartame in effervescent tablets, *Acta Pharm. Pharmacy*, **26**, 59–62.

Poctova, M. & Kakac, B. (1982). Fluorimetry in pharmaceutical analysis, III. Fluorimetric determination of L-aspartyl-L-phenylalanine methyl ester hydrochloride, *Cesk. Farm.*, **31**, 113–15 (*Anal. Abstr.*, **43**, 6E70, 1982).

Prudel, M. & Davidkova, E. (1980). Determination of Usal (aspartame) sweetener in dairy products, *Prum potravin*, **31**, 329–30 (*Anal. Abstr.*, **40**, 1F27, 1981).

Sastry, C. S. P., Rao, B. G. & Reddy, B. S. (1981). Spectrophotometric determination of pharmaceutically important primary aryl amines using metol and NBS, *J. Ind. Chem. Soc.*, **58**, 655.

Scherz, J. C., Monti, J. C. & Jost, R. (1983). Analysis of peptide sweetener aspartame by liquid chromatography, *Z. Lebensm. Unters. Forsch.*, **177**, 124–8.

Skarka, P., Sestakova, I. & Turza, P. (1979). Semi-quantitative determination of synthetic sweetener MEFA (aspartame) in the feed supplement DB SOL, *Biol. Chem. Zivocisne Vyroby-Vet.*, **15**, 39–43 (*Anal. Abstr.*, **37**, 3G21, 1979).

Skarka, P., Sestakova, I., Smolek, P. & Entlicher, G. (1980), Determination of artificial sweetener MEFA (aspartame) in feed supplement, *Biol. Chem. Zivociene Vyroby-Vet.*, **16**, 45–47 (*Anal. Abstr.*, **39**, 5G19, 1980).

Tamase, K., Kitada, Y., Sasaki, M., Ueda, Y. & Takeshita, R. (1985). Determination of aspartame in foods by high-performance liquid chromatography with a fluorescence detector, *Shokuhin Eiseigaku Zasshi*, **26**, 515–18 (*Anal. Abstr.*, **48**, 7F16, 1985).

Terada, H. & Sakabe, Y. (1983). Analysis of food additives by high performance liquid chromatography, *Eisei Kagaku*, **29**, 394–9 (*Anal. Abstr.*, **46**, 10F19, 1984).

Tsang, W. S., Clarke, M. A. & Parrish, F. W. (1985). Determination of aspartame and its breakdown products in soft drinks by reverse phase chromatography with U.V. detection, *J. Agri. Food Chem.*, **33**, 734–8.

Tyler, T. A. (1984). Liquid chromatographic determination of sodium saccharin, caffeine, aspartame and sodium benzoate in cola beverages, *J. Assoc. Off. Anal. Chem.*, **67**, 745–7.

Vechek, J. (1984). Spectrophotometric determination of *N*-L-aspartyl-L-phenylalanine methyl ester, *Cesk. Farm.*, **33**, 217–19 (*Anal. Abstr.*, **47**, 4E86, 1985).

Veerabhadra Rao, M., Narayan, M. S., Kapur, O. P. & Sastry, C. S. P. (1987). Reverse phase liquid chromatographic determination of some food additives, *J. Assoc. Off. Anal. Chem.*, **70**, 578–82.

Verzella, G., Bagnasco, G. & Mangia, A. (1985). Ion-pair high-performance liquid

chromatographic analysis of aspartame and related products, *J. Chromatogr.*, **349**, 83–89.

Verzella, G. & Mangia, A. (1985). High-performance liquid chromatographic analysis of aspartame, *J. Chromatogr.*, **346**, 417–22.

Vesely, Z., Davidkova, E. & Prudel, M. (1980). Determination of L-aspartyl-L-phenylalanine methyl ester (aspartame) hydrochloride in soft drinks, *Nahrung*, **24**, 525–8.

Webb, G. N. & Beckman, D. D. (1984). Reversed-phase liquid chromatographic determination of aspartame in beverages and beverage mixes, *J. Assoc. Off. Anal. Chem.*, **67**, 510–13.

WHO Food Additives Series (1980). *Toxicological evaluation of certain food additives*, WHO, Geneva, No. 15, 18.