Applications of High-Field Asymmetric Waveform Ion Mobility Spectrometry for the Certification of Reference Materials.

Speaker: Margaret McCooeye,
National Research Council of Canada,
1200 Montreal Rd.
Ottawa, Ontario Canada, K1A 0R6.
Telephone: (613) 993-2521
Fax: (613 993-2451
Email: peggy.mccooeye@nrc-cnrc.gc.ca

Authors: Margaret McCooeye, National Research Council of Canada
Zoltán Mester, National Research Council of Canada

Abstract

High-field asymmetric waveform ion mobility spectrometry, FAIMS, is a novel gas-phase separation technique, developed at Institute for National Measurement Standards, INMS, and commercialized by Ionaltyics Corporation, ON, Canada. The FAIMS device operates at ambient conditions and is installed between an atmospheric pressure ion source and the mass spectrometer. The first use of FAIMS data in the certification of a reference material was in support of the NIST project to certify ephedrine alkaloids in dietary supplements. As well, FAIMS technology has been applied to the quantitation of amino acids in a yeast matrix with a view to producing a certified reference material.

1.0 Introduction

In chemical metrology, a reference material (RM) is defined as a material or substance, one or more of whose property values are sufficiently homogeneous and well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials. Similarly, a certified reference material (CRM) is defined as a reference material accompanied by a certificate, one or more of whose property values are certified by a procedure which establishes its traceability to an accurate realization of the unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence. The chemical metrology group of the Institute for National Measurement Standards has been producing certified reference materials (CRMs) to verify procedures for the determination of organic and inorganic measurands since 1976. These materials are natural matrices such as seawater, river water, sediments, tissues and most recently plant materials. Matrix reference materials are introduced at the beginning of the analytical process and can therefore be used to assess the quality of the whole analytical process, including sample extraction, clean up and concentration as well as the final measurement step. Recently, the chemical metrology group of the Institute for National Measurement Standards has pioneered

the use of an emerging analytical technology, high field asymmetric waveform ion mobility spectrometry (FAIMS) for the measurement of some analytes. Several reports of quantitative measurements using FAIMS including the separation and quantitation of the sterioisomers of ephedrine appear in the literature[1-5].

2.0 FAIMS

FAIMS takes advantage of the dependence of gas-phase ion mobility, K, on applied electric field, E. In Figure 1, the electric field strength is plotted against the ratio of ion mobility at high electric field, K_h, to ion mobility at low electric field, K. Ions of type A show a small increase in ion mobility as the field increases to about 20,000 V/cm resulting in a change in the ratio, K_h/K, of 5%. Ion C shows a decrease in ion mobility with increasing field strength and ion B shows varying ion mobility with increasing field strength[6]. The change in ion mobility depends on the nature of the ion and the bath or carrier gas used. FAIMS takes advantage of these small changes in ion mobility with changing electric fields in a specific carrier or bath gas.

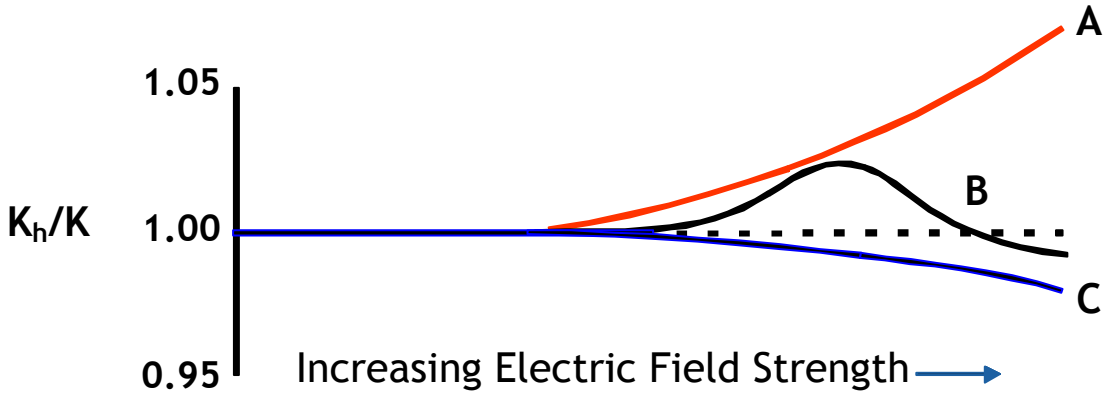

Figure 1 – Theoretical dependence of ion mobility on electric field strength for three different types of ions

