**Analytical Methods**

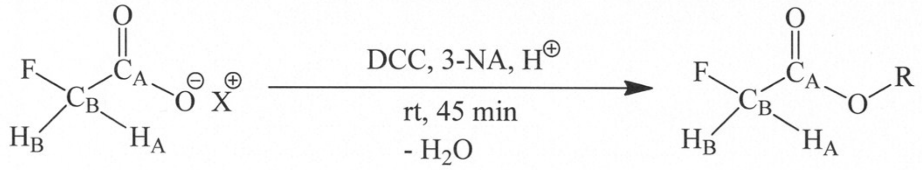
**Electronic Supplementary Information**

# Ultra-trace determination of sodium fluoroacetate (1080) as monofluoroacetate in milk and milk powder by GC-MS/MS and LC-MS/MS

Yiu-Tung Wong*, Wing-Ki Law, Shirley Sau-Ling Lai, Siu-Pan Wong, Kong-Chi Lau and Clare Ho

Analytical and Advisory Services Division, Government Laboratory,
7/F, Ho Man Tin Government Offices, Ho Man Tin, Kowloon,
Hong Kong Special Administrative Region, China

*Corresponding author: Dr. Yiu-Tung Wong, Tel.:+852 27623833
E-mail address: ytwong@govtlab.gov.hk



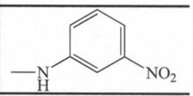
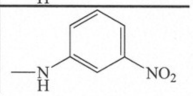

| Compound | $C_A$ | $C_B$ | $H_A$ | $H_B$ | X | R |
|---|---|---|---|---|---|---|
| 1080 | $^{12}C$ | $^{12}C$ | H | H | $Na^{\oplus}$ | - |
| MFA | $^{12}C$ | $^{12}C$ | H | H | - | - |
| MFAA | $^{12}C$ | $^{12}C$ | H | H | $H^{\oplus}$ | - |
| $^{13}C_2D_2$-MFA | $^{13}C$ | $^{13}C$ | D | D | $Na^{\oplus}$ | - |
| MFA-3NA | $^{12}C$ | $^{12}C$ | H | H | - |  |
| $^{13}C_2D_2$-MFA-3NA | $^{13}C$ | $^{13}C$ | D | D | - |  |

**Fig. S1** Structures of 1080, its related compounds and derivatization conditions for MFA

**Table S1** Types of milk and milk powder samples used in this study

| Matrix type | Sample type | Category | Age group | Ingredient type | No. of brands used | Country of origin |
|---|---|---|---|---|---|---|
| Milk | A | Infant milk liquid formula | 0 – 6 months | Cow's milk based | 2 | Malaysia |
| | | | | | | USA |
| | B | Fresh milk | -- | Cow's milk based | 1 | Hong Kong |
| Milk powder | C | Infant formula | 0 – 6 months | Cow's milk based | 2 | Spain |
| | | | | | | Germany |
| | D | Stage 2 | 6 – 12 months | Cow's milk based | 2 | USA |
| | | | | | | Netherlands |
| | E | Stage 3 | 1 – 3 years | Cow's milk based | 2 | New Zealand |
| | | | | | | USA |
| | F | Stage 4 | > 3 years | Cow's milk based | 2 | New Zealand |
| | G | Growing up formula | > 12 months | Goat's milk based | 1 | New Zealand |
| | | Goat whole milk powder | 7 years or above and adults | Goat's milk based | 1 | New Zealand |
| | H | Stage 2 | 6 – 12 months | Soy milk based | 1 | USA |
| | I | Whey protein powder | > 3 years | Pure whey protein | 1 | Switzerland |

