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Effect of methanol quality on the ionisation of herbicides, insecticides and fungicides using gradient elution liquid chromatography

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ABSTRACT

This paper explores the changes in the electrospray signal response of 39 structurally different compounds caused by the quality of the methanol, when used as a component in a gradient elution mobile phase. When three batches of LC–MS grade methanol from one manufacturer were evaluated, the largest variation in the electrospray signal responses of the 39 compounds examined was 18%. However, significant enhancement of the electrospray signals of up to 106% were observed among different brands of LC–MS grade methanol from different manufacturers. The effect of methanol quality on signal response was found to be compound dependent. This study also demonstrated that the senescence of the methanol was important. Using an expired batch of LC–MS grade methanol, electrospray signals were suppressed by as much as 95% for all compounds measured using positive mode electrospray. Conversely, the negative mode electrospray signals of compounds such as 4-octyl benzoic acid showed an enhancement of up to 96% when using the same batch of methanol. Linuron was used as a model compound to examine the change in the electrospray response, during gradient elution, when the proportion of an expired batch of methanol was varied. An infusion experiment showed that the linuron signal intensity decreased as the proportion of expired methanol increased in the mobile phase, which was in direct contrast to the increase in linuron signal observed with a non-expired batch of methanol. A series of isocratic experiments also showed that the linuron signal decreased as the proportion of expired methanol increased in the mobile phase. The ion ratios of several of the compounds studied changed significantly when using the expired batch of LC–MS methanol. The change in the ion ratios accentuates the difficulty of identifying compounds from in-source spectral libraries. A protocol is recommended for assessing the quality of methanol for LC–MS applications.

1. Introduction

The influence of the sample constituents on signal response is the Achilles' heel of atmospheric pressure ionisation (API) liquid chromatography interfaces [1]. This is especially true for quantitative analysis where the response of an analyte can deviate significantly from the true response [2]. A positive deviation from the expected response is commonly referred to as ion enhancement and a negative deviation, ion suppression [3–7]. If ion suppression is severe enough, false negative results may arise and alternatively, significant ion enhancement may yield false positive results.

Ion suppression and enhancement effects are encountered with all types of mass spectrometer analysers, even with tandem mass spectrometers. This is because ion suppression and enhancement occurs solely in the ion source. Post column infusion [8,9] and post extraction spiking [10,11] are indirect methods often used to evaluate signal changes.

Divergence in API responses has been observed when two or more substances are present concurrently in the electrospray ionisation (ESI) ion source [12]. Beaudry and Vachon [13] proposed a physical process for ion suppression by which the co-eluting substances in the mobile phase eluent altered the conductivity, viscosity and surface tension of the droplets produced in the ESI process. The change in those physical properties resulted in a perturbation of the electrospray which subsequently changed the efficiency of gas phase ion formation [14–16].

Both Enke [17] and Kebarle and Tang [18] proposed an alternative theory of a chemical process in which the competition for the surface of the droplets by different ions in an API source was the cause of the matrix effect. Cech and Enke [19,20] further proposed that the ESI signal response was related to the non-polar portion of a compound. A compound with a larger non-polar component would then have an "enhanced affinity" for the droplet surface and yield a higher ESI signal response.

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The divergence in these proposals, as presented in the references above, suggests that the cause of matrix interferences and the resulting impacts on ESI signal response are unlikely to be solely due to a single physical or chemical factor, but a combination of processes. Definitive elucidation of the mechanism for matrix interferences on signal response may take many years' of research.

The constituents of the mobile phase are often overlooked as a component of the matrix. The quality and suitability of all the mobile phase constituents must be considered because they all enter the ion source and influence ion generation. Reproducibility of an analyte peak using LC–MS therefore depends critically on the purity of the mobile phase so that its constituents do not cause undesired signal changes.

Contaminants in LC–MS mobile phases can have a significant impact on the magnitude of ESI responses as previously reported by Annesley et al. [21]. In evaluating the response of 32-desmethoxyrapamycin using 9 brands of methanol from 5 different manufacturers, they [21] demonstrated that the MS/MS peak area could vary by at least an order of magnitude (10-fold). The same group also observed a variation in the ESI response when they used different batches of methanol from a single manufacturer [21].

Napoli et al. [22] also reported that a change in ESI response was observed with different brands of methanol for the analysis of immunosuppressive drugs such as tacrolimus, cyclosporine and sirolimus. A change in ESI response was particularly evident in a sirolimus assay for sirolimus and the internal standard ascomycin.

From literature [23], the process of methanol manufacturing appears to be reasonably direct and involves the reformation of natural gas and steam into a mixture of carbon monoxide, hydrogen and carbon dioxide. The gas mixture is then compressed and reacted in the presence of a copper catalyst to form crude methanol.

It is difficult to clearly identify a possible source of contamination that can influence the electrospray response of analytes. A number of clean up steps are employed, including a series of distillation steps that purify crude methanol and remove gases, water and other by-products. Potentially, sources of contamination could be introduced at any stage of production and includes raw materials, the manufacturing process, the packaging process and even from contaminants in the air.

LC–MS grade methanol is available from many manufacturers. A review of the quality control specifications of several brands of LC–MS grade methanol has shown that they undergo a more rigorous and tighter testing regime than methanol used for other applications. Organic solvents that are produced and targeted for LC–MS analysis should meet the following requirements [24]:

1. yield low mass spectrometric noise, especially for scan applications;
2. contain a low metal content to minimise alkali metal adduct formation;
3. contain minimal amounts of organic contaminants so that chromatographic artefact peaks do not elute when using gradient elution; and
4. provide an optimal and consistent signal for a standard analyte.

Some manufacturers have taken the additional precaution against the formation of sodium adduct ions by bottling LC–MS grade methanol in borosilicate glass containers [25].

It is noteworthy to point out that methanol quality has rarely been assessed for LC–MS applications that employ gradient elution chromatography. Generally detection sensitivity is assessed by the direct infusion of one or two compounds into the mass spectrometer. This work investigates the impact on electrospray ionisation from possible interferences in the methanol when it is used as a component in the mobile phase both at constant (isocratic) and changing mobile phase compositions (gradient). A mixture containing 39 structurally different compounds was chosen to investigate the suppression and enhancement phenomena caused by unknown contaminants in the mobile phase. These compounds were selected in this study for their response and chromatographic characteristics.