The FAIMS device used in the Chemical Metrology Laboratory is a prototype Ionalytics Selectra (Ionalytics, Ottawa, Canada) shown schematically in figure 2. It consists of two axially symmetric steel cylinders mounted inside a PEEK holder and is fastened to the orifice plate of a Sciex API 300 mass spectrometer (Sciex, Toronto, Canada). A high-voltage asymmetric waveform supplies 4000 V, referred to as the dispersion voltage (DV), to the inner cylinder of the FAIMS device. A dc voltage referred to as the compensation voltage (CV) was also supplied to the inner cylinder of the device to overcome the tendency of an ion to drift toward one electrode under the influence of the alternating high and low electric fields supplied by the asymmetric waveform. The CV reflects the difference in ion mobility at high and low electric fields for a particular ion and can either be set to a specific voltage or scanned over a voltage range. The magnitude of the CV depends on properties of both the ion and the curtain/carrier gas. Flow injection analyses are performed using a liquid chromatographic pump and autosampler to deliver the sample in a flowing stream of buffer to the electrospray needle. The FAIMS apparatus is operated at atmospheric pressure and room temperature.

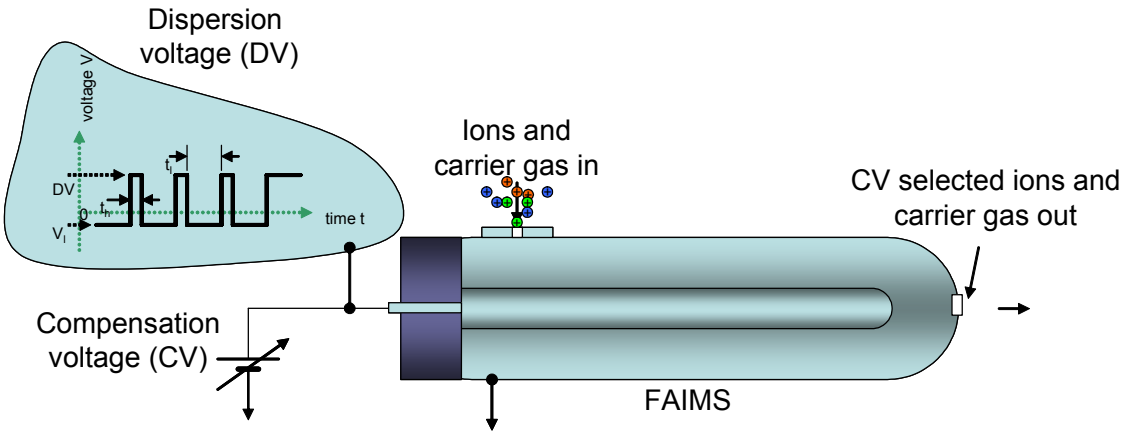

Figure 2 Schematic of a domed FAIMS device

3.0 Applications

3.1 Ephedra-

Ephedra-containing products once represented a large share of the North American market for dietary supplements until concern about their safety resulted in a voluntary recall of some products in Canada [7] and an FDA ruling in the USA that declared dietary supplements that contain ephedrine alkaloids to be adulterated[8]. The International Olympic Committee has put ephedrine on their list of banned substances[9] and Ephedra alkaloids, primarily pseudoephedrine, are also used as starting materials for clandestine laboratories producing methamphetamine for the illegal drug trade[10]. The herb Ephedra Sinica or Ma Huang is a common source of two physiologically active compounds, ephedrine (E) and pseudoephedrine (PE) and their metabolites norephedrine (NE), norpseudoephedrine (NPE), methylephedrine (ME) and methylpseudoephedrine (MPE). Reports of adverse effects of these alkaloids include stroke, heart attacks, heart rate irregularities, seizures, psychoses and deaths[11].

In late 2001, the US Food and Drug Administration began working with the National Institute of Standards and Technology (NIST) and the National Institutes of Health's Office of Dietary Supplements to produce a suite of five ephedra-containing standard reference materials against which analytical methods could be validated and the accuracy of analytical results could be judged. The Chemical Metrology Group of the Institute for National Measurement Standards was invited to participate in this project and the opportunity to use FAIMS in a certification exercise presented itself.