**Table S2** A series of EWG substituted anilines evaluated for the MFA derivatization

| EWG substituted aniline | Molecular formula of the derivative | Nominal mass (m/z) | Most abundant precursor ion (m/z) | In-source fragmentation | Remarks |
|---|---|---|---|---|---|
| 2,4-DCA | $C_8H_6Cl_2FNO$ | 221 | 201 | YES | -- |
| 3,4-DCA | $C_8H_6Cl_2FNO$ | 221 | 201 | YES | -- |
| 3,5-DCA | $C_8H_6Cl_2FNO$ | 221 | 201 | YES | -- |
| 2,4-DFA | $C_8H_6F_3NO$ | 189 | 169 | YES | -- |
| 3,4-DFA | $C_8H_6F_3NO$ | 189 | 169 | YES | -- |
| 3,5-DFA | $C_8H_6F_3NO$ | 189 | 169 | YES | -- |
| 2-NA | $C_8H_7FN_2O_3$ | 198 | 198 | NO | -- |
| 3-NA | $C_8H_7FN_2O_3$ | 198 | 198 | NO | -- |
| 3,5-DNA | $C_8H_6FN_3O_5$ | 225 | -- | -- | No reaction |

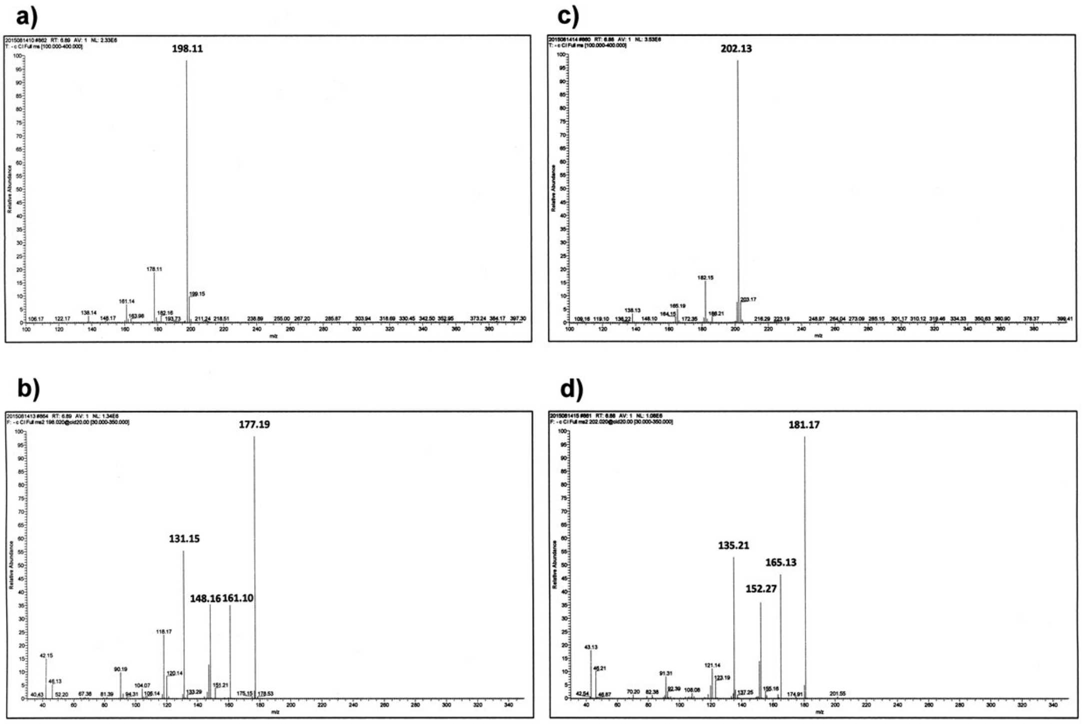



**Fig**. **S2** a) GC-NCI-MS full scan spectra of MFA-3NA; b) GC-NCI-MS/MS product ion scan spectra of MFA-3NA; c) GC-NCI-MS full scan spectra of $^{13}C_2D_2$-MFA-3NA and d) GC-NCI-MS/MS product ion scan spectra of $^{13}C_2D_2$-MFA-3NA.