In particular, this work used different batches of methanol from a single manufacturer, methanol from multiple manufacturers, and multiple brands from a single manufacturer to evaluate the electrospray response of 39 different compounds using gradient elution liquid chromatography. Both changes in signal intensity and ion ratios were assessed. The effect on the electrospray response when using an aged/expired bottle of a certain brand of methanol was also examined. Finally, a robust procedure is recommended here for assessing the suitability of methanol by analysing a multi-compound mixture with gradient elution LC–MS.

2. Experimental

2.1. Chemicals and reagents

The following compounds which were analyzed using positive mode electrospray (ESI(+)) were obtained from Sigma–Aldrich Pty Ltd. Castle Hill NSW Australia: Pestanal® grade aldicarb, atrazine, bensulfuron methyl, carbaryl, carbendazim, carbofuran, chlorsulfuron, cyanazine, dimethoate, diuron, fenuron, fluometuron, linuron, methabenzthiazuron, metolachlor, metsulfuron methyl, molinate, monuron, neburon, pirimiphos-ethyl, prochloraz, prometryn, propyzamide, siduron, simazine, sulfometuron methyl, terbutryn, thifensulfuron methyl, triasulfuron and triphenyl phosphate (TPP). Ametryn and hexazinone (velpar) were obtained from ChemService Inc., West Chester, PA, USA.

Different compounds were also included in the mixture for evaluating the signal response when using negative mode electrospray (ESI(−)) and these were obtained from Sigma–Aldrich Pty Ltd., Castle Hill, NSW, Australia: 2,4-dichlorophenoxyacetic acid (2,4-D), 4-(2,4-dichlorophenoxy)butyric acid (2,4-DB), bentazone, dichlorprop, 4-chloro-2-methylphenoxyacetic acid (MCPA), mecoprop and 4-octyl benzoic acid. The stock standard solution was prepared as described in Section 2.

Merck Lichrosolv Gradient Grade Methanol, Merck Hypergrade LC–MS methanol was obtained from Merck Pty Ltd., Kilsyth, VIC, Australia. JT Baker Ultra Resi Analyzed Methanol, JT Baker Analyzed LC–MS Reagent Methanol and JT Baker Analyzed Ultra LC–MS Methanol were obtained from Mallinckrodt Baker Inc., Phillipsburg, NJ, USA. Fluka LC–MS Chromasolv Methanol was obtained from Sigma–Aldrich Pty Ltd., Castle Hill, NSW, Australia. Honeywell Burdick and Jackson LC–MS Grade Methanol was obtained from Honeywell International Inc., Morristown, NJ, USA. Each batch of methanol was used as received from each supplier and opened only on the day of testing.

An SG high purity water system was obtained from SG Water USA, LLC Nashua, NH, USA and was used to supply 0.2 μm filtered, UV treated water (18 MΩ cm^{-1} and <2 ppm TOC) for analysis. Fluka Formic Acid Puriss P.A., Eluent Additive for LC–MS ~98% and Fluka Ammonium Hydroxide, Puriss P.A., Eluent Additive for LC–MS were obtained from Sigma–Aldrich Pty Ltd., Castle Hill, NSW, Australia. The same batch of formic acid and ammonium hydroxide was used for all experiments.

2.2. Standard solutions

A stock standard solution containing a mixture of pesticides and herbicides was prepared at various concentrations in 96% methanol (v/v)/4% acetone (v/v). A working standard solution was prepared for this study by dilution of the stock standard solution with 50%

Table 1
Normalised peak areas for 39 compounds using three different batches of Merck LC–MS Methanol.

Name	Rt (min)	Batch I513435950	Batch I496935933	Batch I522535006
ESI(+) mode				
Carbendazim	12.04	100	100	101
Fenuron	13.35	100	88	103
Dimethoate	14.79	100	99	100
Aldicarb	20.11	100	95	98
Cyanazine	23.43	100	95	109
Thifensulfuron methyl	24.35	100	91	115
Monuron	23.95	100	95	97
Simazine	24.74	100	100	100
Triasulfuron	25.05	100	96	107
Metsulfuron methyl	25.31	100	97	112
Hexazinone	25.7	100	97	103
Carbofuran	25.66	100	95	102
Sulfometuron methyl	26.27	100	99	107
Chlorsulfuron	27.23	100	98	116
Carbaryl	28.19	100	93	102
Fluometuron	30.02	100	103	99
Atrazine	30.85	100	105	104
Methabenzthiazuron	31.42	100	104	104
Diuron	33.38	100	112	102
Ametryn	34.73	100	110	102
Bensulfuron methyl	35.69	100	97	108
trans-Siduron	36.83	100	102	112
Linuron	36.91	100	98	105
Molinate	38.92	100	97	102
Propyzamide	38.88	100	98	108
Prometryn	39.62	100	115	109
Terbutryn	40.49	100	113	104
Metolachlor	42.02	100	104	100
Neburon	44.24	100	100	107
Triphenyl phosphate	46.03	100	94	103
Prochloraz	47.08	100	100	103
Pirimiphos-ethyl	51.31	100	104	103
ESI(−) mode				
Bentazone	19.18	100	93	84
2,4-D	28.65	100	103	107
MCPA	30.78	100	96	111
Dichlorprop	34.58	100	98	111
Mecoprop	36.15	100	103	118
2,4-DB	42.08	100	102	105
OBZA	57.49	100	108	105

methanol/50% water (v/v). The methanol used here was Merck Lichrosolv Gradient Grade Methanol.

The stock standard solution was stored at 4 °C in a dark amber bottle and was used for six months. The surface of the storage bottle was deactivated using 5% dimethyldichlorosilane (DMDCS) in toluene followed by methanol exposure and washing. The working standard solution was freshly prepared prior to the start of each set of experiments and was used for a single day only.