Figure 3 shows the structure of the six ephedrine alkaloids measured as well as the FAIMS separation of the six target analytes. Ephedrine and pseudoephedrine, shown in red, are diastereoisomers, that is, they have exactly the same mass but different molecular spatial geometry due to differing arrangements of functional groups surrounding a chiral carbon atom within the molecule. They also have the same fragmentation pattern when using tandem mass

spectrometry and thus must be separated before they can be measured using mass spectrometry. The red trace shows the mass spectrometer response gained when scanning the compensation voltage of the FAIMS interface while monitoring the mass/charge of ephedrine. Clearly two peaks can be seen at different compensation voltages due to ephedrine and pseudoephedrine. The same can be seen when scanning the compensation voltage and monitoring the methylephedrine ion. A second peak can be seen at a slightly lower compensation voltage for methylpseudoephedrine. Similarly, when monitoring norephedrine, a second peak due to norpseuodephedrine can be seen when compensation voltage is scanned.

NIST provided us with an opportunity to apply this FAIMS separation scheme by including us in their ephedrine certification project. Samples of four candidate SRMs, ephedra sinica stapf, aerial parts, ephedra-containing solid oral dosage form, ephedra-containing protein powder and a commercial extract were supplied.

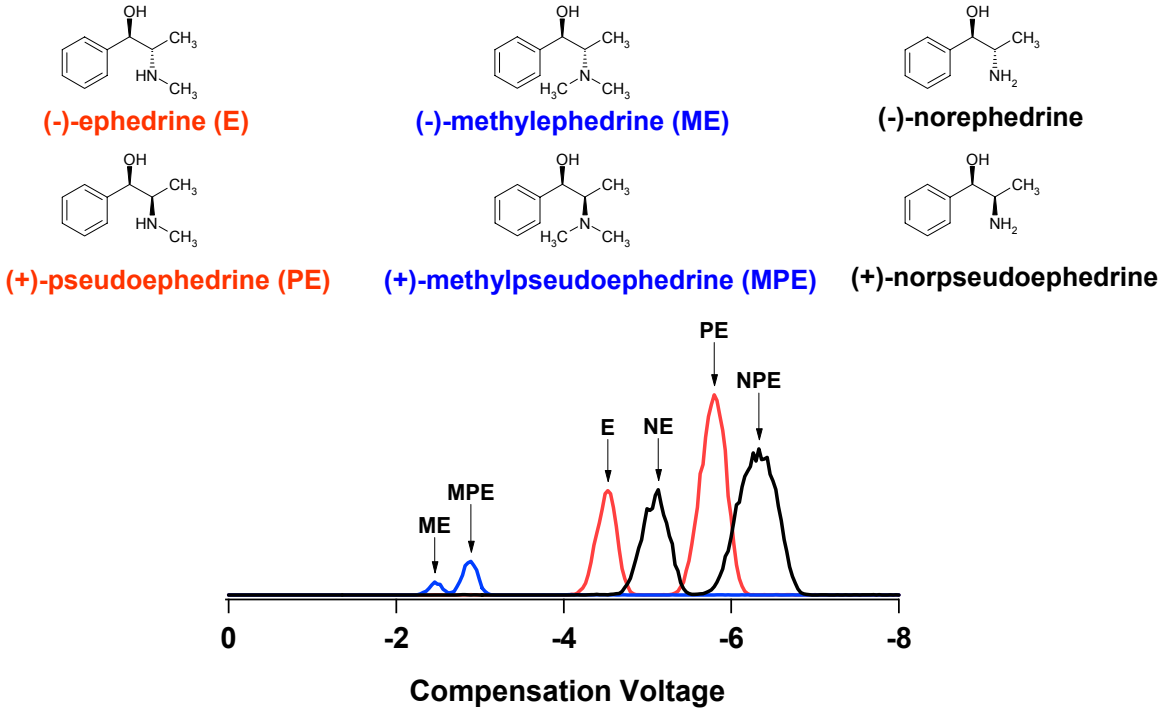

Figure 3 Separation of the sterioisomers of ephedrine

The extraction and sample preparation for FAIMS analysis was straight forward. The sample was subjected to mechanical shaking for 15 min in 25 mL of water containing 3% methanol and 10mM ammonium formate, followed by 45 minutes of sonication. The sample was allowed to cool and topped up to 50 mL total volume for convenience. The mixture was allowed to settle

overnight and an aliquot of the supernatant was filtered. A portion of this filtered sample was weighed and a deuterated ephedrine spike was added. The sample was then diluted with methanol/water containing 0.1 mM ammonium acetate as needed to maintain the mass spectrometer signal within the linear range of the instrument and to reduce signal suppression effects in the electrospray source.

