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FTIR Determination of Aspartame and Acesulfame-K in Tabletop Sweeteners

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Two different strategies for sweeteners determination in tabletop samples by Fourier transform middle-infrared (FTIR) spectrometry, an off-line and a fully mechanized extraction of Aspartame and Acesulfame-K with different mixtures of chloroform and methanol, have been developed. The off-line method involves the extraction of both active principles by sonication of samples with 25:75 v/v $CHCl_3$/CH_3OH and direct measurement of the peak height values at 1751 cm^{-1}, corrected using a baseline defined at 1850 cm^{-1} for Aspartame, and measurement of the peak height at 1170 cm^{-1} in the first-order derivative spectra, corrected by using a horizontal baseline established at 1850 cm^{-1}, for Acesulfame-K. Limit of detection values of 0.10 and 0.9% w/w and relative standard deviations of 0.17 and 0.5% were found for Aspartame and Acesulfame-K, respectively. The time needed for the sweeteners determination is reduced from 35 min for the HPLC method to 7 min by FTIR. On the other hand, the fully mechanized on-line extraction avoids the contact of the operator with toxic solvents and differentiates between samples that contain Aspartame and Acesulfame-K and those that include only Aspartame, reducing the time needed for the analysis of the last kind of samples to 5 min.

KEYWORDS: Sweeteners; Aspartame; Acesulfame-K; FTIR; on-line FTIR

INTRODUCTION

Aspartame, *N*-L-*a*-aspartyl-L-phenylalanine methyl ester, is an artificial sweetener used throughout the world in foods and beverages. It contains two amino acids, aspartic acid and phenylalanine. Studies in a number of animal species indicate that aspartame is quickly and extensively metabolized to its constituent amino acids and methanol. Aspartame is reported to have low toxicity in experimental model systems. The oral LD_{50} of aspartame in rats and mice is >10 g/kg per day (*1*).

Aspartame is coformulated in combination with Acesulfame-K in tabletop commercial sweeteners due to their synergistic sweetening effect (*2*).

Acesulfame-K, a potassium salt of 3,4-dihydro-6-methyl-1,2,3-oxathiazine-4-one-2,2-dioxide, is a high-intensity and noncaloric sweetener. It is not metabolized by the body and is excreted unchanged. Acesulfame-K is currently used in food, beverage, oral hygiene, and pharmaceutical products in about 90 countries. The oral LD_{50} reported is >7 g/kg in rats (*2*).

High-performance liquid chromatography (HPLC) is the most commonly used method nowadays for Aspartame and Acesulfame-K determination, based on isocratic reversed-phase (RP) chromatographic separation and ultraviolet absorbance detection (*3*). Moreover, ion chromatography (IC) with UV (*4*) and electrochemical (*5*) detection offers an attractive alternative to traditional HPLC methods. In recent years, micellar electrokinetic chromatography (MEKC) has been applied to the determination of several sweeteners in foods (*6*). Other methods less commonly used for sweetener determination are amperometry based on the use of bilayer lipid membranes (*7*), spectrophotometry based on the complexation of Aspartame with Cu (*8*), and a biosensor for Aspartame determination (*9*).

Although there are no precedents in the literature on the use of FTIR spectrometry for sweetener determination, it is clear that direct spectrometric measurements on chloroformic solutions could be an alternative to HPLC procedures in quality control processes of active principles in commercial formulations due to their relatively simple matrix and high analyte content (*10*, *11*).

The main objective of this work is the development of an FTIR method for the simultaneous determination of Aspartame and Acesulfame-K in tabletop formulations, and thus a comparison has been made between different strategies in order to be able to carry out these analyses in the best conditions from both aspects, the analytical figures of merit and the environmental side effects.

EXPERIMENTAL PROCEDURES

Apparatus and Reagents. A Bruker Tensor 27 (Bremen, Germany) spectrometer equipped with a temperature-stabilized deuterated lanthanum triglycine sulfate (DLATGS) detector was employed for spectral measurements, using a 0.11 mm path length microflow cell (Graseby-Specac, Orpington, U.K.) with ZnSe and CaF_2 windows. Spectra treatment and data manipulation have been carried out using Omnic