2.3. Liquid chromatography–mass spectrometry

Liquid chromatographic separations were performed on a Waters (Waters Corporation, Rydalmere, NSW, Australia) LC–MS system that comprised a Waters 2795 Alliance HT Separation Module, Waters 2996 photo-diode array detector and a Waters ZQ2000 single quadrupole mass spectrometer. The interface was a Z-Spray electrospray design. MassLynx version 4.0 SP4 was used to control the LC–MS instrument and acquire data.

Low adsorption Chromacol Gold Type-33 glass vials were used for all analysis and were obtained from Chromacol Ltd., Welwyn Garden City, Herts, Great Britain. Separations were carried out using a thermostated (30 °C) 250 mm × 4.6 mm, 5 μm Thermo (Thermo Electron Corporation, Waltham, MA, USA) Hypurity C_{18} column and 10 mm × 4 mm and 5 μm Hypurity C_{18} guard column. The same guard column and analytical column was used for all experiments.

Unless specified otherwise, a linear mobile phase gradient was used that changed from 20% (v/v) methanol/75% (v/v) water/5% (v/v) 20 mM formic acid + ammonia (pH 3.5) to 90% (v/v) methanol/5% (v/v) water/5% (v/v) 20 mM formic acid + ammonia (pH 3.5) over sixty minutes. A 1/4 post-column split was placed before the mass spectrometer that allowed approximately 250 μL/min of eluent to enter the ion source and 750 μL/min to waste.

The following ion source settings were employed: capillary voltage 3.5 kV, cone voltage of 30 V for positive ESI and 20 V for negative ESI, extractor 3 V, RF lens 0.5 V, source temperature 120 °C, desolvation gas temperature 350 °C, and desolvation gas of flow rate at 350 L/h. The mass spectrometer was set to scan over the range of 100–450 amu for 1 s. The scan between 100 and 450 amu continuously alternated between positive and negative ESI.

2.4. Procedures

The LC–MS instrument, described in Section 2.3, was set-up and equilibrated for 20 min before use. The ion source entrance cone and shield were cleaned prior to the start of each set of experiments. The HPLC solvent line and frit that was used for methanol delivery was carefully dried and then primed for two minutes when changing brands of methanol. The only variable in each set of experiments was the brand of methanol used in the mobile phase. Any difference in the electrospray response for any compound was solely due to

Table 2
Normalised peak areas for 39 compounds when using four different brands of LC–MS grade methanol.

Name	Merck Hypergrade B# I496935933	JT Baker Ultra Analyzed B# H35E18	JT Baker Analyzed B# H12E22	Fluka Chromasolv B# SZE9335S
ESI(+) mode				
Carbendazim	100	109	99	99
Fenuron	100	95	112	65
Dimethoate	100	100	92	89
Aldicarb	100	108	104	109
Cyanazine	100	178	96	150
Thifensulfuron methyl	100	198	71	178
Monuron	100	89	110	88
Simazine	100	113	91	98
Triasulfuron	100	167	83	161
Metsulfuron methyl	100	203	74	184
Hexazinone	100	111	111	72
Carbofuran	100	110	106	67
Sulfometuron methyl	100	128	112	80
Chlorsulfuron	100	206	83	199
Carbaryl	100	100	123	70
Fluometuron	100	95	116	98
Atrazine	100	111	128	91
Methabenzthiazuron	100	111	114	68
Diuron	100	98	114	103
Ametryn	100	95	141	88
Bensulfuron methyl	100	148	79	135
trans-Siduron	100	120	88	109
Linuron	100	113	84	104
Molinate	100	110	112	88
Propyzamide	100	125	79	115
Prometryn	100	104	134	94
Terbutryn	100	99	130	87
Metolachlor	100	106	123	95
Neburon	100	114	102	108
Triphenyl phosphate	100	118	85	108
Prochloraz	100	113	86	99
Pirimiphos-ethyl	100	101	97	92
ESI(−) mode				
Bentazone	100	95	98	91
2,4-D	100	127	88	121
MCPA	100	121	86	114
Dichlorprop	100	145	94	140
Mecoprop	100	135	94	130
2,4-DB	100	116	92	114
OBZA	100	94	103	88

the brand of methanol used. No significant change in the retention time (>0.1 min) of each compound was observed among different batches of methanol during each comparative study.

All experiments were conducted as experimental sets which are presented as individual tables in Section 3. Each set of experiments took approximately ten or less hours to complete and each analysis was conducted in either duplicate or triplicate to ensure analytical precision. The experimental results presented in Tables 1–3 were performed in the order as presented from left to right. Experimental trends for the results shown in Tables 2–4 were reconfirmed on multiple occasions and also by running the comparisons in a different order. The average % relative standard deviation of the replicate peak area measurements for the data presented in Tables 1–3 was between 3% and 4% and between 8% and 10% for ion ratio replicates in Table 4.

3. Results and discussion

3.1. Variations in signal response using different batches of methanol from a single manufacturer

In order to put the proceeding results into perspective, it is important to initially outline acceptable or normal variations in electrospray response that can be encountered among different batches of LC–MS grade methanol. Table 1 shows the results for three batches of Merck LC–MS Methanol that have been accepted to be used for gradient LC–MS analysis in the authors' laboratory. The results in Table 1 are normalised peak areas for the compounds measured where the data were normalised according to the following formula:

$$\text{Normalised peak area} = \frac{\text{Area}_{B2}}{\text{Area}_{B1}} \times 100$$

where Area_{B1} is the peak area of a compound using the reference batch of methanol and Area_{B2} is the peak area obtained using a new batch of methanol. The peak areas of different batches, shown in Table 1, were normalised against batch I513435950 (Area_{B1}).

An acceptable variation in the normalised peak areas when comparing different batches of methanol is ±30%, which is based on experience in the authors' laboratory. Table 1 shows that the three batches from Merck exhibited acceptable similarity because all of the normalised peak areas for all 39 compounds were within the normalised range of 70–130%. The largest deviation was recorded for mecoprop (118%) using batch I522535006. This result indicates that the reproducibility among batches from the same manufacturer can be relied upon to give a consistent response.

