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Analysis of semicarbazide in baby food by liquid chromatography tandem mass spectrometry (LC–MS–MS)—In-house method validation

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Abstract

A method for detection of semicarbazide (SEM) in baby food was validated. SEM was extracted with hydrochloric acid and derivatised with 2-nitrobenzaldehyde, using [$^{15}N_2$,^{13}C] semicarbazide as internal standard. The extract was neutralised, purified on a solid phase extraction cartridge and SEM was determined by reversed phase LC–MS–MS. Linearity was demonstrated in the ranges from 0.1 ng ml^{-1} to 1 ng ml^{-1} and from 2 ng ml^{-1} to 80 ng ml^{-1}. Matrix effects were non significant for meat-based and significant for apple and rice-based baby foods, in both ranges. Mean recoveries ranged from 87.8% to 107.2% with relative standard deviation from 0.2% to 9.1%, considering both ranges. Limits of detection and quantification were 0.1 μg kg^{-1} and 0.25 μg kg^{-1}, respectively. The results of the validation process demonstrated the method suitability for use in food control.

Keywords: Semicarbazide; Baby food; Liquid chromatography tandem mass spectrometry; In-house validation

1. Introduction

Semicarbazide (SEM), a metabolite of nitrofurazone, has been used as a marker residue for the illegal use of this drug in animal food production [1,2]. However, it has been shown that SEM in food may originate from other sources including environmental and those associated with food processing and packaging materials [3,4]. The origin of SEM in some foods was suspected to be structurally-related to azodicarbonamide (AZDC), a chemical blasting agent in the production of plastic seals for lids on glass jars [5], also used in some countries as a flour improving agent [6].

There are few data on concentrations of SEM in food packed in glass jars and bottles. The levels of SEM in other foods have been found to be variable in the range non-detectable (less than 1 μg kg^{-1}) up to 25 μg kg^{-1}. Baby foods have been reported with higher concentrations, perhaps because of the higher ratio of gasket area to food mass for these small pack sizes [7,8].

SEM belongs to a family of hydrazines which are known to cause cancer in laboratory animals. However, SEM has not been extensively tested for toxic effects. It has weak genotoxic activity in vitro and weak carcinogenic activity in female but not male mice. Due to the limited data available, it is not possible to conclude whether SEM may pose risks to humans [8].

The methods of analysis used to detect SEM in food involve acid hydrolysis and a derivatisation step. These steps are to extract and measure SEM bound to protein in meat as a marker for nitrofurazone [1,2,9]. Considering that SEM is known to react with chemicals such as carbonyl compounds and that these functional groups are present in food, some or all SEM in food may be bound and not free. It would

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be necessary to base any analytical method on the hydrolysis and derivatisation procedure to determinate both free and reversible-bound residues of SEM in food. As there is a wide variety of baby food available, a method capable of determining SEM in a wide range of matrices is required.

Irrespective of the method employed, the reliability of results concerning exposure of SEM, including occurrence data should be guaranteed using validated procedures [10,11]. Method validation providing the performance figures that indicate fitness-for-purpose have come to dominate the practical use of validation. However, considering that validation studies are based on statistical hypothesis testing, a method validation procedure also needs to provide a basic check that the assumptions made with regard to the principles of the tests are not seriously flawed [12].

Considering that validation by interlaboratory assays mainly evaluates trueness, repeatability and reproducibility [13], in-house validation covering performance parameters such as linearity, matrix effects, selectivity and limits is fundamental to define the analytical procedures and to check suitability of the method for further collaborative trial. In-house validation is appropriate to ensure the viability of the method before the costly exercise of a formal collaborative trial and to provide evidence of the reliability of analytical methods if collaborative trial data are not available [12].

The work reported here represents the application of a purposed approach to in-house validation in a quantitative method for analysis of SEM in baby food by liquid chromatography tandem mass spectrometry (LC–MS–MS).

2. Experimental

2.1. Reagents, solvents and materials

Ethyl acetate (LC grade), methanol (LC grade), water (LC fluorescence grade) and hydrochloric acid concentrate were obtained from Fisher Scientific (Loughborough, Leicestershire, UK). 2-Nitrobenzaldehyde (2-NBA) and ammonium formate were supplied by Sigma–Aldrich Co. Ltd. (Gillingham, Dorset, UK). Di-potassium hydrogen orthophosphate anhydrous and sodium hydroxide were obtained from BDH (Poole, Dorset, UK). Solid phase extraction (SPE) cartridges (200 mg, 3 ml) strata styrene divinylbenzene polymer SDB-L (100 μm, 260A) were Phenomenex (Macclesfield, Cheshire, UK).