An Agilent series 1100 autosampler was used to inject 10 ul samples into a running buffer supplied by an Agilent series 1100 LC pump(Agilent Technologies, Inc., Palo Alto, CA, USA) The running buffer was 90% methanol, 10% water containing 0.2 mM ammonium acetate. The stream was split to deliver about 5 ul/min to the electrospray needle. The electrospray source was laboratory-built and operated in positive mode with 4000 Volts applied to the needle. The FAIMS interface used in this study was the prototype Ionalytics Selectra (Ionalytics, Ottawa, Canada) with the domed geometry shown earlier. The mass spectrometer is an Applied Biosystems API 300 (Sciex, Toronto, Canada). The FAIMS device was operated at dispersion voltage +4000 volts and compensation voltage was scanned with mass. The curtain/carrier gas was nitrogen. The mass spectrometer software allowed us to set individual CVs while scanning m/z. Table 1 lists the concentration values obtained using ESI-FAIMS-MS for the ephedrine alkaloids and the results of the intercomparison exercise for (a) Ephedra sinica Stapf-aerial parts, SRM 3240 and (b) Ephedra containing solid oral dosage form, SRM 3243. The intercomparison values were assigned using a total of nine sets of measurements from four independent laboratories employing conventional analytical methods. The FAIMS data is generally in good agreement with the intercomparison values obtained using more conventional approaches including liquid chromatography with UV, mass spectrometric and tandem mass spectrometric detection as well as capillary electrophoresis with UV detection and has been included in the determination of the certified values for the ephedrine alkaloids in NIST SRMs 3240 and 3243.

Table 1- Concentration values for ephedrine alkaloids in (a) Ephedra sinica Stapf-aerial parts SRM 3240 and (b) ephedra-containing solid oral dosage form SRM 3243

(a)

	ESI-FAIMS-MS (mg/g)	Intercomparison value (mg/g)
Ephedrine	9.85±0.57	11.31±0.76
Pseudoephedrine	3.30±0.20	3.53±0.26
Norephedrine	0.46±0.07	0.44±0.09
Norpseudoephedrine	0.72±0.11	0.65±0.14
Methylephedrine	1.34±0.11	1.18±0.14
methylpseudoephedrine	0.10±0.01	0.046±0.015

(b)

	ESI-FAIMS-MS (mg/g)	Intercomparison value (mg/g)
Ephedrine	10.83±0.31	11.21±0.42
Pseudoephedrine	2.63±0.10	2.81±0.11

Norephedrine	0.14±0.02	0.16±0.026
Norpseudoephedrine	0.22±0.03	0.186±0.029
Methylephedrine	0.24±0.01	0.323±0.031
methylpseudoephedrine	n.d	0.020±0.011

3.2 Amino Acids

Trace analysis of free amino acids in biological matrices is of interest in the biotechnology industry owing to their significant role in biological function and processes. FAIMS has been used to measure 20 proteinogenic amino acids in a yeast matrix without derivatization with a view to producing a certified reference material in the future. Included in the list of 20 amino acids are two pairs of entries which have the same molecular mass and identical fragmentation patterns in MS/MS. Leucine and Isoleucine, *m/z* $(M-H)^-$ =130, and Lysine and Glutamine, *m/z* $(M+H)^+$ =147. It is necessary, therefore, to separate these ions from each other as well separating all of the ions of interest from the background chemical noise in order to quantify these analytes. Three separate sets of FAIMS conditions were found to provide all of the critical separations. The twenty amino acids and the FAIMS conditions for separation are listed in Table 2.