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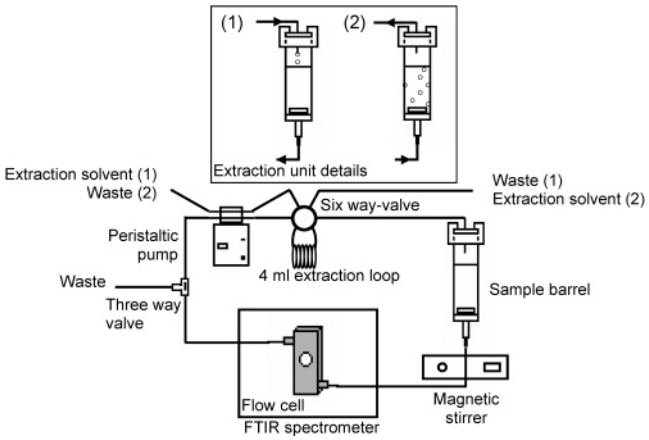

Figure 1. Manifold employed for on-line FTIR extraction of Aspartame and Acesulfame-K in tabletop sweetener samples: (**1**) configuration used in strategies A and B; (**2**) design employed in strategy C (air is circulated).

2.1 and OmnicMacros 2.1 software from Nicolet (Madison, WI). To carry out the on-line extraction of sweeteners, the manifold depicted in **Figure 1** was built by employing one six-way Rheodyne 5041 injection valve (Cotati, CA) and a Gilson Minipuls 2 peristaltic pump (Villiers-le-Bel, France) furnished with Viton (isoversinic) tubes (1 mm i.d. and 3 mm o.d.). The connecting tubes employed were made of PTFE of 0.8 mm i.d.

A Hewlett-Packard series 1050 (Palo Alto, CA) high-performance liquid chromatograph, equipped with a variable wavelength UV−vis detector and a Kromasil C-18 column (250 × 4.6 mm i.d. and 5 μm particle diameter), was also used for the analysis of tabletop sweetener samples, this methodology being employed as a reference for the validation of the FTIR determination procedures.

A J.P. Selecta ultrasonic water bath (Barcelona, Spain) was employed to improve a fast sweetener extraction in off-line experiments.

An SBS A-01 magnetic stirrer (Barcelona, Spain), working at medium rotation speed, was employed to improve the sweetener extraction in the manifold depicted in **Figure 1**.

Guinama (Barcelona, Spain) supplied Aspartame standard (99% w/w) and Fluka (Buchs, Switzerland) provided Acesulfame-K standard (98.9% w/w). Analytical grade chloroform stabilized with 150 mg L^{-1} amylene and HPLC grade methanol (Scharlau, Barcelona, Spain) were employed for the preparation of FTIR samples and standards. KH_2PO_4 and HPLC grade acetonitrile supplied by Scharlau (Barcelona, Spain), were used for the preparation of HPLC samples and standards. Commercial sweeteners employed in this study, containing Aspartame and/or Acesulfame-K, also contain lactose, sodium carboxymethylcellulose, L-leucine, and glycine as excipients. Samples analyzed were obtained directly from the Spanish market.

HPLC-UV Reference Procedure. A whole sweetener tablet (60 mg) was accurately weighed (±0.1 mg) inside a 25 mL volumetric flask, diluted to the volume with 0.02 M KH_2PO_4/acetonitrile (90:10 v/v), and sonicated during 5 min in an ultrasonic water bath to extract Aspartame and Acesulfame-K from the matrix. Half a milliliter of the extract was diluted to 10 mL with the same solvent mixture and filtered through a 0.22 μm nylon membrane; 20 μL of this latter solution was directly injected in a 0.02 M KH_2PO_4/acetonitrile (90:10 v/v) mobile phase using a constant 0.75 mL min^{-1} carrier flow, and Aspartame and Acesulfame-K were determined in the isocratic mode by absorbance measurements at 205 nm peak area values of the chromatogram obtained at 28.5 and 5.9 min, for Aspartame and Acesulfame-K, respectively, and were interpolated in the corresponding calibration lines obtained from six standards of the two studied compounds (from 10.49 to 41.97 mg g^{-1} for Aspartame and between 6.83 and 54.65 mg g^{-1} for Acesulfame-K). Each sample or standard solution was injected three times.

Calibration lines obtained in the aforementioned conditions were $A_1 = (0.6 \pm 0.8) + (42.58 \pm 0.04)C_{AS}$ with $R^2 = 0.99992$ and $A_2 = (-0.3 \pm 0.5) + (35.58 \pm 0.02)C_{AK}$ with $R^2 = 0.99998$ for Aspartame (C_{AS}) and Acesulfame-K (C_{AK}), respectively, A_1 and A_2 being the area values found at the aforementioned elution times and C being the concentration in mg L^{-1}.