2.2. Standards

Semicarbazide hydrochloride and internal standard [$^{15}N_2$,^{13}C] semicarbazide ([$^{15}N_2$,^{13}C] SEM) were supplied by Sigma–Aldrich Co. Ltd. (Gillingham, Dorset, UK) and Witega (Berlin, Germany), respectively. Stock solutions (1 mg ml^{-1}) of SEM and [$^{15}N_2$,^{13}C] SEM free were prepared in methanol. Intermediate solutions (10 μg ml^{-1}) of SEM and [$^{15}N_2$,^{13}C] SEM were prepared by dilution of the respective stock solutions with methanol. Spike solutions of SEM (20 ng ml^{-1} and 200 ng ml^{-1}) and [$^{15}N_2$,^{13}C] SEM (100 ng ml^{-1}) were prepared by dilutions of the respective intermediate solutions in methanol. The stock, intermediate and spike solutions were stored at 2 °C to 8 °C and prepared at each year, monthly and weekly, respectively.

2.3. Instrumentation

LC–MS–MS analyses were performed using a LC Waters 2695 Separations Module (Milford, Massachusetts, USA) coupled via an electrospray interface to a mass spectrometer Quattro Ultima Pt Micromass (Wythenshawe, UK).

2.4. Samples

Sample blanks of apple based puree (from plastic containers), meat based meal and rice pudding (from metal cans) were obtained from supermarkets. These three types of baby food were studied representing groups of acidic, fatty/protein and carbohydrate-rich products, respectively. These samples were identified and stored frozen at −18 °C, until the moment of the analyses.

2.5. Analytical procedure

The validated method was based on previously described procedures [1,9,14,15]. Aliquots of homogenised baby food (2.00 g ± 0.03 g) at room temperature were weighed into 50 ml falcon tubes. Solvent blanks, omitting any sample, were prepared for the calibration curves preparation. Extraction solution (10 ml ± 5% of the 0.2 mol l^{-1} hydrochloric acid), internal standard (100 μl ± 2% of the 100 ng ml^{-1} [$^{15}N_2$,^{13}C] SEM spike solution) and derivatisation solution (240 μl ± 5% of the 2-NBA 10 mg ml^{-1} in methanol) were added to all tubes. The tubes were sealed securely and placed in a shaking water bath at 40 °C ± 3 °C during 15 h. The samples were allowed to cool to room temperature and pH was adjusted with 10 ml ± 5% of 0.2 mol l^{-1} di-potassium hydrogen orthophosphate and 800 μl ± 5% of 2 mol l^{-1} sodium hydroxide. The tubes were sealed securely, vortex for 30 s and centrifuged at 3900 × *g* for 15 min.

SPE cartridges were at room temperature prior to conditioning. For conditioning 3 ml ± 5% of ethyl acetate were applied to the top of the SPE tubes and passed through under gravity. This washing with ethyl acetate was a precautionary step to ensure there are no co-extractives that might be eluted later with the sample and interfere with the subsequent analysis. This procedure was repeated applying 3 ml ± 5% of methanol and 5 ml ± 5% of water, sequentially. A small portion (0.5 ml) of water was remained on the cartridges until the application of sample extracts.

The sample and solvent blank extracts were transferred with a pipette to the SPE tubes, dripping through under gravity. The cartridges were washed with 5 ml ± 5% of water, dripping through under gravity. SPE cartridges were dried un-

Table 1
MS–MS conditions for multiple reaction monitoring

Component	Molecular ion $[M+H]^+$ (*m*/*z*)	Product ion (*m*/*z*)	Collision energy (eV)	Dwell time (s)
SEM	209 ± 0.5	134 ± 0.5	10	0.10
SEM	209 ± 0.5	166 ± 0.5	10	0.10
SEM	209 ± 0.5	192 ± 0.5	10	0.10
[$^{15}N_2$,^{13}C] SEM	212 ± 0.5	168 ± 0.5	10	0.10

der vacuum for at least 2 min. The 2-NBA derivative of SEM was eluted with 3 ml ± 5% of ethyl acetate to 4 ml vials, using mild vacuum to obtain a flow rate 1 ml min^{-1}. The solvent was evaporated to dryness at 40 °C ± 3 °C under nitrogen and re-dissolved in 1000 μl ± 2% of water:methanol (60/40, v/v). The purified extracts were filtered through a 0.45 μm syringe filter.

LC analyses were performed on a C-18 reversed-phase column (50 mm × 2.1 mm i.d., 3 μm particle size, from Thermo Hypersil-Keystone) at 30 °C ± 5 °C. The autosampler was maintained at 15 °C ± 5 °C and the injection volume was 10 μl. The mobile phase was composed of solvent A (ammonium formate 5 mmol l^{-1}) and solvent B (methanol). The gradient program started at 20% solvent B and increasing to 95% solvent B (over 7 min). This proportion was maintained for 1 min and then returned to the initial condition. The total run time was 16 min and the flow rate was set at 0.2 ml min^{-1}.