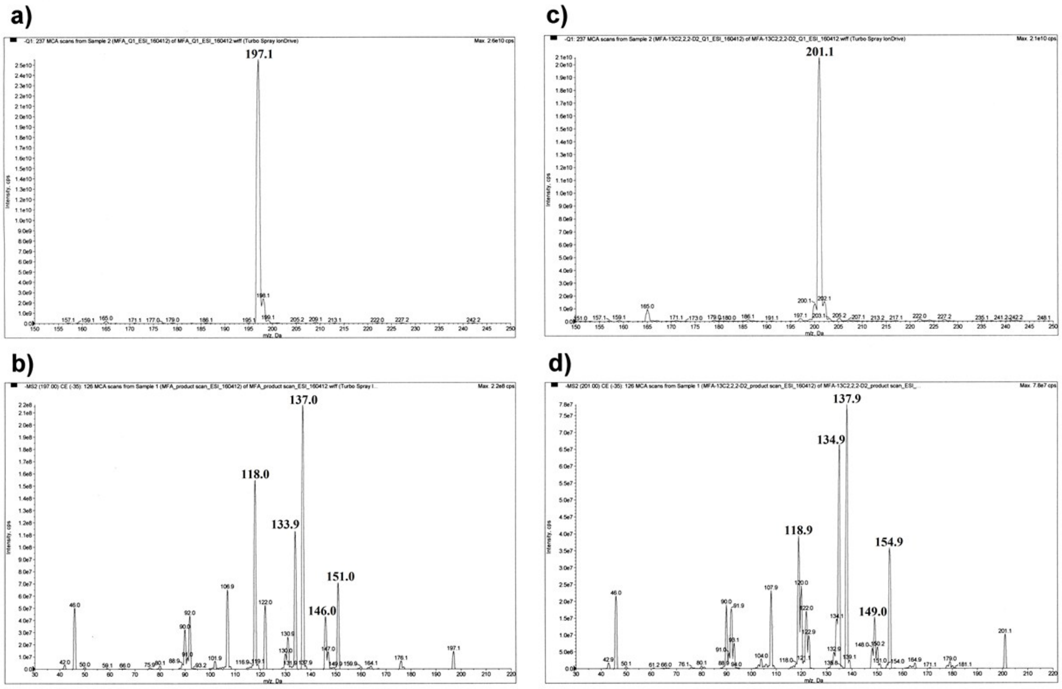



**Fig. S3** a) Negative ion ESI full scan spectra of MFA-3NA; b) Negative ion LC-ESI-MS/MS product ion scan spectra of MFA-3NA; c) Negative ion ESI full scan spectra of $^{13}C_2D_2$-MFA-3NA and d) Negative ion LC-ESI-MS/MS product ion scan spectra of $^{13}C_2D_2$-MFA-3NA.