The repeatabilities, established as the relative standard deviation, were 0.1 and 0.03% of five independent analyses of a 10.5 mg L^{-1} Aspartame standard and 6.8 mg L^{-1} Acesulfame-K, respectively, and limit of detection values (established as $3s_{blank}$/calibration slope) of 0.016 and 0.03% w/w, for Aspartame and Acesulfame-K, respectively, were achieved by this procedure.

Off-Line Extraction FTIR Procedure. A whole tablet of sample (60 mg) was accurately weighed (±0.1 mg) and diluted with 4 g of a mixture of $CHCl_3$ and methanol in a ratio of 25:75 (v/v). The mixture was sonicated for 5 min in an ultrasonic water bath. Sample extract was passed through a 0.22 μm nylon filter and introduced into the FTIR measurement cell by using a peristaltic pump. The spectra were obtained per triplicate in the stopped-flow mode at 4 cm^{-1} nominal resolution and accumulating 25 scans per spectrum from 4000 to 850 cm^{-1} using a background of the cell filled with the solvent mixture.

Two individual external calibration sets of Aspartame (six standards from 1.26 to 4.28 mg g^{-1}) and Acesulfame-K (six standards from 1.32 to 3.45 mg g^{-1}) were prepared and their FTIR spectra obtained under the same conditions employed for samples. A calibration line was established for Aspartame by measuring peak height values at 1751 cm^{-1}, corrected using a baseline defined at 1850 cm^{-1}. For Acesulfame-K determination, measurements of the peak height at 1170 cm^{-1} in the first-order derivative spectra, corrected by using a horizontal baseline established at 1850 cm^{-1}, were employed. The concentrations of Aspartame and Acesulfame-K in samples were calculated by interpolation of the absorbance data in the above calibration plots.

On-Line Extraction FTIR Procedure. For on-line extraction of Aspartame and Acesulfame-K three different strategies, which all provide quantitative and comparable results, were used.

Strategy A. A whole tablet of sample (60 mg) was accurately weighed (±0.1 mg) and introduced inside a homemade glass syringe barrel of 6 mL internal volume that includes a porous glass filter. The syringe was placed in the manifold of **Figure 1**. A 25:75 mixture of $CHCl_3$ and methanol was introduced through a 4 mL loop. The solvent was circulated in the closed system for 7 min with the magnetic stirrer on at medium speed level. After that, the spectrum was recorded per triplicate, accumulating 25 scans and employing a nominal resolution of 4 cm^{-1}.

The concentrations of Aspartame and Acesulfame-K in samples were calculated by interpolation of the absorbance data obtained for samples in the spectral conditions indicated before in the calibration plots obtained from standard solutions in $CHCl_3$ and methanol as indicated for off-line extraction.

The manifold includes a three-way valve, as can be seen in **Figure 1**, to clean the closed system before the introduction of other sample.

Strategy B. A whole sample tablet (60 mg) was weighed (±0.1 mg) and introduced inside a glass syringe barrel; after that, the syringe was placed in the manifold of **Figure 1**. A 50:50 mixture of $CHCl_3$ and methanol was introduced through the 4 mL loop. The solvent was circulated in the closed system during 5.5 min (magnetic stirrer on), and after that, the FTIR spectrum was recorded per triplicate using a nominal resolution of 4 cm^{-1} and accumulating 25 scans. Under these conditions, the concentration of Aspartame in samples was calculated by interpolation of the peak height of the absorbance spectra at 1751 cm^{-1} corrected with a horizontal baseline located at 1850 cm^{-1} in a calibration line established from standard solutions in 50:50 $CHCl_3$/methanol as indicated before.

For sample spectra that present two intense bands located at 1600 and 1150 cm^{-1} indicating the presence of Acesulfame-K, the loop was filled again with pure methanol, which was introduced in the glass syringe. The resulting solvent mixture was circulated for 7 min, and the spectrum was acquired in the aforementioned conditions but employing a background registered for a solvent mixture of 25:75 chloroform/methanol. Acesulfame-K was determined by using the first-order derivative spectra and the conditions indicted before.