Table 2(a) FAIMS conditions for amino acid analysis. (a) Amino acids transmitted in FAIMS condition A, DV=4000V, positive ions, N_2 bath gas at 2.5 L/min

Amino Acid	m/z	Compensation voltage
Glycine	76	-21
Alanine	90	-17.1
Serine	106	-16
Proline	116	-15
Threonine	120	-13.9
Valine	118	-12.2
Methionine	150	-9.3
Histidine	156	-7.6
Phenylalanine	166	-6.8
Tryptophan	205	-2.7
Tyrosine	182	-6.4
Arginine	175	-2.7
Cysteine	122	-12.8

Asparagine	133	-12.4
^{13}C Methionine	151	-9.3

Table 2(b) Amino acids transmitted in FAIMS condition B, DV=-4000 V, negative ions, bath gas is air at 2.5 L/min.

Amino Acid	m/z	Compensation voltage
Leucine	130	11.3
Isoleucine	130	12.2
Trans hydroxyl L-proline	130	14.2
Aspartic acid	132	17.5
Asparagine	131	16.5
^{13}C Methionine	149	9.9

Table 2(c) Amino acids transmitted in FAIMS condition C, DV=-4000V, negative ions, bath gas is N_2 containing 2% CO_2 at 2.5 L/min

Amino Acid	m/z	Compensation voltage
Glutamine	145	15.7
Lysine	145	8.5
Glutamic acid	146	13.6
^{13}C Methionine	149	11.4

DV – dispersion voltage

The impact of the FAIMS separation may be seen by comparing the spectra shown in Figure 4. The mass spectrum of a hydrolysed yeast sample in is shown in Figure 4(a) and the FAIMS mass spectrum of the same sample is shown in Figure 4(b). Note that the

target analyte is proline, *m/z* 116. Use of the FAIMS device results in a striking reduction in background chemical noise.