3.2. Change in electrospray response associated with different methanol brands

Table 2 shows the normalised peak areas of all the 39 compounds studied in this work. JT Baker LC–MS Methanol, JT Baker

Table 3
Comparison of the normalised peak areas for 39 compounds when using expired and non-expired batches of methanol.

Name	Merck Gradient Grade B# K39586207907	Honeywell LC–MS B# CX768	Merck Hypergrade B# I489035925	JT Baker Analyzed LC–MS (expired) B# C05E79
ESI(+) mode				
Carbendazim	100	105	96	10
Fenuron	100	88	79	22
Dimethoate	100	94	93	10
Aldicarb	100	93	89	27
Cyanazine	100	112	92	15
Thifensulfuron methyl	100	122	87	41
Monuron	100	84	73	8
Simazine	100	98	100	9
Triasulfuron	100	109	90	33
Metsulfuron methyl	100	121	87	36
Hexazinone	100	97	98	12
Carbofuran	100	94	97	12
Sulfometuron methyl	100	86	75	25
Chlorsulfuron	100	115	89	33
Carbaryl	100	86	86	14
Fluometuron	100	78	69	6
Atrazine	100	89	84	5
Methabenzthiazuron	100	93	89	14
Diuron	100	88	78	6
Ametryn	100	95	93	13
Bensulfuron methyl	100	109	88	28
trans-Siduron	100	104	96	6
Linuron	100	100	95	7
Molinate	100	91	91	22
Propyzamide	100	102	99	9
Prometryn	100	94	92	14
Terbutryn	100	94	96	14
Metolachlor	100	100	82	7
Neburon	100	101	96	6
Triphenyl phosphate	100	104	99	11
Prochloraz	100	98	91	14
Pirimiphos-ethyl	100	97	96	20
ESI(−) mode				
Bentazone	100	97	103	128
2,4-D	100	117	109	133
MCPA	100	110	92	143
Dichlorprop	100	110	83	99
Mecoprop	100	104	94	164
2,4-DB	100	95	62	85
OBZA	100	82	81	196

Ultra LC–MS Methanol, and Fluka LC–MS Methanol were compared against Merck LC–MS Methanol. Generally, the majority of the compounds normalised responses were within the range of 70–130%. However, a significant enhancement (i.e. normalised peak area >130%) was observed for cyanazine (150–178%) and the sulfonyl urea herbicides (135–206%) when using JT Baker Ultra LC–MS Methanol and Fluka LC–MS Methanol in the mobile phase.

When different brands of methanol from JT Baker were compared, the ESI signal response for some compounds was observed to be considerably different. For example, Table 2 shows that when JT Baker Ultra LC–MS Methanol and JT Baker LC–MS Methanol were used, the normalised peak areas for cyanazine were 178% and 96% respectively.

Table 2 also shows that, with the exception of a half dozen compounds, there were also similarities in the electrospray response between JT Baker Ultra LC–MS Methanol and Fluka LC–MS Methanol when compared to Merck LC–MS and JT Baker LC–MS methanol brands. For example, both JT Baker Ultra LC–MS Methanol and Fluka LC–MS Chromasolv Methanol yielded a large enhancement with the signal response of cyanazine, thifensulfuron methyl, metsulfuron methyl, and chlorsulfuron using ESI(+) as well as for dichlorprop and mecoprop using ESI(−). Compounds measured using both negative and positive mode ESI yielded comparable increases in the normalised peak areas in contrast to the Merck LC–MS and JT Baker LC–MS brands of methanol.

Although signal enhancement has been reported in this experiment, signal suppression could also have been reported if JT Baker Ultra LC–MS Methanol was used as the reference. Therefore reporting of ion suppression or enhancement is dependant upon the point of reference. In light of this, it is important to establish an acceptable benchmark for comparing all future brands and batches.

3.3. Change in electrospray response associated with an expired batch of methanol

A significant electrospray response change was observed when four different batches of methanol were assessed for LC–MS gradient application. Fig. 1 shows the total ion chromatogram results from the injection of the mixture described in Section 2.

The methanol component of the mobile phase was either Merck LC–MS Methanol or an expired batch of JT Baker LC–MS Methanol. The expired JT Baker LC–MS Methanol batch was three years old and past the normal expiry period of two years. Fig. 1(a) shows that the ESI(−) total ion chromatograms appear to be reasonably similar between the two brands of methanol. However, the ESI(+) total ion chromatograms (Fig. 1(b)) were significantly different because the majority of the peaks were suppressed when the expired brand of methanol was used. In fact, as shown in Fig. 1(b), many of the chromatographic peaks almost disappeared into the baseline.

Two examples of this differential response are shown in Fig. 2(a) and (b). Fig. 2(a) shows an overlay of the $m/z = 199$ extracted ion

Table 4
Comparison of the normalised ion ratios of 39 compounds when using expired and non-expired batches of LC–MS methanol.

Name	Primary ion[a]	Secondary ion[b]	Normalised ion ratios (quantification/qualifier)		
			Merck Hypergrade B# I489035925	Honeywell LC–MS B# CX768	JT Baker Analyzed LC–MS (Expired) B# C05E79
ESI(+) mode					
Carbendazim	192	160	100	99	52
Fenuron	165	166	100	103	120
Dimethoate	199	171	100	98	66
Aldicarb[c]	116	213	100	221	19
Cyanazine	241	214	100	104	109
Thifensulfuron methyl	388	167	100	109	120
Monuron	199	201	100	104	106
Simazine	202	204	100	100	97
Triasulfuron	402	424	100	90	149
Metsulfuron methyl	382	167	100	100	117
Hexazinone	171	253	100	99	84
Carbofuran	165	222	100	101	185
Sulfometuron methyl	150	365	100	99	187
Chlorsulfuron	358	141	100	101	67
Carbaryl	145	146	100	100	110
Fluometuron	233	234	100	102	161
Atrazine	216	174	100	94	48
Methabenzthiazuron	165	222	100	97	181
Diuron	233	235	100	101	100
Ametryn	228	186	100	102	99
Bensulfuron methyl	411	149	100	105	105
Siduron	233	137	100	106	38
Linuron	249	251	100	100	97
Molinate	126	188	100	99	266
Propyzamide	256	173	100	97	65
Prometryn	242	243	100	105	108
Terbutryn	242	186	100	103	100
Metolachlor	252	254	100	88	117
Neburon	275	277	100	95	96
TPP	327	359	100	107	111
Prochloraz	340	308	100	98	87
Pirimiphos-ethyl	334	198	100	96	155
ESI(−) mode					
Bentazone (ESI−)	239	240	100	63	72
2,4-D (ESI−)	219	221	100	110	107
MCPA (ESI−)	199	201	100	100	99
Dichlorprop (ESI−)	233	235	100	101	94
Mecoprop (ESI−)	213	215	100	91	80
2,4-DB (ESI−)	161	163	100	96	95
OBZA (ESI−)	233	234	100	100	94