Strategy C. A third strategy was employed, based on the same principle as that described for strategy B but including a step based on the circulation of an air current through the syringe barrel containing both the sample and the solvent mixture. In this case air bubbles provide

Table 1. Analytical Features of the FTIR Determination of Aspartame and Acesulafame-K Using Different Bands, Baseline Criteria, and Measurement Modes

measurement mode	wavenumber (cm^{-1})	baseline correction	$a \pm s_a$	$b \pm s_b$	R^2	% RSD	LOD[a] ($\mu g\ g^{-1}$)	LOD[b] (% w/w)	Er[c] % w/w
			Aspartame Calibration Curve [$y = a + bC$ (mg g^{-1})]						
height	1751	1850	-0.0005 ± 0.0002	0.01039 ± 0.00002	0.9996	0.17	14	0.10	0.5
area	1756–1746		-0.008 ± 0.001	0.0970 ± 0.0006	0.9996	0.10	5	0.03	6.0
height	1690		-0.0003 ± 0.0005	0.0115 ± 0.0002	0.996	0.16	12	0.08	32
area	1695–1685		-0.005 ± 0.004	0.110 ± 0.002	0.996	0.09	60	0.4	40
height	1606		-0.0010 ± 0.0004	0.0143 ± 0.0002	0.998	0.3	50	0.3	43
area	1611–1601		-0.011 ± 0.004	0.136 ± 0.002	0.998	0.2	30	0.19	48
			Acesulfame-K Calibration Curve [$y = a + bC$ (mg g^{-1})]						
height	1652	1850	0.0004 ± 0.0005	0.0284 ± 0.0004	0.999	0.14	7	0.05	58
area	1657–1647		0.002 ± 0.005	0.246 ± 0.004	0.998	0.17	14	0.10	66
height	1575		0.0000 ± 0.0001	0.01367 ± 0.00008	0.9997	0.7	20	0.13	94
area	1580–1570		-0.002 ± 0.001	0.130 ± 0.001	0.9996	0.6	40	0.3	94
height	1176		0.0009 ± 0.0003	0.0449 ± 0.0002	0.9998	0.4	13	0.09	8.5
area	1181–1171		0.002 ± 0.003	0.401 ± 0.002	0.9997	0.4	18	0.12	11
height	945		0.0009 ± 0.0003	0.0118 ± 0.0002	0.997	3.0	100	0.7	–16
area	950–940		0.005 ± 0.003	0.094 ± 0.002	0.996	1.4	200	1.3	12

[a] Limit of detection values were established for a probability level of 99.6% from the expression $3S_{blank}/b$, S_{blank} being the standard deviation of six measurements of a blank of chloroform/methanol 25:75 solution and b the slope of the calibration line. [b] Limit of detection in tabletop formulations for a sample mass of 60 mg. [c] Accuracy error in percent calculated from three independent determinations of sample 1 that contains 11.88 ± 0.02% (w/w) Aspartame and 11.71 ± 0.02% (w/w) Acesulfame-K.

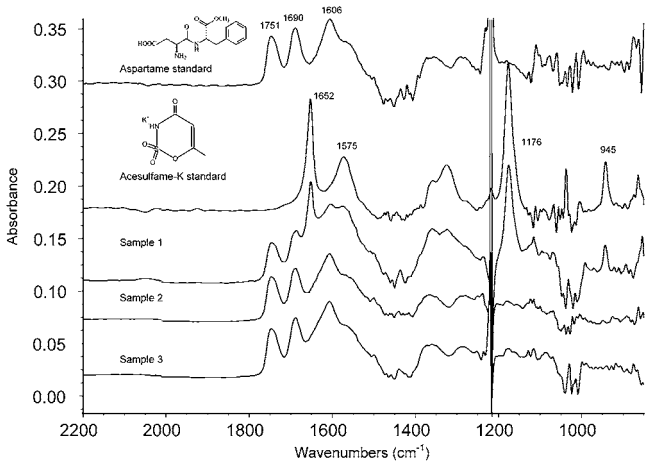

Figure 2. FTIR spectra of Aspartame and Acesulfame-K standard and three tabletop commercial samples extracted with a 25:75 v/v $CHCl_3$/CH_3OH solvent mixture. Spectra are the average of 25 scans using a nominal resolution of 4 cm^{-1}. Concentrations of standards correspond to 3.45 mg g^{-1} Aspartame and 3.13 mg g^{-1} Acesulfame-K. Sample 1 contains 11.88% w/w Aspartame and 11.71% w/w Acesulfame-K, and samples 2 and 3 contain 38.5 and 38.7% w/w of Aspartame, respectively.

a fast and complete extraction of Aspartame and Acesulfame-K from the samples, and FTIR measurements were made as indicated before.