The MS source was maintained at 120 °C and the electrospray capillary voltage to 3 kV. Desolvation temperature was 450 °C. Nitrogen was used as the drying and nebulising gas at a flow rate of 700 l h^{-1} and 100 l h^{-1}, respectively. The collision cell entrance and exit energies were set at 0 eV. The collision gas, argon, was blend into the cell at a pressure of 10^{-4} mbar. Spectra for SEM and [$^{15}N_2$,^{13}C] SEM were over the range *m*/*z* 50 to 300 in the MS mode only. Selected ions were monitored by multiple reaction monitoring (Table 1). Concentrations were calculated by comparing the ratio of *m*/*z* 209 → 166 response SEM with the ratio of *m*/*z* 212 → 168 response [$^{15}N_2$,^{13}C] SEM.

2.6. Validation procedure

The performance characteristics of the method were established by in-house validation procedures employing assays with standard solutions, sample blanks and spiked samples. Linearity, matrix effects, selectivity, trueness, precision, detection and quantification limits were studied. The fitness-for-purpose of this method was assessed based on the results of the established performance characteristics [12]. The validation was carried out in two sets of analysis to cover two concentration ranges.

2.6.1. Linearity and matrix effects

Linearity and matrix effects were assessed by solvent and matrix-matched calibration curves. Apple-based puree, meat-based meal and rice pudding (three different varieties), were used as representative matrices. Six calibration curves (solvent, apple, meat, rice variety 1, rice variety 2 and rice variety 3) were prepared at the levels of 2, 10, 20, 40 and 80 ng ml^{-1} (corresponding to 1, 5, 10, 20 and 40 μg kg^{-1} of SEM in baby food) and run in a random order, on the same day. Blanks were also prepared for each curve as a quality control tool, but not included in regression analysis. These curves were prepared in three different days to obtain independent replicates. Before the extraction step, the solvent or sample blanks were spiked with 100 μl of 20 ng ml^{-1} SEM spike solution and 50 μl, 100 μl, 200 μl and 400 μl of 200 ng ml^{-1} SEM spike solution, respectively.

Calibration curves from 0.1, 0.2, 0.3, 0.4 and 1 ng ml^{-1} were prepared, corresponding to 0.05 μg kg^{-1} to 0.5 μg kg^{-1} of SEM in baby food.

After an exploratory fit with simple linear regression, the residuals were examined for obvious patterns. Jacknife standardised residuals test was applied sequentially until no further outliers were detected [16] or until a drop of 22.2% in the original number of results [13]. Violations of assumptions underlying regression analysis were evaluated: residual normality [17], independence [18] and homoscedasticity [19,20]. *F*-tests were undertaken to check the regression and lack-of-fit significance [21]. The slopes and interceptions obtained for the solvent and each matrix-matched calibration function were compared for significant differences by t test [16]. The hypothesis tests were performed at the $\alpha = 0.05$ level.

2.6.2. Selectivity, trueness and precision

Selectivity, trueness, precision and experimental limits of detection and quantification of the method were defined by assays with apple based puree, meat based meal and rice pudding blanks and spiked at 0.10, 0.15, 0.20, 0.25, 0.50, 1, 10 and 30 μg kg^{-1}, in three independent replicates, before the extraction step. Considering the results of the matrix effects tests, respective matrix matched curves were prepared to calculate the SEM concentration in the spiked samples. The concentrations of spiked samples at 0.1 μg kg^{-1} to 0.5 μg kg^{-1} were calculated using the respective matrix matched calibration curves in the range of 0.1 ng ml^{-1} to 1 ng ml^{-1}, while the concentrations of spiked samples at 1 μg kg^{-1} to 30 μg kg^{-1} were calculated using the respective matrix matched curves in the range of 2 ng ml^{-1} to 80 ng ml^{-1}.

The absence of false positive results for all sample blanks was considered acceptable for selectivity. Trueness was investigated through mean recovery obtained for the three replicates of spiked samples at each level. The minimum trueness criteria ranged from −50% to +20%, −30% to +10% and −20% to +10% for ≤1 μg kg^{-1}, >1 μg kg^{-1} to 10 μg kg^{-1} and ≥10 μg kg^{-1} mass fractions, respectively [22]. Based on

these criteria, the acceptable mean recovery range for spiked samples at 0.10, 0.15, 0.20, 0.25, 0.50 and 1 μg kg^{-1} was 50% to 120% and for spiked samples at 10 μg kg^{-1} and 30 μg kg^{-1} was 80–110%. Intralaboratory precision under repeatability conditions was expressed in terms of relative standard deviation obtained for the replicates of spiked samples at each level. This parameter was considered acceptable when falling within two thirds [22] of the range calculated by the Horwitz function modified by Thompson [23]. Considering that the interlaboratoryal precision estimated by Thompson function [23] was 22%, intralaboratory relative standard deviations ≤14.7% were acceptable.