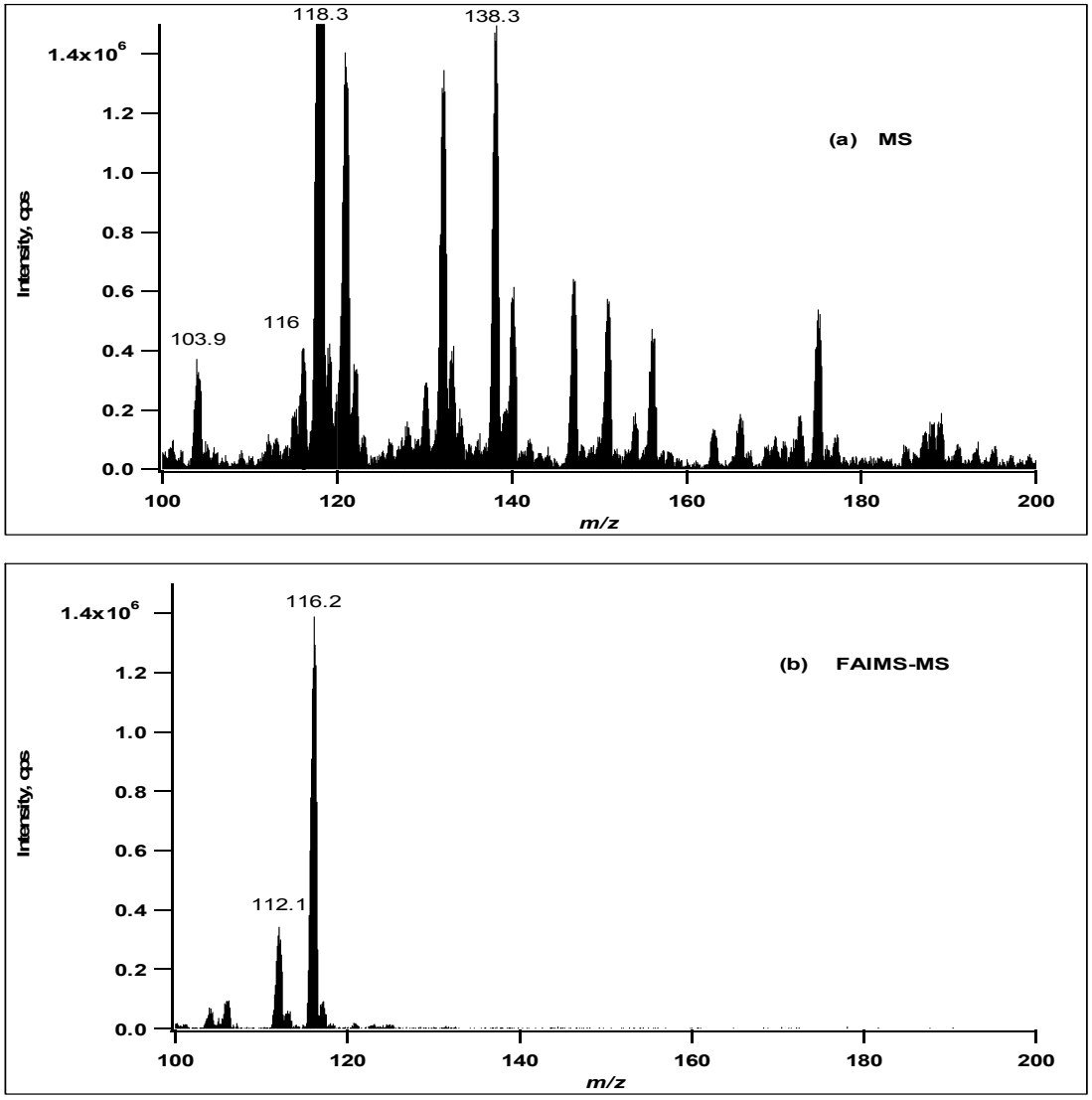

Figure 4 Mass spectra of a hydrolyzed yeast sample dissolved in distilled deionized water and diluted approximately 1:100 with a methanol/water buffer containing 0.2 mM ammonium acetate (m/z 116 is protonated proline): (a) ESI-MS spectrum; (b) ESI-FAIMS-MS spectrum, DV=4000 V, CV=-15 V and carrier gas is nitrogen at 2.5 L/min.

4.0 Conclusion

FAIMS technology, developed at INMS and commercialized by Ionalytics Corporation, is proving to be a valuable tool for the Chemical Metrology Group as it provides an additional independent analytical method. The key benefits of the technology include reduced chemical background, increased selectivity and, in some cases the ability to separate isomers, often leading to reduced limits of detection and faster analysis times.

5.0 References

1. McSheey, S. and Z. Mester, *Arsenic speciation in marine certified reference materials Part 1. Identification of water-soluble arsenic species using multidimensional liquid chromatography combined with inductively coupled plasma, electrospray and electrospray high-field waveform ion mobility spectrometry with mass spectrometric detection.* J. Anal. At. Spectrom., 2004. **19**: p. 373-80.
2. McCooeye, M.A., et al., *Quantitation of morphine and codeine in human urine using high-field asymmetric waveform ion mobility spectrometry (FAIMS) with mass spectrometric detection.* J. Anal. Toxicol., 2001. **25**: p. 81-87.
3. McCooeye, M., et al., *Quantitation of amphetine, methamphetamine and their methylenedioxy derivatives in urine by solid-phase microextraction coupled with electrospray ionization-high-field asymmetric waveform ion mobility spectrometry-mass spectrometry.* Anal. Chem., 2002. **74**: p. 3071-3075.
4. McCooeye, M., et al., *Separation and Quantitation of the Sterioisomers of Ephedra Alkaloids in Natural Health Products Using Flow Injection-Electrospray Ionization-High Field Asymmetric Waveform Ion Mobility Sprectrometry-Mass Spectrometry.* Anal. Chem., 2003. **75**: p. 2538-2542.
5. Ells, B., et al., *Detection of nine chlorinated and brominated haloacetic acids at part-per-trillion levels using ESI-FAIMS-MS.* Anal. Chem., 2000. **72**(19): p. 4555-4559.
6. Mason, E.A. and E.W. McDaniel, *Transport properties of ions in gases*. 1988, New York: John Wiley& Sons, Inc.
7. Canada, H., *Advisory*. 2002, Health Canada.
8. *Food and Drug Administration.* 2004. p. 6787-854.
9. Committee, I.O. and W.A.-D. Agency, *Olympc movement anti-doping code*. 2002.
10. Andrews, K.M., *Ephedra's role as a precursor in the clandestine manufacture of methamphetamine.* J. of Forenssic Sciences, 1995. **40**(4): p. 551-560.
11. Health Canada Website, www.hc.sc.gc.ca/english/protection/warnings/2002