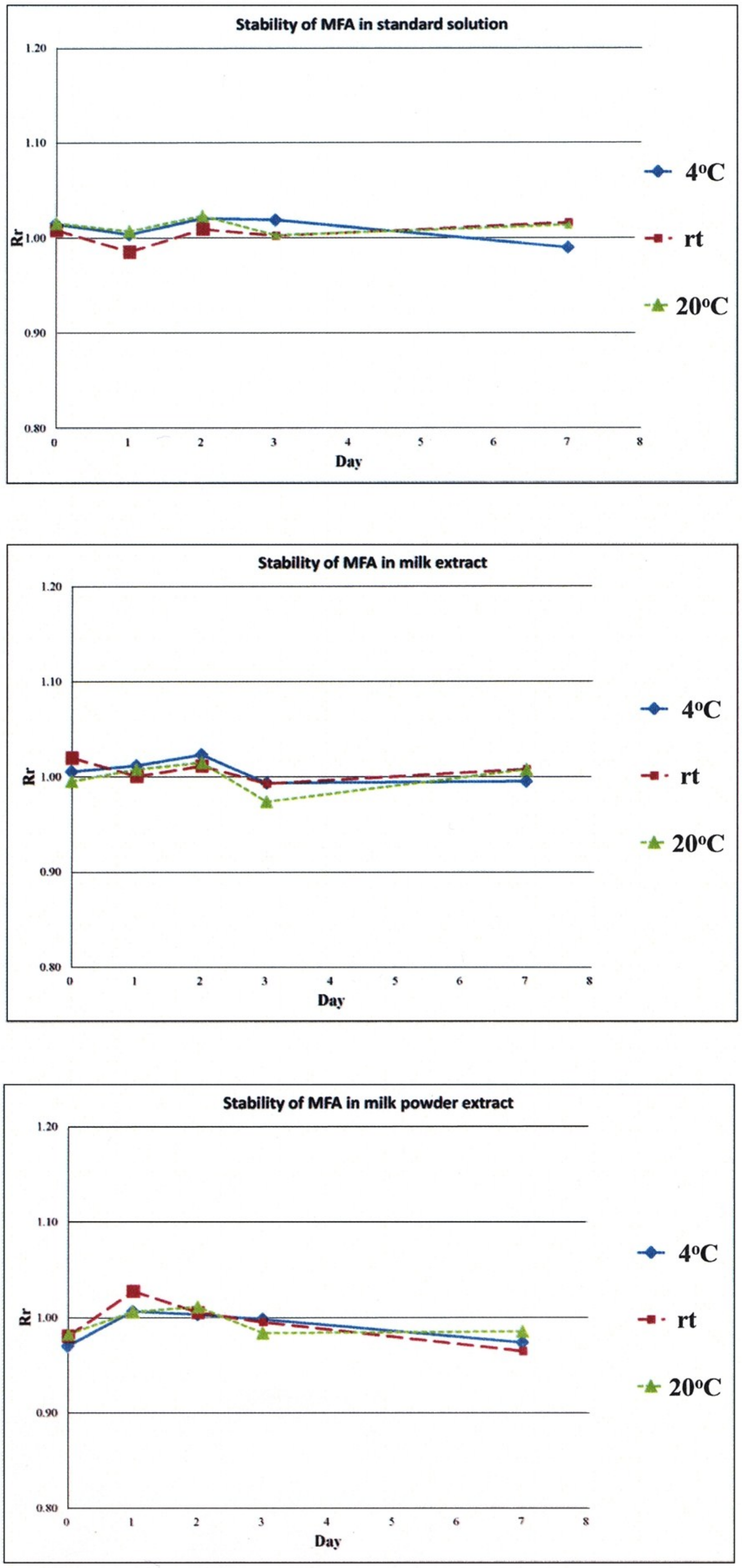



**Fig. S4** Stability of MFA-3NA in different matrices and under different storage temperatures

**Table S3 a)** MRM transitions monitored in GC-MS/MS analysis (CI, -ve mode).

| Compound | Precursor Ion (m/z) | Product Ion (m/z) | CE (eV) | Remark |
|---|---|---|---|---|
| MFA-3NA | 198 | 131 | 25 | Qualification |
| | **198** | **161** | **10** | **Quantitation** |
| | 198 | 177 | 15 | Qualification |
| $^{13}C_2D_2$-MFA-3NA | 202 | 135 | 25 | Qualification |
| | **202** | **165** | **10** | **Quantitation** |
| | 202 | 181 | 10 | Qualification |

**b)** MRM transitions monitored in LC-MS/MS analysis (ESI, -ve mode).

| Compound | Precursor Ion (m/z) | Product Ion (m/z) | DP (V) | CE (V) | Remark |
|---|---|---|---|---|---|
| MFA-3NA | **197** | **118** | **-44** | **25** | **Quantitation** |
| | 197 | 134 | -80 | -24 | Qualification |
| | 197 | 137 | -80 | -24 | Qualification |
| | 197 | 146 | -51 | -33 | Qualification |
| | 197 | 151 | -80 | -28 | Qualification |
| $^{13}C_2D_2$-MFA-3NA | **201** | **119** | **-66** | **-28** | **Quantitation** |
| | 201 | 135 | -40 | -35 | Qualification |
| | 201 | 138 | -30 | -27 | Qualification |
| | 201 | 149 | -30 | -32 | Qualification |
| | 201 | 155 | -60 | -35 | Qualification |