2.6.3. Limits of detection and quantification

The limit of quantification was stated as a concentration below which the method could not operate with an acceptable precision and trueness. The limit of detection was the lowest concentration of SEM that was detectable in all replicates but not necessarily quantified, distinguished from zero (signal/noise ≥3). These limits were established based on the mean recovery and relative standard deviation results obtained for the replicates of spiked samples.

3. Results and discussion

3.1. Linearity and matrix effects

The residual plots and outliers removed for the solvent and matrix-matched curves at the upper range are shown in Fig. 1. The assumption that the residuals are normally distributed was confirmed. Ryan–Joiner correlation coefficients were 0.9575, 0.9667, 0.9754, 0.9855, 0,9793 and 0.9486 for solvent, apple, meat, rice variety 1, rice variety 2 and rice variety 3 curves, respectively, indicating no significant ($p > 0.10$) deviation of normality. No autocorrelation was observed and Durbin–Watson statistics were 2.04, 2.24, 1.69, 1.59, 2.05 and 2.17 for solvent, apple, meat, rice variety1, rice variety 2 and rice variety 3 curves, respectively, showing that residuals were statistically independent ($p > 0.05$). Homoscedasticity was confirmed. The residual variability across all concentration levels was constant, since Levene *t*-statistics were not significant ($p > 0.05$) (Table 2). The high significance ($p < 0.001$) of the regression can be seen in Table 3, while the lack-of-fit was not significant ($p > 0.05$) for the solvent and matrix matched curves, indicating linearity in the range from 2 ng ml^{-1} to 80 ng ml^{-1} (corresponding to 1 μg kg^{-1} to 40 μg kg^{-1} of SEM in baby food). The solvent, apple, meat, rice variety 1, rice variety 2 and rice variety 3 curves are demonstrated in Fig. 2.

All the regression assumptions were tested and confirmed for the 0.1 ng ml^{-1} to 1 ng ml^{-1} curves indicating linearity in this range. No outliers were detected in solvent, apple and meat curves by Jacknife standardised residuals test. One outlier was detected in rice curve at 0.4 ng ml^{-1} level. Ryan-Joiner correlation coefficients were 0.9849, 0.9747, 0.9645 and 0.9763 for solvent, apple, meat and rice curves, respectively, demonstrating normal distribution of the residuals ($p > 0.10$). Durbin Watson statistics obtained for these curves were 2.58, 2.89, 2.52 and 1.97 respectively, showing the residuals independence ($p > 0.01$). Levene $t_{18\ DF}$ statistics were 1.61×10^{-1}, 3.11×10^{-1}, -1.17 and -9.98×10^{-1}, respectively, confirming homoscedaticity ($p > 0.05$). No lack of fit ($p > 0.05$) and significant regression ($p < 0.001$) were obtained for these curves.

The intercepts were not significantly different from zero ($p > 0.05$) and no significant differences were observed between the intercepts of the solvent and matrix matched curves ($p > 0.05$), in both ranges. No matrix effects were detected for meat. However, when the slopes from the solvent standard curves were compared with that from apple and rice matrix-

Table 2
Residual homoscedasticity evaluation by modified Levene test for solvent and matrix-matched calibration curves

Statistic	Solvent	Apple	Meat	Rice 1	Rice 2	Rice 3
n	13	13	12	14	13	12
s_p^2	5.38×10^{-4}	6.02×10^{-4}	3.65×10^{-4}	1.09×10^{-3}	1.57×10^{-3}	1.23×10^{-3}
t^*	7.72×10^{-1}	-1.71×10^{-3}	-1.12	-1.80	-1.53	7.34×10^{-1}
p	4.56×10^{-1}	9.99×10^{-1}	2.89×10^{-1}	9.67×10^{-2}	1.53×10^{-1}	4.80×10^{-1}

n: number of observations, s_p^2: pool variance, t^*: Levene *t*-statistic, *p*: significance.

Table 3
ANOVA statistics for regression including lack of fit test of solvent and matrix-matched calibration curves

Statistic	Solvent	Apple	Meat	Rice 1	Rice 2	Rice 3
Regression						
F	2.69×10^4	1.89×10^4	3.26×10^4	2.67×10^4	1.04×10^4	1.60×10^4
p	5.49×10^{-20}	3.79×10^{-19}	6.64×10^{-19}	1.86×10^{-21}	1.00×10^{-17}	2.34×10^{-17}
Lack-of-fit						
F	2.71	1.08	2.83	3.81	3.27	0.269
p	1.15×10^{-1}	4.12×10^{-1}	1.16×10^{-1}	5.16×10^{-2}	8.02×10^{-2}	8.46×10^{-1}

F: variance ratio, *p*: significance.