[a] The m/z value of the largest peak in a compounds mass spectrum.
[b] The m/z value of a characteristic ion in a compounds mass spectrum that is different to the primary ion.
[c] Results not reliable for aldicarb due to poor reproducibility among assay replicates.

chromatograms for dimethoate that were obtained using Merck LC–MS Methanol, Honeywell LC–MS Methanol and an expired batch of JT Baker LC–MS Methanol. The peaks from the Merck LC–MS Methanol and Honeywell LC–MS Methanol brands almost coalesced, whilst the peak from the expired batch of JT Baker LC–MS Methanol was reduced by approximately 90%. The same observation can also be seen in the extracted ion chromatograms for diuron as presented in Fig. 2(b). It is important to point out here that although the two results shown in Fig. 2(a) and (b) present an almost identical trend, the percentage of methanol in the mobile phase at the time of elution was 37.4% (v/v) and 59.0% (v/v) respectively.

For comparative purposes, Table 3 shows the normalised peak area results of the 39 analytes using four brands of methanol. There were two methanol brands from Merck, one from Honeywell and also the expired batch of JT Baker LC–MS Methanol. As shown in Table 3, use of the expired JT Baker LC–MS Methanol resulted in comparatively severe signal suppression when using positive ESI mode. Notably, the normalised peak areas for all of the compounds were within the range of 5–41%. The normalised peak areas were between 78–122% for the Honeywell LC–MS Methanol and between 69% and 100% for Merck LC–MS Methanol.

When negative ESI mode was used, the normalised peak area results were between 85% and 196%, 82% and 117%, and 62% and 109% for the expired JT Baker, Honeywell and Merck LC–MS brand methanol respectively. The normalised peak area of 196% reported for 4-octylbenzoic acid (OBZA) using the expired JT Baker LC–MS methanol was especially notable considering the severe signal suppression encountered for all compounds acquired with the ESI(+) mode.

A review of the data in Table 3 shows that the magnitude of ion suppression and enhancement associated with the use of the expired JT Baker Methanol was unpredictable. Specifically, there was no discernible trend regarding the magnitude of the ion suppression with positive mode electrospray, nor enhancement with negative mode electrospray as the percentage of methanol increased over the course of the gradient run. The lack of any predictable trend with ion suppression or enhancement effects was most likely due to the difference in the structures of the compounds contained in the mixture.

This unpredictable behaviour was particularly notable when comparing the ESI(−) response for the two structurally related compounds, dichlorprop and mecoprop using the expired batch of methanol. The only difference between the two compounds is that