RESULTS AND DISCUSSION

FTIR Transmission Spectrum of Artificial Sweeteners. Figure 2 shows the FTIR spectra of an Aspartame standard solution with a concentration of 3.45 mg g^{-1}, an Acesulfame-K standard solution with a concentration of 3.13 mg g^{-1}, and three commercial tabletop sweeteners, one containing Aspartame and Acesulfame-K and two only Aspartame.

The most intense bands present in the Aspartame spectra are the C═O band in the ester group at 1751 cm^{-1}, that of 1700 cm^{-1} due to the C═O stretch in carboxylic acids, and the band at 1650 cm^{-1} due to the C═O stretch in amides.

The most intense bands of Acesulfame-K are those located at 1680 and 1150 cm^{-1} due to C═O stretch and NH_2 rock in amides. Other less intense absorption bands are located at 1600 and 1356 cm^{-1} due to SO_2 stretch and C═C stretch in cyclohexenes, respectively (*12*).

Selection of FTIR Absorbance Bands for Aspartame and Acesulfame-K Determination. To choose the best analytical conditions for Aspartame and Acesulfame-K determination in tabletop sweeteners by FTIR, different bands and baseline criteria were evaluated. In every case, the use of peak height and peak area absorbance measurement was considered.

Table 1 summarizes the main analytical features of the FTIR determination of Aspartame and Acesulfame-K using a typical stopped-flow approach for sample extraction in $CHCl_3$/methanol. As can be seen, the sensitivity found by peak area measurements was in all the cases 1 order of magnitude better than that obtained by using peak height. However, the limit of detection (LOD) values obtained (see footnote of **Table 1** for details) varied from 0.03 to 0.3% w/w and from 0.05 to 1.6% w/w for Aspartame and Acesulfame-K, respectively, and this latter parameter strongly depends on the stability of absorbance measurements as well as the sensitivity.

As can be seen in **Figure 2**, the Aspartame bands at 1690 and 1606 cm^{-1} highly overlap with the Acesulfame-K bands at 1652 and 1575 cm^{-1}. Therefore, despite the good regression coefficients and LOD values obtained, these bands could not be used for the determination of the two studied compounds in samples containing a mixture of both.

Therefore, the band selected to carry out Aspartame determination was that presented at 1751 cm^{-1}. From a comparison of peak height and peak area measurements it can be concluded that the use of peak area provides in general 1 order of magnitude greater sensitivity than the use of peak height. However, the mean relative errors achieved when peak area measurements were used were higher than those obtained for peak height values, so the latest measurement mode was selected for the Aspartame determination in tabletop sweeteners.

On the other hand, the Acesulfame-K band at 945 cm^{-1} does not overlap with any Aspartame band, but the low signal-to-noise (S/N) ratio in this region when compared with that achieved in the 2000−1100 cm^{-1} range increases the LOD

Table 2. Analytical Features of the FTIR Determination of Acesulafame-K by Using First-Order Derivative Spectra and Evaluating Different Bands and Measurement Modes

			Acesulfame-K calibration curve [$y = a + bC$ (mg g^{-1})]						
measurement mode	wavenumber (cm^{-1})	baseline correction	$a \pm s_a$	$b \pm s_b$	R^2	% RSD	LOD[a] (μg g^{-1})	LOD[b] (% w/w)	Er[c] % w/w
height	1647		0.00006 ± 0.00005	0.00282 ± 0.00002	0.9996	0.17	40	0.3	–20
area	1652–1642		–0.0001 ± 0.0004	0.0185 ± 0.0001	0.9995	0.08	50	0.4	–21
height	1170	1850	0.00008 ± 0.00006	0.00225 ± 0.00002	0.9992	0.5	140	0.9	1.0
area	1175–1165		0.0004 ± 0.0002	0.01839 ± 0.00008	0.9998	0.6	70	0.5	–4.3
height	938		0.00002 ± 0.00008	0.00122 ± 0.00003	0.994	1.4	200	1.3	–12
area	943–933		0.0004 ± 0.0004	0.0071 ± 0.0001	0.996	3.3	300	2.2	–18

[a] Limit of detection established for a probability level of 99.6% from the expression $3S_{blank}/b$, S_{blank} being the standard deviation of six measurements of a blank of chloroform/methanol 25:75 solution and b the slope of the calibration line. [b] Limit of detection in tabletop formulations for a sample mass of 60 mg. [c] Accuracy error in percent calculated from three independent determinations of sample 1 containing 11.71 ± 0.02% (w/w) Acesulfame-K.

values obtained from peak height measurement. Therefore, for Acesulfame-K determination the band located at 1176 cm^{-1} corrected with a single-point baseline located at 1850 cm^{-1} was selected. In these conditions, an accuracy relative error of the order of 8% w/w was obtained for the analysis of a commercially available sample containing Aspartame and Acesulfame-K.