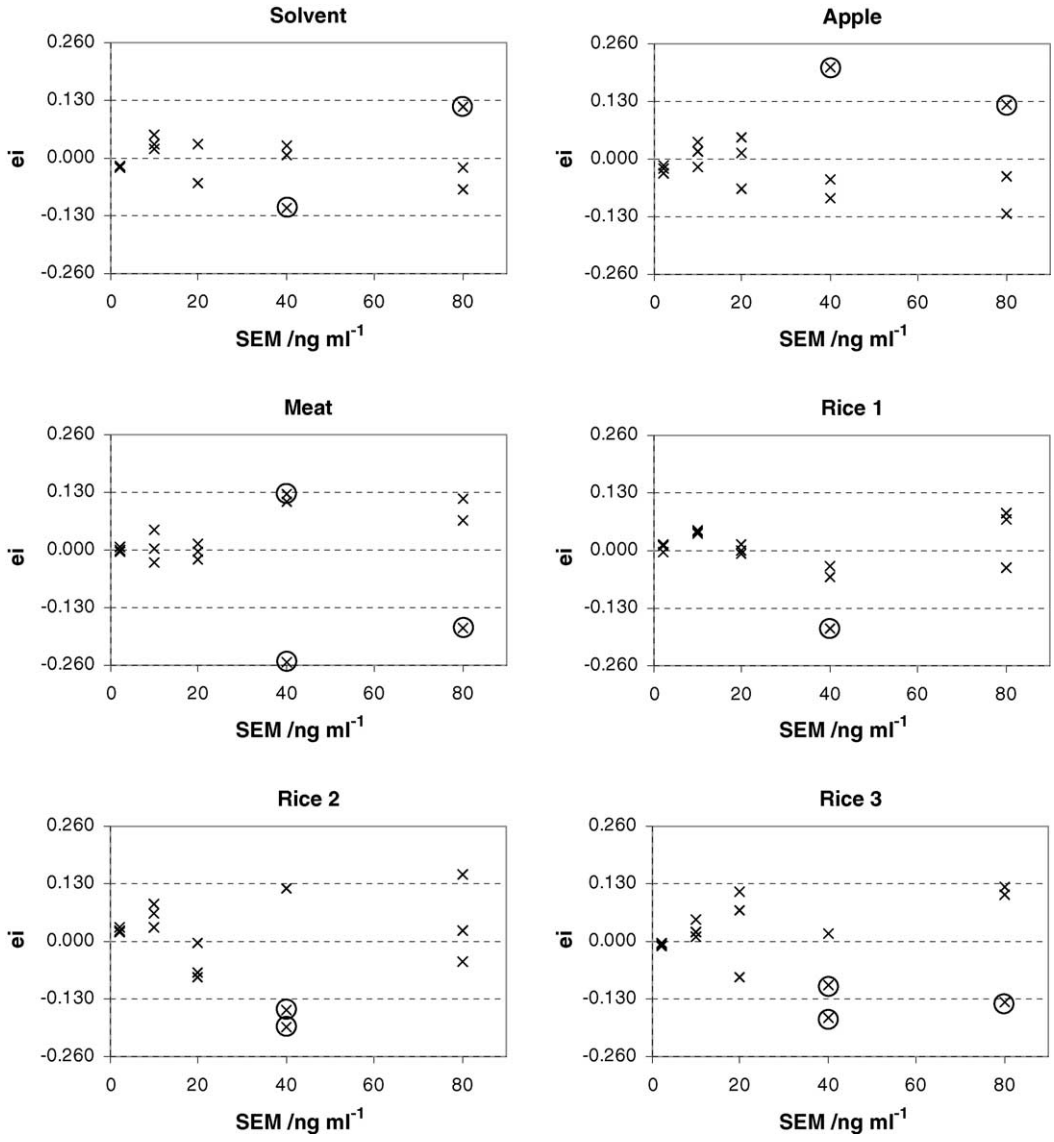

Fig. 1. Residual plots for outlier diagnostics by Jacknife standardised residuals test in solvent and matrix-matched calibration curves. e_i = residual; × = non-outlier; ⊗ = outlier.

matched curves significant differences ($p < 0.05$) were observed for the two studied ranges. Table 4 shows the matrix effects results for the curves from 2 ng ml^{-1} to 80 ng ml^{-1} range. Based on these results it was possible to conclude that SEM solvent curve analysed as described in this method gave the same signal as meat-based baby food samples containing the same concentration of SEM. In the other hand, matrix matched curves need to be used to calculate SEM concentrations for apple and rice-based baby foods. Considering the wide variety of baby food available, it is important to evaluate each specific case that will require new tests of matrix effects.