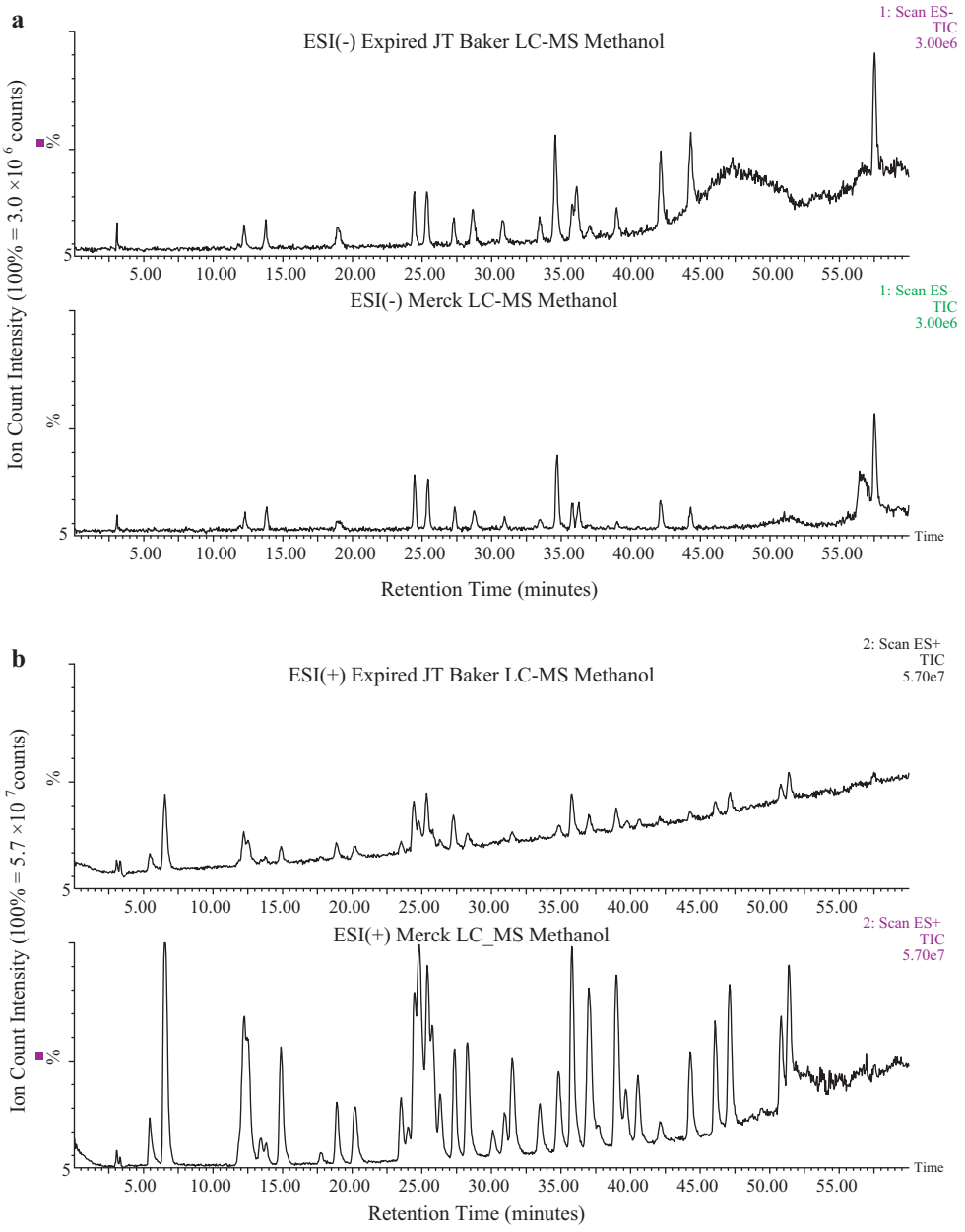

Fig. 1. Total ion chromatograms of a mixture of herbicides, fungicides and insecticides using two different brands of methanol using (a) ESI(−) mode and (b) ESI(+) mode.

one of the two chlorine atoms of dichlorprop has been replaced by a methyl group in mecoprop. Whilst no significant suppression or enhancement was observed for the former, a normalised enhancement of 64% was observed for the latter.

The difference in the observed response for dichlorprop and mecoprop is consistent with the proposal for ion enhancement given by Cech and Enke [19,20]. The substitution of the chlorine atom by a methyl group in mecoprop decreases the polarity of mecoprop compared to dichlorprop. Consequently, according to Cech and Enke [19,20], the mecoprop ion would have a higher affinity for the ESI droplet surface compared to the dichlorprop species. This polarity difference may be the reason that relative signal enhancement was observed for mecoprop and no significant change was observed for dichlorprop.

As indicated by the results in the last column of Table 3, because ion suppression was encountered with the ESI(+) mode and enhancement with the ESI(−) mode, there must be a change to the dynamics of ion evaporation and/or ejection. It is also speculative that the properties of the droplets formed through ESI(+) and ESI(−) are different and as a consequence, suppression was observed with ESI(+) and enhancement with ESI(−). The substance in the expired JT Baker solvent causing ion suppression and enhancement must contain different functional groups in order to affect both modes of electrospray ionisation. However, there is no definitive data in supporting such hypothesis and further investigation is required.

3.4. Extent of ion suppression with respect to %methanol in mobile phase

As noted in the previous section, no suppression trend could be discerned along the course of the mobile phase gradient. If there was one, it is probably hidden among the varying magnitude of signal responses of the structurally different compounds contained in the mixture.

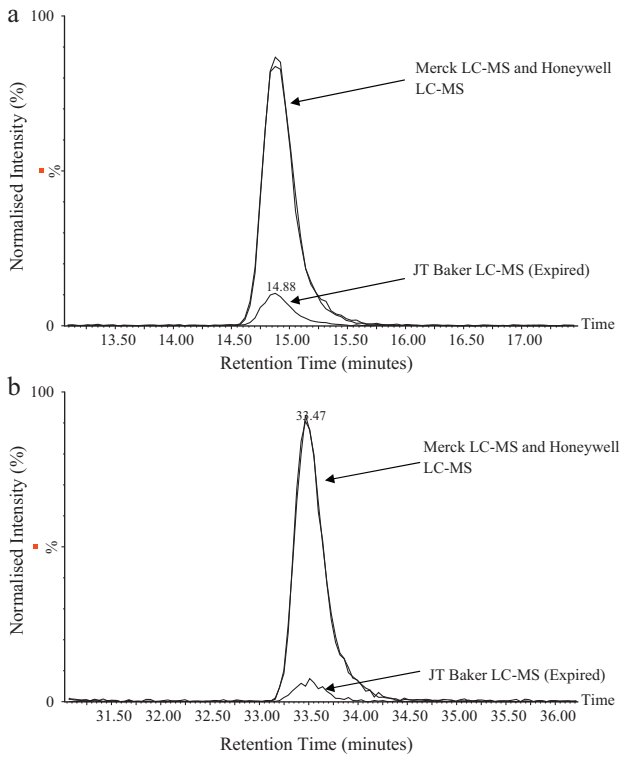

Fig. 2. ESI(+) extracted ion chromatograms of (a) dimethoate at $m/z = 199$ and (b) diuron at $m/z = 233$ using different brands of methanol as a mobile phase component.

Two experiments were therefore conducted to examine the ion suppression trend with an increasing proportion of an expired lot of GC grade methanol (JT Baker Ultra Resi Analyzed Methanol) in the mobile phase. This batch of methanol was chosen because it yielded ESI(+) ion suppression similar to the JT Baker LC–MS Methanol that was totally consumed in the experiment that produced the results presented in Table 3.

Using the setup shown in Fig. 3(a), the first experiment involved the introduction of a mobile phase gradient eluent directly into the mass spectrometer at a rate of 250 μL/minute. A 500 ppb standard solution of linuron was infused continuously into the mobile phase stream after a C_{18} guard column during the gradient, at approximately 10 μL/min. The linear gradient, as shown in Fig. 3(b), started at two minutes from 10% (v/v) and finished at 15 min with 90% (v/v) methanol.

In order to produce a more pronounced trend concerning the ion suppression effect from the expired JT Baker solvent, the JT Baker solvent was effectively diluted with Merck LC–MS Methanol during the gradient experiment. Use of the JT Baker solvent alone did not show a clear trend because the ion suppression effect was too strong, even at the start of the mobile phase gradient. The linear gradient was the same as is shown in Fig. 3(b), but consisted of 1% JT Baker v/v/9% Merck v/v for two minutes followed by a linear ramp to 50% JT Baker v/v/40% Merck v/v at 15 min.

Fig. 3(b) shows that when Merck LC–MS Methanol was used in the gradient run, an increase in the linuron SIR signal was observed as the amount of methanol increased in the mobile phase eluent. This is in agreement with previous observations [26], because an increase in the droplet volatility and a decrease in droplet surface tension occur in the ion source as the percentage of methanol increases. Consequently, both ion desolvation and ion generation efficiency have been enhanced.