To improve the Acesulfame-K determination in tabletop samples, the first-order derivative spectra were obtained and, as can be seen in **Table 2**, different bands and measurement modes were tested.

As can be seen, the limits of determination achieved are worse than those obtained using zero-order spectra, being between 0.3 and 2.2% w/w. Peak height values at 1170 cm^{-1} corrected with a single-point baseline located at 1850 cm^{-1} were selected because a mean accuracy relative error of 1.0% w/w was achieved on the analysis of actual samples.

Study of Interferences. From the manufacturer's information the main excipient present in the samples studied is lactose. This compound is very slightly soluble in alcohol and insoluble in $CHCl_3$, and it remains undissolved after the complete extraction of Aspartame and Acesulfame-K. Other excipients present, at a low level, are sodium carboxymethylcellulose (insoluble in alcohol), glycine (soluble, 0.06 g/100 mL of alcohol), and L-leucine (soluble, 0.07 g in 100 mL of alcohol). Because of that, spectral interferences expected were simply the mutual ones between both studied sweeteners.

To ensure the accuracy of the Aspartame and Acesulfame-K determination in commercial formulations containing both products, a series of studies was carried out on the influence of the concentration of each of these compounds on the other. For a fixed concentration of 0.88 mg g^{-1} Aspartame the effect of increasing amounts of Acesulfame-K, from 0.97 to 3.05 mg g^{-1}, was studied, and for a fixed concentration of 0.86 mg g^{-1} Acesulfame-K the effect of Aspartame, from 1.01 to 2.31 mg g^{-1}, was also evaluated.

The presence of Acesulfame-K does not interfere with the Aspartame determination when peak height values at 1751 cm^{-1}, corrected with a baseline defined at 1850 cm^{-1}, were used.

However, Aspartame clearly interferes with Acesulfame-K determination when peak height values at 1176 cm^{-1} (corrected with a baseline defined at 1850 cm^{-1}) in the zero-order spectra were used. Therefore, to avoid this interference, first-order derivative spectra were employed for Acesulfame-K determination. When peak height values at 1170 cm^{-1} with a baseline defined at 1850 cm^{-1} were used, the interference of Aspartame on Acesulfame-K determination was avoided.

Off-Line Extraction of Sweeteners. To reduce the time needed to carry out the extraction of Aspartame and Acesulfame-K from solid samples, different sonication times from 2 to 12 min were applied to extract both compounds with a $CHCl_3$/methanol (25:75 v/v) mixture; it was found that 5 min of sonication is enough for the quantitative recovery of the two compounds under study.

Effect of Experimental Variables on the On-Line Extraction of Aspartame and Acesulfame-K. To develop a fully mechanized procedure for FTIR determination of Aspartame and Acesulfame-K in tabletop sweeteners, the on-line approach described in the experiment set was assayed. Different parameters, such as carrier flow rate, direction of flow, or geometry of the extraction cell, were evaluated to improve the extraction by reducing the time required.

Carrier flow rate was modified between 0.5 and 2.75 mL min^{-1}. As can be seen in **Figure 3**, this parameter is not decisive because the time needed for the dissolution of the sweeteners in the sample cartridge is higher than the recirculation solvent time.

Another parameter evaluated was the solvent recirculation direction. Two different configurations based on the solvent recirculation in a closed system and the air bubbling through the mixture of the sample and solvent, remaining all the time inside the syringe barrel, were assayed. However, as can be seen in **Figure 4** both air and solvent circulation provided a complete Aspartame extraction, but the use of air bubbles seems to be more effective, requiring 6 min instead 7 min for a complete extraction.

Different sample extraction cell geometries were tested to minimize the extraction time: a 35 mL volume, 7.78 cm length, and 2.67 cm i.d. plastic syringe barrel; two glass cells of 6 mL volume, 5.48 cm length, and 1.14 cm i.d.; and a 9 mL volume with 3.70 cm length and 1.69 cm i.d. As can be seen in **Figure 5**, a syringe barrel of 6 mL internal volume was selected because it facilitates the homogenization and avoids the deposition of solid particles on the sample cell internal surface.