Table 4
Slope and intercept comparisons of apple, meat, rice 1, 2 and 3 curves with solvent standard curve

Statistic	Apple	Meat	Rice 1	Rice 2	Rice 3
Slope comparisons with solvent curve					
t	4.95	1.11	2.87	5.41	5.89
p	5.94×10^{-5}	2.80×10^{-1}	8.65×10^{-3}	1.94×10^{-5}	7.61×10^{-6}
Intercept comparisons with solvent curve					
t	6.77×10^{-2}	2.05	1.54	1.40	1.13
p	9.47×10^{-1}	5.35×10^{-2}	1.37×10^{-1}	1.75×10^{-1}	2.72×10^{-1}

t: *t*-statistic for the contrasts of the matrix-matched curves with the solvent curve, *p*: significance.

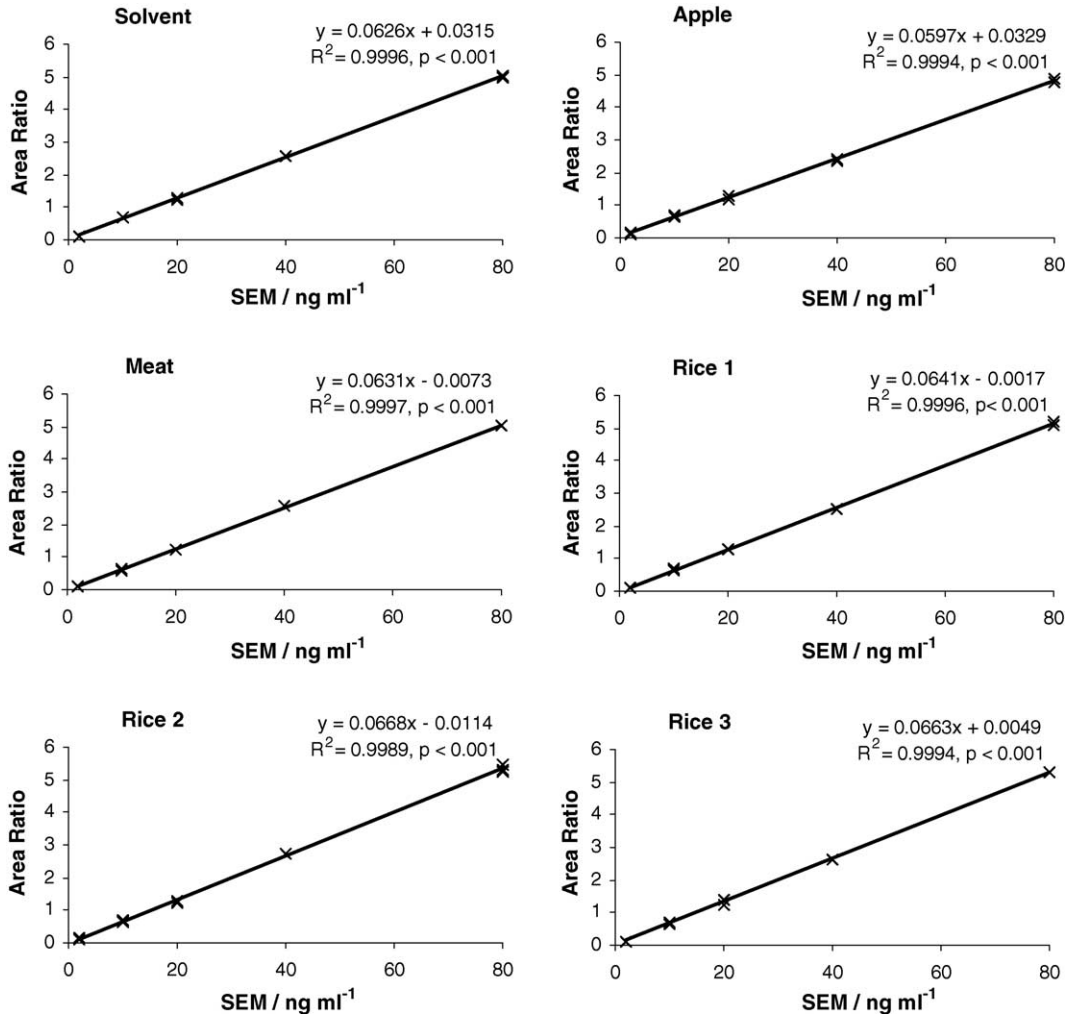

Fig. 2. Solvent and matrix-matched calibration curves with respective regression equations, determination coefficients (R^2) and significances of regression (p). Area ratio = area SEM/area [$^{15}N_2$,^{13}C] SEM.

3.2. Selectivity, trueness and precision

Some typical multiple reaction monitoring ion chromatograms are shown in Fig. 3 illustrating the m/z 209 → 192 transition for a solvent standard (used for confirmation) together with the m/z 209 → 166 transition used for quantification for a typical baby food. The mean retention time for SEM was 8.08 min ± 0.03 min. In all cases for the foods examined the ion chromatograms were free from interferences and peak shapes were sharp and essentially indistinguishable in profile from standards of comparable concentrations.