**Table S4** LODs, LOQs, slopes and intercepts of the solution and matrix-matched calibration curves of MFA with regression statistics by GC-MS/MS and LC-MS/MS

| **Matrix** | **Technique** | **a** | **b** | $\mathbf{r^2}$ | $\mathbf{s_b}$ | $\mathbf{s_{y/x}}$ | ***F*** | ***p*** | **LOD** | **LOQ** |
|---|---|---|---|---|---|---|---|---|---|---|
| Solution | GC-MS/MS | $1.59 \times 10^{-3}$ | 0.0137 | 0.999 | $9.07 \times 10^{-5}$ | 0.0165 | $5.1 \times 10^{-4}$ | 1.00 | -- | -- |
| | LC-MS/MS | $1.30 \times 10^{-3}$ | 0.0233 | 1.00 | $3.04 \times 10^{-5}$ | $5.54 \times 10^{-3}$ | 0.66 | 0.63 | -- | -- |
| Milk | GC-MS/MS | $4.03 \times 10^{-4}$ | 0.0133 | 1.00 | $2.95 \times 10^{-5}$ | $5.38 \times 10^{-3}$ | 0.64 | 0.65 | 0.0013 µg/L | 0.0042 µg/L |
| | LC-MS/MS | $-2.43 \times 10^{-3}$ | 0.0241 | 1.00 | $4.08 \times 10^{-5}$ | $7.43 \times 10^{-3}$ | 2.19 | 0.13 | 0.00010 µg/L | 0.00033 µg/L |
| Milk powder | GC-MS/MS | $6.83 \times 10^{-3}$ | 0.0127 | 1.00 | $3.22 \times 10^{-5}$ | $5.87 \times 10^{-3}$ | 0.24 | 0.91 | 0.0025 µg/kg | 0.0085 µg/kg |
| | LC-MS/MS | $4.84 \times 10^{-4}$ | 0.0242 | 1.00 | $3.86 \times 10^{-5}$ | $7.03 \times 10^{-3}$ | 0.027 | 1.00 | 0.0026 µg/kg | 0.0088 µg/kg |

Remarks: The linear regression equations of the calibration curves were expressed in the form of y = bx + a (where y = area of analyte divided by the area of internal standard, b = slope of the regression line, x = concentration of analyte, a = intercept of the regression line, $r^2$ = square of correlation coefficient, $s_b$ = standard deviation of slope, $s_{y/x}$ = standard error of estimate). F = *F*-statistic and *p* = p-value of the lack-of-fit test.

**Table S5** Comparison of the recovery data of MFA at different I.S. fortification levels based on Student's *t*-test

| | | | **Low I.S. spike** | | **High I.S. spike** | | **Student's *t*-test** | |
|---|---|---|---|---|---|---|---|---|
| **Matrix Type** | **Sample Type** | **Measurement Technique** | **Mean Recovery (%)** | **SD (%)** | **Mean Recovery (%)** | **SD (%)** | ***t*-value** | ***p*** |
| Milk Powder | Cow's milk based | GC-MS/MS | 99 | 4.2 | 97 | 1.4 | 0.89 | 0.43 |
| | Whey protein | GC-MS/MS | 100 | 6.1 | 94 | 2.6 | 1.7 | 0.17 |
| | Soy milk based | LC-MS/MS | 98 | 1.6 | 96 | 0.32 | 1.4 | 0.24 |
| | Goat's milk based | LC-MS/MS | 99 | 2.3 | 99 | 1.6 | 0.50 | 0.64 |
| Milk | Infant milk liquid formula | GC-MS/MS | 100 | 5.3 | 99 | 1.8 | 0.22 | 0.83 |
| | Fresh milk | LC-MS/MS | 92 | 5.1 | 100 | 2.6 | 2.4 | 0.075 |

Remarks: Mean recovery was calculated from three independent determinations (N = 3). Critical *t*-value = 2.78 ($\alpha = 0.05$ with degree of freedom = 4).