ANALYTICAL FIGURES OF MERIT

To verify the absence of matrix effect on the FTIR determination of Aspartame and Acesulfame-K, external calibration and standard addition lines were compared, employing in both cases the previously selected measurement conditions.

The typical calibration line obtained by the standard addition for Aspartame was $A = (0.01820 \pm 0.00008) + (0.01040 \pm 0.00006)C$ (mg g^{-1} Aspartame added), with $R^2 = 0.9997$, which is comparable to the external calibration line $A = (-0.0005 \pm$

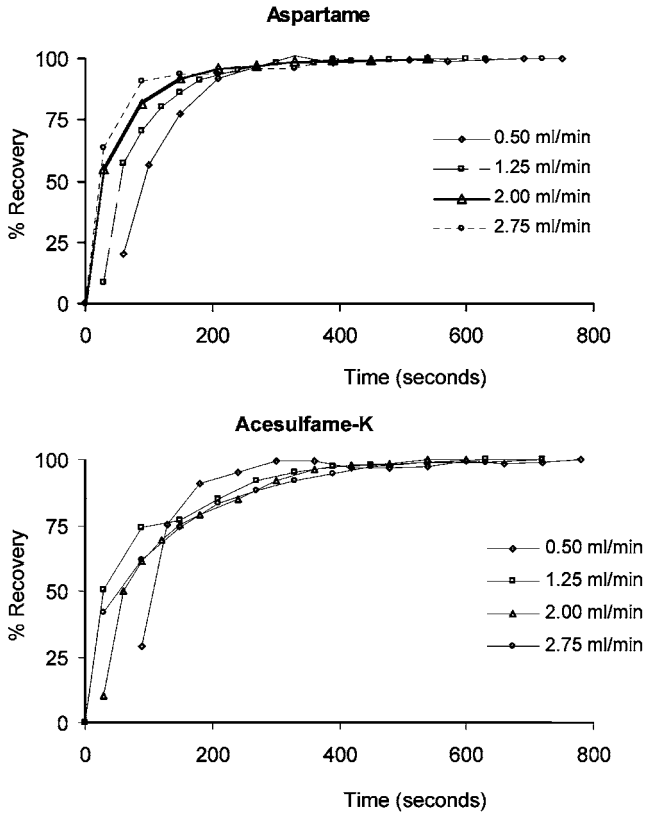

Figure 3. Effect of carrier flow rate and extraction time on Aspartame and Acesulfame-K recovery. For Aspartame peak height values at 1751 cm^{-1} corrected using a baseline defined at 1850 cm^{-1} were used, and for the Acesulfame-K peak height values at 1170 cm^{-1} with a baseline defined at 1850 cm^{-1} in the first-order derivative spectra were employed. The percentage recovery was calculated using the concentration of both sweeteners in sample 1 found by the HPLC reference procedure.

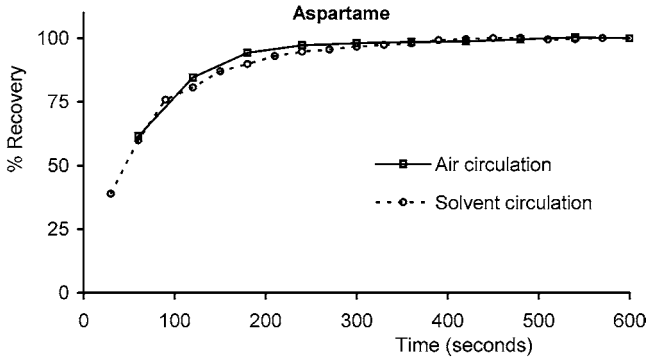

Figure 4. Effect of solvent circulation on Aspartame extraction by using air (□) and solvent (○) circulation with a flow of 2.00 mL min^{-1}. Measurement conditions were as indicated in **Figure 3**.

0.0002) + (0.0104 ± 0.0001)C (mg g^{-1} Aspartame), with R^2 = 0.9990.

On the other hand, in the case of Acesulfame-K, the calibration line obtained from first-derivative spectra by standard addition was A = (0.00188 ± 0.00006) + (0.00224 ± 0.00005)C (mg g^{-1} Acesulfame-K added), with a regression coefficient of R^2 = 0.998, which is comparable to the external calibration line A = (0.00008 ± 0.00004) + (0.00225 ± 0.00002)C (mg g^{-1} Acesulfame-K), with R^2 = 0.9995.