All the sample blanks analysed had non-detected results for SEM (signal/noise <3). The mean recovery values ranged from 93.8% to 107.2%, 96.6% to 104.1% and 87.8% to 112.9% for apple, meat and rice spiked samples, respectively. Relative standard deviations obtained under repeatability conditions were between 0.7% and 24.6% for apple, 0.2% and 9.3% for meat and 0.7% and 24.7% for rice (Table 5). Acceptable mean recoveries were obtained in the range from 0.1 μg kg^{-1} to 30 μg kg^{-1} for apple, meat and rice spiked samples. Relative standard deviations were above 14.7% for apple spiked samples at 0.1 μg kg^{-1} and 0.15 μg kg^{-1} and for rice spiked samples at 0.15 μg kg^{-1} and 0.2 μg kg^{-1}. Precision and no lack of trueness were observed between 0.25 μg kg^{-1} and 30 μg kg^{-1} with mean recoveries varying from 87.8% to 107.2% and relative standard deviation from 0.2% to 9.1%.

3.3. Limits of detection and quantification

The whole spiked samples at 0.1 μg kg^{-1} was detected for SEM and this concentration level was established as the method limit of detection, adopting the criterion for detection signal/noise ≥3. The limit of quantification of this method was 0.25 μg kg^{-1}, the lowest concentration level that trueness and precision results were appropriate (mean recovery between 50% and 120% and relative standard deviation ≤14.7%, respectively). The pro-

Table 5
Mean recovery and relative standard deviation for apple, meat and rice spiked samples at different levels

Level ($\mu g\,kg^{-1}$)	Apple		Meat		Rice	
	Rm (%)	RSD (%)	Rm (%)	RSD (%)	Rm (%)	RSD (%)
0.10	104.6	24.6	97.4	9.3	97.0	9.1
0.15	93.8	19.2	103.5	9.2	104.0	16.2
0.20	102.3	3.5	98.7	8.8	112.9	24.7
0.25	94.1	2.3	104.0	3.0	96.0	5.9
0.50	99.7	7.7	96.6	7.6	106.5	0.7
1	102.8	0.7	103.7	0.2	98.9	8.1
10	105.1	2.7	104.1	6.0	102.2	7.1
30	107.2	3.5	101.0	9.1	87.8	5.1

Rm: mean recovery, RSD: relative standard deviation. Rm criteria: −50% to +20% (for spiked samples at $\leq 1\,\mu g\,kg^{-1}$); −30% to +10% (for spiked samples at $>1\,\mu g\,kg^{-1}$ to $10\,\mu g\,kg^{-1}$); −20% to +10% (for spiked samples at $\geq 10\,\mu g\,kg^{-1}$). RSD criterion: ≤14.7%.

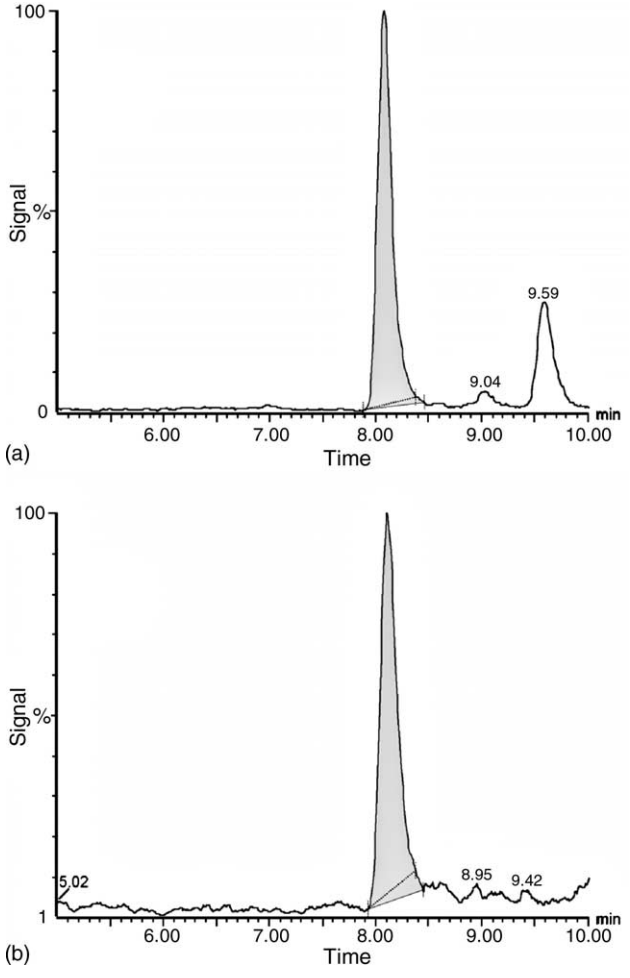

Fig. 3. MRM chromatogram obtained after the LC–MS–MS traces for (a) *m/z* 209 → 192 transition for 2 ng ml^{-1} solvent and (b) *m/z* 209 → 166 transition for 1 $\mu g\,kg^{-1}$ apple baby food.

file of distribution of the individual recovery values for spiked samples between 0.1 $\mu g\,kg^{-1}$ and 0.5 $\mu g\,kg^{-1}$ (Fig. 4) shows the transition from the detection to the quantification range.