Fig. 3(b) also shows the corresponding linuron SIR profile when the expired JT Baker and Merck solvent combination was used in the gradient run. The profile shows a decrease in signal intensity with an increase in the percentage of methanol, which resulted from a competition between the enhancement associated with increasing methanol and ion suppression caused by the suppressing agent within the JT Baker solvent component. However, Fig. 3(b) shows that the linuron infusion signal decreased when the amount of suppressing agent was increased during gradient elution.

The results of the second experiment are shown in Fig. 4. This experiment was conducted under isocratic conditions where the mobile phase consisted of 80% (v/v) methanol/20% (v/v) water with 1 mM formic acid/NH_4OH (pH 3.5). The injection volume of the 500 ppb linuron solution was 25 μL. The methanol mobile phase component was composed of different proportions of the Merck LC–MS Methanol and the expired JT Baker Ultra-Resi Analyzed Methanol. Fig. 4 shows that the linuron peak size was largest when the mobile phase only contained methanol from Merck. A decrease in the linuron peak area was observed as the proportion of the expired JT Baker solvent was increased to approximately 40% (v/v), where the profile plateaued.

In summation, the results shown in Fig. 4 are in general agreement with those shown in Fig. 3(b). That is, ion suppression increased initially with an increase in the amount of the suppressing mobile phase component and then flattened out afterwards.

3.5. Effects on ion spectra

The ion ratios of two ions from each of the 39 compounds were evaluated to discern any change from the use of different methanol brands and the results are shown in Table 4. It must be pointed out here that the ion ratio for aldicarb was not stable, even when using one batch of methanol. Consequently, the ion ratio results for this compound should be ignored for now and this will be investigated further.

The methanol batches included in this evaluation were Merck LC–MS, Honeywell LC–MS and the expired batch of JT Baker LC–MS Methanol. As previously noted, the expired batch of JT Baker LC–MS Methanol caused ESI(+) signals to be suppressed and ESI(−) signals to be enhanced. A review of the results presented in Table 4 shows that the normalised ion ratios for some of the compounds varied by more than 30% when the expired JT Baker solvent was used compared to either the Merck or Honeywell solvents. The ion ratios (quantifier ion/qualifier ion) of the 39 compounds studied were normalised in a similar way as for the peak areas as discussed in the sections above.

The enhancement/suppression effect on the normalised ion ratios is dependent on whether or not the m/z value of the quantifier ion was larger than the m/z value of the qualifier ion. The normalised ion ratio for carbendazim, as presented in Table 4, was 100% and 99% when using the Merck and Honeywell solvents but was noted to decrease to 52% when using the expired JT Baker solvent. Likewise, the same trend was observed with dimethoate (100, 98 and 66% respectively), chlorsufuron (100, 101 and 67% respectively), siduron (100, 106 and 38% respectively), atrazine (100, 94 and 48% respectively) and propyzamide (100, 97 and 65% respectively). A reduction of the normalised ratios was due to either a decrease in the quantifier ion or an increase in qualifier ion, or both.

When the m/z value of the quantifier ion was lower than the qualifier ion, the normalised ion ratios of some compounds were noted to increase significantly when using the expired JT Baker solvent. This observation is exemplified by the normalised ion ratio results for triasulfuron (100, 90 and 149%), carbofuran (100, 101 and 185%), sulfometuron methyl (100, 99 and 187%), fluometuron (100, 102 and 161%), methabenzthiazuron (100, 97 and 181%) and molinate (100, 99 and 266%). The effect of the expired JT Baker LC–MS Methanol on the ion ratios would accentuate the difficulty in