Therefore, on the basis of these results it can be concluded that the selected conditions for Aspartame and Acesulfame-K determination are free from matrix interferences, and thus external calibration can be employed.

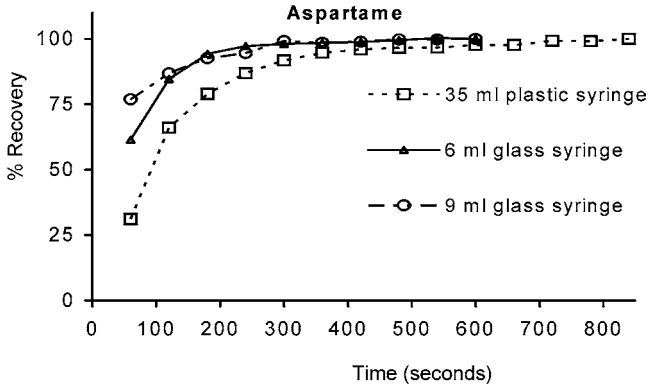

Figure 5. Effect of sample syringe geometries on Aspartame extraction time. The conditions employed are the same as those indicated in **Figure 3**. The percentage recovery was calculated using the concentration of Aspartame in sample 1 found by the HPLC reference procedure.

Table 3. Determination of Aspartame and Acesulfame-K Content (Percent w/w) in Commercially Available Tabletop Formulations

sample	active substance	HPLC[a]	FTIR off-line[a]	t_{exp}[b]	FTIR on-line[a]	t_{exp}
1	Aspartame	11.88 ± 0.02	11.83 ± 0.08	1.36	11.9 ± 0.2	0.22
	Acesulfame-K	11.71 ± 0.02	11.74 ± 0.14	0.47	11.8 ± 0.3	0.52
2	Aspartame	38.5 ± 0.2	38.4 ± 0.2	0.79	38.6 ± 0.3	0.62
3	Aspartame	38.7 ± 0.2	38.70 ± 0.10	0.00	38.9 ± 0.4	1.00

[a] Average of three independent analyses ± standard deviation. [b] t_{tab} = 2.132, for a probability level of 95% and 4 freedom degrees.

Table 4. Comparison of the Analysis Time and Organic Solvent Consumption for the Measurement Step among the Developed Procedures

	Aspartame	Acesulfame-K	total	
	analysis time (min)	analysis time (min)	time (min)	volume (mL)
HPLC	30	7	35	6.5
FTIR off-line	7	7	7	4
FTIR on-line				
strategy A	6.5	7	7	4
strategy B	5.5	7	12.5	4
strategy C	5	6	11	4

COMPARISON AMONG THE DIFFERENT METHODOLOGIES DEVELOPED

Off-line and on-line FTIR procedures developed in this work provide statistically comparable results (for a probability level of 95%) with those obtained by the HPLC reference method, as can be seen in **Table 3**.

The precision obtained by off-line FTIR (in terms of the relative standard deviation of results) is of the same order of magnitude as that found by the reference method, the results obtained by on-line FTIR being less precise than those determined by HPLC.

The limits of detection achieved by FTIR procedures were higher than those found by HPLC, being for Aspartame and Acesulfame-K 0.09 and 0.9% w/w and 0.016 and 0.03% w/w, respectively. However, due to the high concentration of these two compounds present in commercial formulations, FTIR procedures are sufficiently sensitive for their analysis.

FTIR strategies involve a strong reduction of the time of analysis as compared with the chromatographic procedures, as can be seen in **Table 4**. The time needed for the determination

of Aspartame and Acesulfame-K in tabletop samples was reduced from 35 to 7 min by using FTIR.

On the other hand, in this paper, a fully mechanized extraction procedure has been developed, in which the contact of the operator with the solvents is avoided. This strategy provides a clear differentiation between samples that contain only Aspartame and those that include Acesulfame-K. In the first case, Aspartame can be extracted and filtered on-line in only 5 min, and it could be determined by FTIR measurements. In tabletop samples containing Aspartame and Acesulfame-K, a second extraction step is necessary, increasing the time of analysis to 11 min.

FTIR procedures developed in this work are an alternative to the chromatographic procedures in the quality control of sweeteners in tabletop formulations.

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