The performance characteristics of the method presented in this paper indicate its fitness for use in food control.

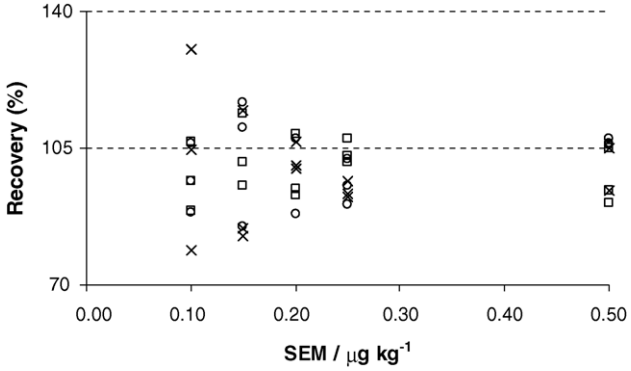

Fig. 4. Distribution of the individual recovery values for spiked samples between 0.1 $\mu g\,kg^{-1}$ and 0.5 $\mu g\,kg^{-1}$, including detection (0.1 $\mu g\,kg^{-1}$) and quantification (0.25 $\mu g\,kg^{-1}$) limits. ○ = rice; □ = meat; × = apple.

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References

[1] A. Leitner, P. Zöllner, W. Lindner, J. Chromatogr. A 939 (2001) 49.
[2] A. Connely, A. Nugent, M. O'Keefe, P.P.J. Mulder, J.A. Van Rhijn, L. Kovacsics, A. Fofor, R.J. Mc Cracken, D.G. Kennedy, Anal. Chim. Acta 483 (2003) 91.
[3] J.A. Van Rhijn, D.G. Kennedy, P.P.J Mulder, I.J.W. Elbers, T. Zuidema, P.B. Young, in: Proceedings of Euro Food Chem XII, Brugges, 2003.
[4] A.S. Pereira, L.J. Donato, G. De Nucci, Food Addit. Contam. 21 (2004) 63.
[5] R.H. Stadler, P. Mottier, P. Guy, E. Gremaud, V. Varga, S. Lalljie, C.L. Schultz, R. Whitaker, J. Kintscher, V. Dudler, L. Castle, W.A. Read, Analyst 129 (2004) 276.
[6] R. Joiner, F. Vidal, H. Marks, Cereal Ind. 40 (1963) 539.
[7] EFSA—European Food Safety Authority, Panel on food additives, flavourings, processing aids and materials in contact with food, Additional advice on semicarbazide, in particular related to baby food, Press release 9 October 2003: http://www.efsa.eu.int/p_foodadd_en.html.
[8] EFSA—European Food Safety Authority, Press release 15 October 2003: http://www.efsa.eu.int/pdf/pressreal20031015_en.pdf.

[9] E. Horne, A. Cadogan, M. O'Keefe, L.A.P. Hoogenboom, Analyst 121 (1996) 1463.
[10] Eurachem, The fitness for purpose of analytical methods, A laboratory guide to method validation and related topics, 1998, 61 pp.
[11] ISO—International Standard Organization, ISO/IEC 17025, General requirements for the competence of testing and calibration laboratories, 1999, 26 pp.
[12] M. Thompson, S.L.R. Ellison, R. Wood, J. Pure Appl. Chem. 74 (2002) 835.
[13] W. Horwitz, Pure Appl. Chem. 67 (1995) 331.
[14] M. Gips, M. Bridzy, S. Barel, S. Soback, Euro Residue II, Veldhoven, Holland, 1993, p. 313.
[15] L.A.P. Hoogenboom, T.H.H.G. Polman, Euro Residue II, Veldhoven, Holland, 1993, p. 376.
[16] P. Armitage, G. Berry, Statistical Methods in Medical Research, third ed., Blackwell Science Ltd., Osney Mead, 1994.
[17] T.A. Ryan, B.L. Joiner, Am. Stat. 27 (1973) 222.
[18] J. Durbin, G.S. Watson, Biometrika 38 (1951) 159.
[19] H. Levene, Contributions to Probability and Statistics, Stanford University Press, Stanford, 1960, p. 278.
[20] M.B. Brown, A.B. Forsythe, J. Am. Stat. Assoc. 69 (1974) 364.
[21] N. Draper, H. Smith, Applied Regression Analysis, Wiley, New York, 1981.
[22] European Commission, Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning performance of analytical methods and the interpretation of results, Official Journal of the European Communities, 2002, L 221/8.
[23] M. Thompson, Analyst 125 (2000) 385.