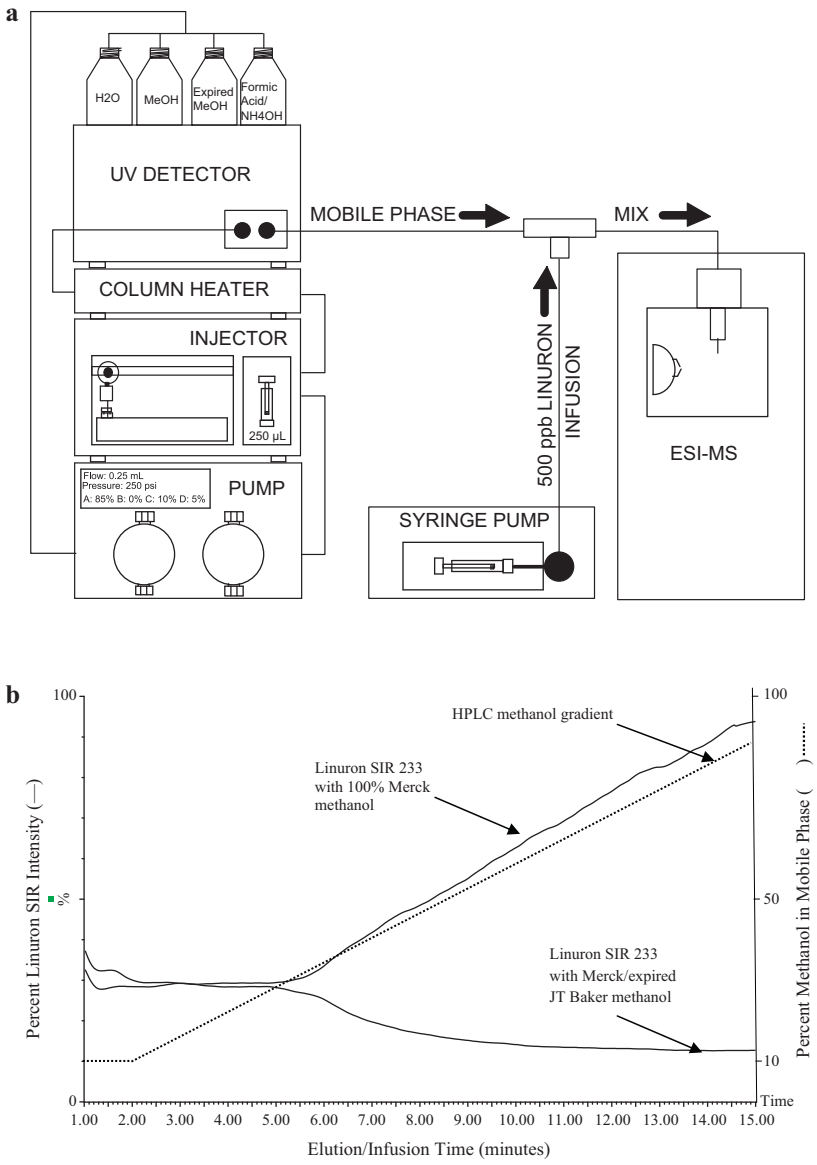

Fig. 3. (a) Schematics for the linuron infusion experiment. (b) ESI(+) SIR infusion profiles at $m/z = 233$ of a 500 ppb linuron solution when using methanol gradient elution.

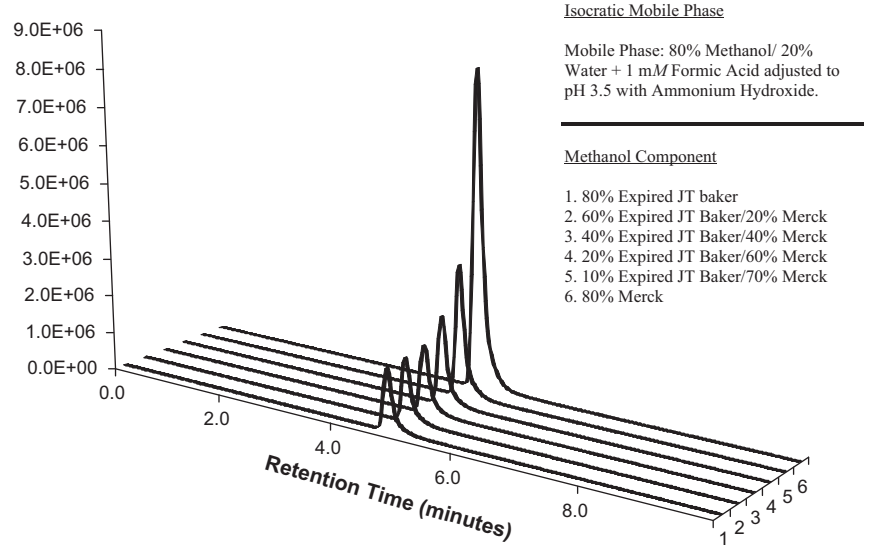

Fig. 4. The changing ESI(+) signal response of linuron with a change in the ratio of expired JT Baker LC–MS Methanol to Merck LC–MS Methanol.

positively identifying compounds using an in-source spectral library and also identification with quantitative methods.

3.6. In-house methanol quality acceptance test

The suppression and enhancement problems associated with methanol quality demonstrated in this work can be extended to other organic solvents used for LC–MS applications. Because of this, a simple testing regime is required to evaluate their suitability for LC–MS work when using gradient elution chromatography. To ensure consistency in quality, a batch of an LC–MS solvent should always be ordered in sufficient quantities to last 6–12 months. A bottle of solvent with proven performance should be used as a reference when assessing the performance of a new batch. The acceptance criteria used in-house for this laboratory are:

1. A gradient elution analysis of a mixture containing at least 10 different compounds must be performed in duplicate. The mix of compounds must be selected so that half are sensitive to ESI(+) and the other half to ESI(−). The retention time of compounds within each group should be spread over the course of the gradient.
2. The normalised peak areas of the compounds in the mixture are to be compared to those obtained using the reference solvent and they should not deviate outside of the normalised range of 70% to 130%.

   A constant level of ion suppression has a more detrimental effect on method performance than a constant level of ion enhancement. Constant ion suppression will increase the method detection limit, whereas constant ion enhancement will improve method sensitivity. Any quantitative result that is below the quantitative reporting limit will still be below the reporting limit, regardless of whether constant ion enhancement or suppression is encountered. A new solvent batch may be accepted if an enhancement is obtained.
3. An overlay of the total ion chromatograms (TIC) from a blank injection, obtained using the reference batch and new batch, must be compared using an absolute scale. The ion count of the baseline, preferably at the start and end of the gradient program, should not be larger than a factor of 5 for the new solvent as experienced in this author's laboratory.
4. The TICs from the reference and new methanol should be compared in order to identify organic contaminants in the new methanol batch. Organic contaminants in methanol are normally only visible in LC–MS chromatograms under gradient elution. Generally, those organic contaminants focus on the inlet side of the HPLC column at the start of the mobile phase gradient and elute as a large chromatographic interference at some latter point.

Acceptance or rejection based on this test is dependent upon the time that the organic contaminant elutes. The organic contaminant may elute as a continuum, right up to the point of the maximal percentage of methanol in the gradient. Therefore, ion suppression can then occur over a large portion of the gradient. New solvents can be accepted if the contaminants do not negatively affect the electrospray intensity.

4. Conclusion

The quality of the methanol, as a component of the mobile phase, can have a significant effect on ESI signal response, particularly when it is used with gradient elution chromatography. The change in quality can be due to brand differences or ageing of the solvent. Interferences are manifested by significant enhancement or suppression of the electrospray signal response as well by a change of the ion ratios. The extent of suppression or enhancement is largely unpredictable and may occur under either positive or negative ESI modes.

For LC–MS applications using gradient elution chromatography, it is imperative to evaluate the suitability of a new batch of methanol based on a laboratory's own set of performance indicators. Such an evaluation must include a mix of standard compounds that are amenable to ESI(+) and ESI(−), as well as having retention times that are spaced along the course of the gradient. LC–MS methods, based on gradient elution, are increasingly being used for the analysis of multi component mixtures, particularly in environmental fields. In environmental forensics, rapid scanning for a vast array of pesticides or herbicides is often required for containment and/or regulatory purposes. Interferences from organic components in the mobile phase may yield false negative results with disastrous environmental consequences. Alternatively, such interferences could also adversely affect method performance parameters such as standard curve linearity and gradient.

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